Multivariate Discrimination of Body Shape Plasticity In The Long Whiskers Catfish (*Mystus Gulio*) Collected From Different Sources And Salinity Gradient Habitats

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Roll No: 0122/05

Registration No: 1105

Session: January-June'2022

The thesis submitted in the partial fulfilment of the requirements for the degree of Master of Science in Marine Bioresource Science

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June, 2023

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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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Acknowledgments

The author is indebted to Almighty Allah who enabled him to complete the research work and write up the dissertation successfully for the degree of Master of Science (MS) in Marine Bioresource Science under the Department of Marine Bioresource Science, Chattogram Veterinary and Animal Sciences University (CVASU).

The author expresses his sincere thanks and gratitude to his supervisor ***Dr. Md Asaduzzaman****, Associate Professor, Department of Marine Bioresource Science, CVASU for his valuable supervision and guidance. It was really a great pleasure and an amazing experience for him to work under his supervision.*

The author expresses his profound gratitude to his co-supervisor, ***Nayeema Ferdausy Hoque,*** Assistant Professor*, Department of Marine Bioresource Science, CVASU for his valuable advice, scholastic guidance, suggestions, and inspiration. His dynamism, vision, and confidence inspired him and gave him confidence and strength.*

The author sincerely expresses his deepest sense of gratitude and indebtedness to honorable Vice-chancellor ***Prof. Dr. A S M Lutful Ahasan*** *and* ***Prof. Dr. Mohammed Nurul Absar Khan****, Dean, Faculty of Fisheries, CVASU for their supportive administrative co-ordination to fulfill his research.*

The author is greatly indebted to his senior ***Fahmida Sultana*** *for her encouragement and cooperation at every stage of this study from its inception to completion.*

Last but not least, the author expresses his deepest sense of gratitude to his beloved parents ***Md Jakar Islam*** *and* ***Aktara Begum*** *for their sacrifice, blessings, and encouragement.*

The Author

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ABBREVIATION

DFA	Discriminant Function Analysis
PCA	Principle Component Analysis
GM	Geometric Morphometrics
GMA	Geometric Morphometric Analysis
ANOVA	Analysis of Variance
SPSS	Statistical Package for Social Science
F Value	Variation between sample means / variation within the samples
P	Level of Significance
SD	Standard Deviation
NS	Not Significant
DF	Degrees of freedom
LDA	Linear Discriminant Analysis
i.e	Examples
et al.	Associates

ABSTRACT

The study was carried out to investigate the body shape variation of the *Mystus gulio* collected from three different salinity gradient habitats (high-salinity brackish water, low-salinity brackish water, and freshwater) and two different sources (wild and hatchery) using traditional morphometrics (linear and truss-networking distances) and landmark-based geometric morphometrics. A total of 436 individuals of *M. gulio* were collected from the high-salinity (8-15 ppt), low-salinity (2-6 ppt), and freshwater bodies (0 ppt). For wild versus hatchery sources body shape variation, 430 individuals of *M. gulio* were collected from various coastal rivers and hatcheries. *M. gulio* from different salinity gradient habitats and sources exhibited negligible sexual dimorphism. Principal component analysis (PCA) indicated that high and low salinity populations appeared to form overlapping clusters with the freshwater populations, but other multivariate analyses discriminated both populations from brackishwater habitats from the freshwater counterparts. The geometric morphometrics displayed that the body shape variation of *M. gulio* from salinity gradient habitats was mainly visible in the width of the dorsal-ventral part, the snout shape, the tail shape, the head region and the eye diameter. PCA demonstrated that multivariate spaces of both the wild and captive populations overlapped each other, although many individuals of the wild population were discriminated from the culture populations. Our results showed that traditional and geometric morphometric methods provide consistent outcomes for body shape discrimination in the *M. gulio* populations.

Keywords: *Mystus gulio*; Morphometric variation; Body shape; Truss networks; Wild population, Salinity; Captive fish

CHAPTER I

INTRODUCTION

The Long Whisker Catfish, scientifically known as *Mystus gulio* (Hamilton-Buchanan, 1822), belongs to the Bagridae family and is commonly referred to as "nona tengra" in the local dialect. This species is an oviparous estuarine fish with euryhaline characteristics, and is primarily distributed along the eastern coast of Bangladesh and India (Rahman et al., 2020; Siddiky et al., 2015; Hossain et al., 2015;). It has also been reported in Sri Lanka, , Java, Indonesia, Pakistan, Nepal, China, Myanmar, and Malaysia (Dong et al., 2012; Roberts, 1993). The primary habitat of *M. gulio* is brackish water, which can also migrate and live in zero-salinity water bodies like rivers, canals, haoars, and lakes (Shafi, 2001). From 1960 to 2000, the catch of this species decreased by 33.6 % in the southern coastal area area (Patra et al., 2005). Moreover, this species is listed as a state of least concern (IUCN Bangladesh, 2015), vulnerable (IUCN, 2000; Mukherjee et al., 2002), and near threatened (Patra et al., 2005) at different times. Even though *M. gulio* is not endangered and is naturally caught in substantial quantity, its annual catch rate is decreasing day by day due to indiscriminate overexploitation, climate change, destruction of habitat, high fishing pressure, and various ecological and anthropological impacts (Hossain et al., 2015; Alam et al., 2006). This small indigenous species has both national and export demand due to its delicacy in taste, high nutrient content, better consumer acceptance, high market price, and good ornamental value (Begum et al., 2010; Gupta and Banerjee, 2014; Haniffa, 2009). The adaptability of this species to varying salinity levels and its robust physiological characteristics render it a viable option for cultivation in both coastal and freshwater environments within Southeast Asia. (Begum et al., 2008, 2009; Ross et al., 2003; Haniffa, 2009). Artificial breeding technology for this species was developed by the Bangladesh Fisheries Research Institute (Alam et al., 2006). Therefore, the captive culture practice is recently gaining popularity in polyculture with other fish species in both coastal and inland waterbodies due to the abundance of seed production from hatchery sources (Alam et al., 2007). A deeper understanding of the broodstocks of this species with unique and desirable morphological characteristics is essential for improved quality seed production in hatcheries, facilitating improved management practices and devising conservation strategies for the wild population.

The morphometric study is one of the most commonly employed techniques that is both economical and straightforward in its implementation for identifying and characterizing fish stocks (Siddik et al., 2016) in establishing the arrangement of fish varieties. (Cheng et al., 2005) and differentiating within or between the closely related fish populations (Siddik et al., 2015). Even though genetic identification is more trustworthy, truss networking and geometric morphometrics methods can also be reliable and subtle in doing these tasks (Costa et al., 2003). On the contrary, traditional morphometrics has been limited to using linear measurements such as widths, lengths, masses, areas, ratios, and angles (Bonhomme et al., 2014). Truss networking is a landmark-based quantitative method of measuring body distances and shapes through a set of interconnected lines and forming a pattern of adjacent quadrilaterals referred to as trussed boxes over the entire body (Mahfuj et al., 2019). For more likelihood of extracting the morphometric variation, its use has increased in various research studies over the few decades. Another advanced method, geometric morphometrics, is currently regarded as the most precise morphometric tool (Rohlf and Slice, 1990; Marcus et al., 1996). The geometric approach based on landmarks is proficient in capturing significant information regarding species morphology, without any limitations on the direction of variation or the location of shape alterations. The utilization of diverse image processing tools has considerably contributed to the enhancement of fish morphometric analysis. Moreover, it is feasible to visually represent the modifications and conversions necessary for distinguishing between distinct shapes and reconstructing the shape of a collective agreement and a theoretical shape of a shared progenitor. Multivariate and cluster analyses are utilized to identify the degree of phenotypic variability present in divergent stocks of diverse fish populations (Cavalcanti et al., 1999; Cadrin and Friedland, 1999; Zelditch et al., 2004).

The specialized body shape found in fish and other aquatic organisms is an evolutionary adaptation due to the variations of environmental factors originating from waterbodies where the organisms grow and inhibit (Knouft, 2003; Pflieger, 2004; Moyle and Cech, 2004). When populations inhabit different environments and geolocations, divergent selective forces can lead to morphological differentiation in body shapes and features with a local fitness benefit (Kawecki and Ebert, 2004). Moreover, the isolation of populations for an extended time and interbreeding can also result in morphometric variation between populations (Yamamoto et al., 2006; AnvariFar et al., 2011). Various studies have shown a

strong correlation between the morphometric traits of fish and surrounding environmental factors like temperature, salinity, food availability, water movements, predator abundance, soil types, etc. (Sharker et al., 2015; Nahar et al., 2015). As with other factors, habitat salinity directly or indirectly affects fish growth, survival, metabolism, and body shape (Semra et al., 2013; Styga et al., 2019; Eagderi et al., 2019; Mandal et al., 2020). It may also influence predators' composition, directly affecting selective evolutionary advantage to a species for body shape divergence. Moreover, fish naturally make an effective variation in body shape while living in a diverse saline environment to maintain osmoregulation through their skin (Karnaky, 1998; Styga et al., 2019). However, such salinity-induced body shape divergence is not well documented for the euryhaline long-whiskered catfish, *M. gulio* from the coastal waterbodies of Bangladesh and elsewhere.

In addition to salinity, the captive breeding of wild populations and selective breeding in hatcheries have contributed to the emergence of a distinct captive phenotype of fish that differs phenotypically from their wild counterparts (Teletchea and Fontine, 2014). The captive phenotype is a consequence of domestication, which is brought about by genetic modifications over successive generations and environmental influences during an animal's lifespan. The observed phenotype is primarily a result of the cumulative selection of polygenic variation, which confers adaptive advantages to organisms in anthropogenic environments (Price, 1999). The process of captive breeding and domestication has been influenced by three primary genetic mechanisms, namely genetic drift, inbreeding, and selection. These mechanisms have played a crucial role in the development of captive phenotypes (Pulcini et al.,2013). When fish are reared in a confined environment, they mostly face a captive environment with limited energy expenditure for searching for food, no competition with other species, and no migration required (Huntingford, 2004). Moreover, high fish density, frequent human treatment, and vulnerability to various diseases distinguish wild and captive populations (Huntingford, 2004). Numerous research endeavors have endeavored to delineate the morphological differentiation between captive and wild fish across various fish taxa. (Berejikian et al., 1997; Busack et al., 2007; Fleming, 1994; Siciliano-Martina et al., 2022; Wringe et al., 2015). However, little is known about differences in body shape between native and captive populations of the long whiskers catfish *M. gulio*.

Information on any fish species' biology and population structure is a prerequisite for better understanding the population stock structure and developing management and conservation strategies (Cadrin and Friedland, 1999). Despite having economic and ecological significance, there are very few studies on the morphometric divergence of *M. gulio* elsewhere in the world. Therefore, the present study aims to investigate the body shape variations of *M. gulio* collected from different salinity gradient habitats (high-salinity brackish water, low-salinity brackish water, and freshwater populations) and sources (wild and hatchery populations) using traditional morphometrics (linear and truss-networking distances) and landmark-based geometric morphometrics. The main objectives of this study are knowing the stock structure of the population for better management and the protection of the species for the country's sustainable development.

CHAPTER II

LITERATURE REVIEW

2.1 Necessity of Morphological Study for Stock Discrimination and Fisheries Management

The assessment of the stock status of a given fish species is of the utmost importance in the context of fisheries management. This is because the identification of stocks with distinct life history traits is critical for optimizing yield and implementing effective stock enhancement and overall management programs (Siddik et al., 2016). Morphological variation studies between populations play a crucial role in identifying stock structures whose consistent shape variations may expose to distinct growth patterns, mortality or reproduction (Swain and Foote, 1999; Cadrin, 2000). Morphometric measurements are commonly employed to distinguish between different fish populations (Cheng et al., 2005). The use of morphometrics is extensively accepted in the contemporary biological landscape. Due to its low cost and discrimination-appropriate resolving power, it is increasingly utilized as an indispensable supplement to molecular research (Sen et al., 2011). It may be more pertinent to the study of short-term environmental variation (Begg et al., 1999). Formerly, scientists held the belief that morphometric character variation was solely determined by genetics. However, contemporary research has demonstrated a correlation inbetween such environmental factors and variation, such as habitat, water physico-chemical parameters, and substrate types (Sharker et al., 2015). While molecular markers are deemed more reliable in revealing physiological and genetic differences between stocks, morphometric variations remain a significant parameter in the identification and characterization of stocks (Costa et al., 2003).

2.2 Implication of Truss Networking for Identifying Body Shape Plasticity and Stock Discrimination

The truss network system is regarded as a more advanced approach compared to conventional morphometrics, which utilizes morphometric characteristics to depict the entire shape of fish. This conventional method has been widely employed in the domains of fish taxonomical classification and fisheries management (Francoy et al., 2012; Turan, 2004). The methodology of conventional morphometrics entails the quantification of linear

dimensions, including length, width, and height, which are subsequently subjected to multivariate statistical analyses for investigating the configurations of the shape diversity both within and between populations (Winans, 1984). This approach also facilitates the identification of allometric trends in bodily structures (Góes and Fransozo, 1997), patterns of growth (Chu., 1999), and the evaluation of geographic disparities (Cardoso and Negreiros-Fransozo, 2004). Despite its numerous benefits, conventional morphometrics is limited by the fact that measurements obtained from two distinct shapes may yield identical outcomes. This is due to the absence of information regarding the spatial relationship between the measurements and the high correlation typically observed among linear distance measurements, which poses a challenge for shape analysis (Góes and Fransozo, 1997). The utilization of the truss network technique (Strauss and Bookstein, 1982) in the field of morphometrics is a quantitative approach that enables the comprehensive depiction of the morphology of fish. The present depiction employs precisely delineated morphological features that span the entirety of the fish. The utilization of the truss morphometrics approach has been found to be a proficient technique for obtaining data pertaining to the physical configuration of an organism, as reported by Cavalcanti et al. (1999). The trussed box denotes the expansion of a truss network into a uniform network over a fish (Strauss and Bookstein, 1982). The trussed box, as posited by Turan (1999), is a theoretical framework that aims to capture the shape of fish specimens. Its purpose is to enhance the probability of extracting the morphometric differences between the specimens. Some of the benefits from employing the truss network (Strauss and Bookstein, 1982), entail comprehensive extend of coverage across the outline, as opposed to conventional forms which ensure highly irregular coverage, allowing rebuilding of the original configuration of points. Statistical techniques such as multivariate analyses (Discriminant function analysis and Principal components analysis) and analysis of variance (ANOVA) may be correlated to distinguish between the taxonomic groups and the geometrical descriptions of studied species. Although, morphological traits are susceptible to environmental factors and may not always correspond to genetic variations within a species (Cadrin and Friedland, 1999). Several studies have been conducted globally on the truss network system, examining species such as Caspian lamprey (*Caspiomyzon wagneri*), Roho labeo (*Labeo rohita*), Alewife (*Alosa pseudoharengus*), and black stripe minnow (*Galaxiella nigrostriata*) (Galeotti et al., 2015;

Mir et al., 2012; Solomon, Okomoda, & Ogbenyikwu, 2015). However, its implementation in the coastal areas of Bangladesh remains limited.

2.3 Implication of Geometric Morpheme for Identifying Body Shape Plasticity and Stock Discrimination

Landmark-based geometric morphometrics is a method that is both sophisticated and easily accessible to biologists. This approach involves the collection of data in the form of spatial arrangements of landmarks along a biological structure. This potent methodology has the capability to apprehend dissimilarities in configurations that are not readily discernible through conventional forms of quantification or visual inspection (Park et al., 2013). Landmark-based techniques do not impose limitations on the localization of shape alterations or the orientations of the variation. They are extremely adept at capturing important information on organism shapes. Geometric morphometrics is predicated on the utilization of the shape variables that are statistically comparable. This allows for the reconstruction of both the hypothetical shape of a common ancestor and a group consensus shape. It is feasible to visually represent alterations and conversions that are essential for discriminating between distinct shapes. The utilization of multivariate statistical techniques is regarded as a supplementary approach to the morphometric methods (Zelditch et al., 2004) as it enables the statistical representation of the inherent shape variability. In addition, they are utilized for the purpose of assessing noteworthy correlations between bodily configuration and ecological characteristics, or for appraising the significance of phylogenetic inertia with regards to shape resemblance. It is anticipated that taxa that are closely related would exhibit greater similarity to each other as compared to those that do not share evolutionary history (Rosenberg 2002). In order to establish a dependable connection between a hypothesized process that drives adaptive divergence, it is crucial to incorporate phylogenetic information into geometric morphometric approaches (Linde et al. 2004). Geometric morphometrics is a prevalent methodology employed in the assessment of shape plasticity. The Generalized Procrustes Analysis (GPA) method in Geometric Morphometrics (GM) differs from traditional morphometrics (Adams et al. 2004) in that it employs landmark points (Rohlf and Marcus 1993) to record data. These landmarks are of biological significance (Richtsmeier et al. 2002) and are represented by their coordinates, as opposed to linear measurements, counts, and ratios. The graphical representation of

outcomes in GM can be accomplished through the utilization of either difference-vector diagrams or thinplate spline, as indicated by Slice (2007). The implementation of the image processing methods has significantly augmented morphometric assessments and has substantially ameliorated the identification and differentiation of fish stocks (Cadrin & Friedland, 1999). Genetic markers (GM) have been utilized in numerous research endeavors pertaining to the biology of fish populations, as well as for the purposes of stock identification and distictions.

2.4 Commercial and Ecological Importance of the Long Whiskers' Catfish (*Mystus gulio*)

Mystus gulio, commonly referred to as 'Nona tengra', is a small catfish species that is euryhaline and primarily inhabits estuarine environments. *Mystus gulio*, commonly known as the long whiskers' catfish, is a diminutive autochthonous species belonging to the Bagridae family within the Siluriformes order. The genus in question is indigenous to the coastal waters of the southern Bangladesh and various countries situated in the Indian Ocean, spanning from India to Vietnam, along with Pakistan (Talwar and Jhingran, 1991). This particular species is referred to as nuna tengra in Bangladesh, long whisker catfish in India, Nga-zin in Myanmar, and Sri Lanka (Froese and Pauly, 2018). According to the IUCN report of 2015, the species is classified as of least concern in the water-bodies of Bangladesh. The fish in question are primarily of a coastal water origin, with the ability to migrate into freshwater environments and establish residence therein. Adult *M. gulio* specimens are typically observed inhabiting freshwater environments, particularly in larger bodies of water such as rivers and streams. These habitats are characterised by soil or clay matrices that are less commonly encountered in smaller streams. According to Ng (2010), the exportation of this particular fish as an ornamental species is infrequent. The aforementioned species is significantly contributing to both the commercial and the local fisheries of Bangladesh. According to Haniffa (2009) and Begum et al. (2010), this particular food item is highly favoured by consumers due to its delectable taste, significant nutritional content, and increasing demand in both the domestic and the export markets. Additionally, it commands a high price in the market. The declining trend of its catch can be attributed to a combination of factors, including destructive fishing practises, overexploitation, habitat degradation, and ecological changes, which have had a mutual

effect (Alam et al., 2006). The annual catch of fish has historically been quite substantial; however, it is currently experiencing a gradual decline as a result of various factors, including over-exploitation, destructive fishing practises, habitat loss, and ecological changes (Alam et al., 2006). The aforementioned fish species is experiencing a surge in market demand due to its delectable taste and is increasingly being recognised as a viable option for aquaculture in the coastal regions of Bangladesh. In 2007, the Bangladesh Fisheries Research Institute developed a breeding technology for a certain fish species with the aim of both conservation and augmenting its supply (Alam et al., 2007). The Bangladesh Fisheries Research Institute successfully developed artificial breeding technology for this particular species, as documented by Alam et al. (2006). The practise of captive culture has become increasingly prevalent in polyculture with other fish species in coastal and inland waterbodies. This is largely due to the ample seed production from hatchery sources, as noted by Alam et al. (2007). The development of *M. gulio* culture techniques, which focus on enhancing species enhancement by incorporating other salt tolerant species, presents a potentially viable solution to mitigate the adverse effects of climate change-induced salinity intrusion on fishery species depletion.

2.5 Influence of Habitat Induced Environmental Factors in Body Shape Variation

One of the primary challenges in ecology is comprehending the correlation between an organism's morphology and its surrounding environment (Gaston & Lauer, 2015). The body morphology of fish, both at the individual and population level, is significantly impacted by the intricate and diverse array of biotic and abiotic factors associated with environmental variation. The investigation of morphological alterations triggered by environmental stimuli can facilitate a more comprehensive comprehension of the process of phenotypic plasticity, which arises as a consequence of induced factors (Jalili et al., 2015). The variation in environmental conditions across different spatial locations plays a significant role in inducing divergent selection, which in turn drives the evolution of phenotypic traits, facilitates local adaptation, and may even lead to speciation (Schluter, 2000). The long-term segregation of populations and interbreeding can result in morphometric variations and provide a foundation for population differentiation (Yamamoto et al. 2006; Anvarifar et al. 2011). Geographical isolation and environmental heterogeneity are both influential factors in population connectivity, which encompasses the dispersal of gametes, larvae, and

organisms, as well as population structure. The response of marine species to these processes can result in morphological variations through genetic differentiation, which may be attributed to divergent selection (Grether, 2005). According to Swain and Foote (1999), diverse species exhibit the capacity to produce numerous morphological, physiological, and behavioral variations, as well as different degrees of expression of a specific developmental process. The expression of polymorphous signals along with other phenotypic characteristics can be subject to environmental influences or may result from the interaction between environmental and genetic factors (Klingenberg, 2013). Phenotypic characteristics have demonstrated their usefulness for determining groupings or sub-units, regardless of their source. This is due to the fact that phenotypic variation in the fishes can be versatile, as suggested by previous studies (Webb, 1984; Via et al., 1995). The notion of isolation by distance was first introduced by Wright (1943) in a model of population structure on an island, wherein the dispersal capacity of organisms is limited by gene flow and distance is expected to be highest between the neighboring populations (Planes & Fauvelot, 2002). Simultaneously, the bathymetry-associated vertical dimension exhibits a diverse and intricate function in ecosystem fragmentation by means of its interplay with hydrodynamic consequences, such as turbulent mixing processes, wind-induced circulation, and buoyancy forces, as explicated by Johnston & Merrifield (2003). The impact of abiotic and biotic elements of habitats on body shapes has been studied in relation to the fitness and functional success. This includes factors such as predator avoidance, character displacement and foraging requirements due to competition. Research conducted by Robinson and Wilson (1994), Svanbäck et al. (2008), and Adams and Huntingford (2004) has demonstrated the significance of these factors. In recent studies, it has been demonstrated that there is a morphological divergence in the shape of freshwater fish in habitats that have been altered by human activity. These findings have been reported by Franssen et al. (2013). Consequently, comprehending the manner in which populations acclimatise to diverse habitats may offer a perspective on the implications of anthropogenic alterations to streams and the process of evolution.

2.6 Influence of Salinity Gradient habitats on Body Shape Variation

The evaluation of optimal locations for fish cultivation can be facilitated by analyzing the salinity tolerance of each fish species. Numerous investigations have examined the impact of

this variable on the efficacy of euryhaline fish (Riche & Williams, 2010). In addition to performance evaluation, it is imperative to examine the impact of salinity on the health and organs of the fish (Árnason et al., 2013). The level of salinity is a crucial environmental factor that affects various aquatic organisms, including fish species. It is noteworthy that each fish species exhibits a distinct range of tolerance towards salinity levels. Teleost fish possess the ability to uphold the equilibrium of their body fluids in terms of ionic and osmotic concentration amidst varying environmental salinities through the utilisation of energy-intensive osmoregulatory mechanisms (Sampaio and Bianchini, 2002). Therefore, comprehending the impact of varying salinity conditions is of utmost importance in aquaculture, as it aims to establish an optimal and salubrious milieu to enhance financial profitability. Whilst the precise impact of salinity niche on body morphology remains uncertain, there exist several rationales as to why alterations in body form may be associated with salinity niche in Fundulus species. The habitat salinity has the potential to impact the encountered predator types (such as gape-limited or ambush) and their density for populations of a particular species. This may result in the development of a body shape that is selectively advantageous for that species. As an illustration, it is widely acknowledged that prey fish may derive selective benefits from possessing a broad mid-body depth, as it can potentially reduce the risk of mortality caused by predators with limited gape size (Walker, 1997; Walker and Bell, 2000). The skin of teleosts undergoes passive loss of water and ions, (Perry, 1997). It is plausible that natural selection, motivated by the need for osmoregulatory efficiency, plays a role in the observed variation in body shape between environments with low and high salinity. Freshwater fish exhibits hyperosmotic regulation in response to their external environment, necessitating the need to counteract the perpetual influx of water. Freshwater fish expend a considerable amount of their energy budget on osmoregulation, despite possessing numerous physiological adaptations to combat this phenomenon (Boeuf and Payan, 2001). The selection for osmoregulatory efficiency may have led to the development of body form with reduced surface areas, such as fusiform shapes or cylindrical, in order to minimize passive loss or gain of solutes or water from the external environment.

CHAPTER III

MATERIALS AND METHODS

3.1 Sample collection

To analyze salinity-induced body shape variation, a total of 436 *M. gulio* individuals were collected in the same month from different salinity gradient coastal waterbodies in the Caufaldandi and Khurushkul areas of Cox's Bazar, Bangladesh. Among these 436 individuals of *M. gulio*, 100 individuals were collected from the high-salinity brackish waterbodies (8-15 ppt), 178 individuals from low-salinity brackish waterbodies (2-6 ppt), 158 individuals from freshwater bodies (0 ppt). For captivity-induced body shape variation,

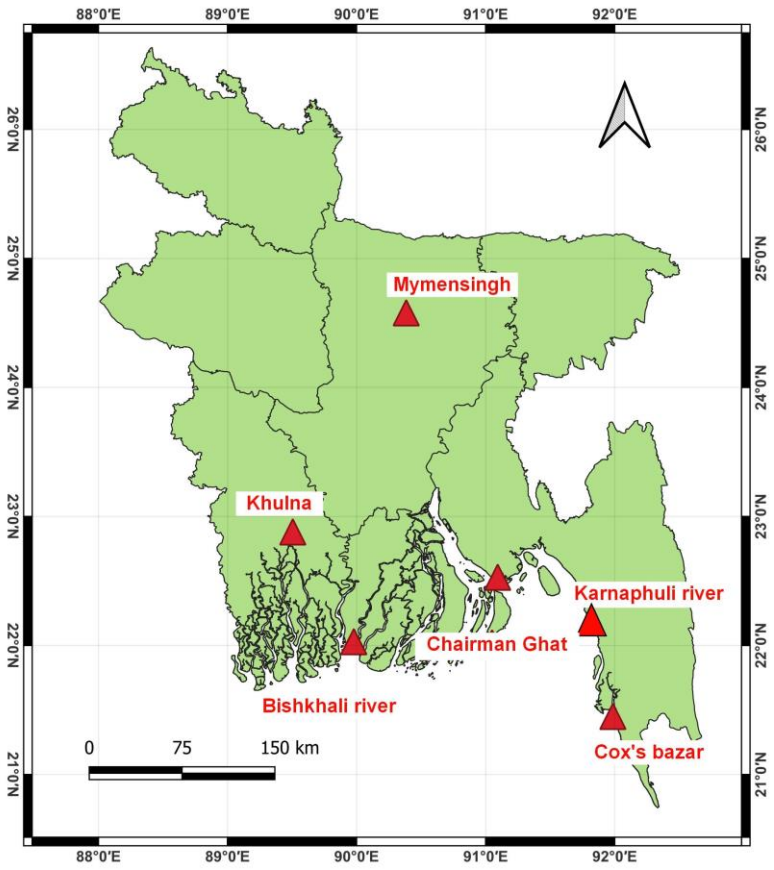

Figure 1 : Map of six sample collection sites for Mystus gulio (Karnaphuli river, Chattogram; Bishkhali river, Barguna; Bakkhali river, Cox's Bazar; Chairman Ghat, lower Meghna River; Hasan Fish Hatchery, Mymensingh & Pona Mach Fisheries Hatchery, Khulna)

a total of 430 individuals of *M. gulio* were collected, including 260 individuals from various coastal rivers (wild population) and 170 individuals from a hatchery-source captive pond system (captive population). Among the 260 wild source individuals, 60 individuals were collected from the bridge ghat area of the Karnaphuli river (22.019069 N and 91.050306 E), 60 individuals from the Bishkhali river of Barguna (22.028998 N and 89.978700 E), 100 individuals from Mazir ghat area of Bakkhali river of Cox's Bazar (21.449582 N and 91.986776 E) and 40 individuals from the Chairman Ghat area (22.5287240 N and 91.0939044 E) of lower Meghna River of Noakhali regions. For 170 hatchery-source individuals, 70 individuals were collected from the Hasan Fish Hatchery, Trisal, Mymensingh, and 100 individuals were collected from the Pona Mach Fisheries Hatchery, Khulna (Figire 1). After collecting samples, further morphological and geometric morphometrics studies were done at the Fisheries Oceanography Laboratory, Department of Marine Bioresource Science, Chattogram Veterinary and Animal Sciences University, Bangladesh.

3.2 Digital imaging

First, samples were washed with clean fresh water and positioned at a fixed point on the surface of graph paper to ensure the same position for all individuals. For the visualization of the fish's original body shape, fin rays were appropriately embellished on the graph paper. Every specimen was assigned a unique code to ensure accurate documentation. A Canon EOS 60D 18.0MP with an 18-55MM lens DSLR camera (USA) (Figure 2) was used to take the digital image of fish, which permitted replication of original fish for morphometric measurements in SigmaScan Pro 5, MorphoJ, and other image processing software (Cardin and Friedland, 1999).

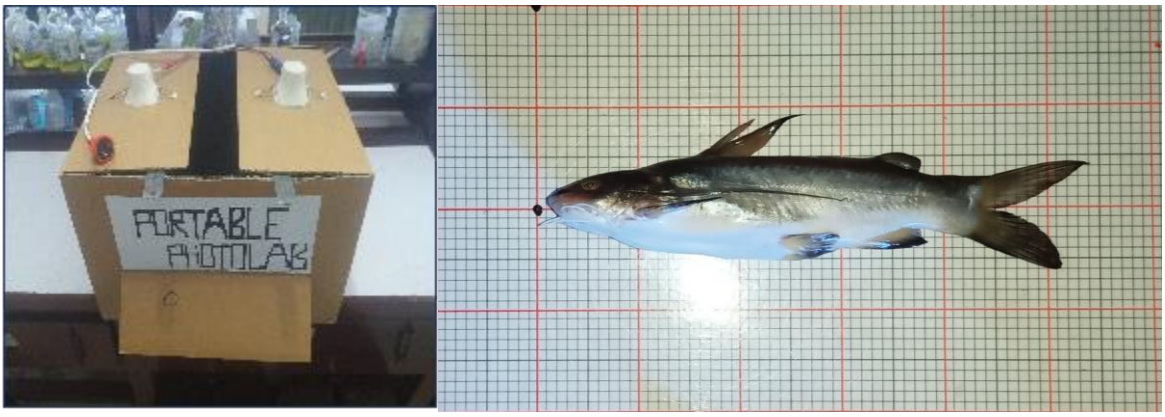

Figure 2 : Photo Lab along with image with captured via camera

3.3 Landmark-based truss networking analysis

Fourteen morphometric distances were measured across the whole fish body from the left to right side, and thirty-three truss distances were constructed by interconnecting seventeen landmark points by using SigmaScan Pro 5 software (Figure 3; Table 1).

Before performing the analysis, size measurements were adjusted to eliminate size effects from datasets to ensure that the morphological plasticity were due to the difference in body shape and not to relative sizes (Elliott et al., 1995). All measurements correlated significantly with the standard length of the collected *M. gulio*. Therefore, size-dependent variations were eliminated by the using method suggested by Elliot et al., 1995:

$$M_{adj} = M\,(SL_{av}/SL_{ob})^{b}$$

where M_{adj} is the size-adjusted measurement, M is the actual measurement, SL_{ob} is the standard length of the *M. gulio*, SL_{av} is the average mean, the standard length for all samples in each analysis, and b is the slope of the regression of Log M on log SL_{ob} for each character. The validity of the allometric method's results was assessed through an examination of the correlation between transformed variables and the standard length of fish (Turan, 1999). The univariate analysis of variance (ANOVA) was conducted using the "car" package (Fox and Weisberg, 2011), followed by Tukey multiple comparison tests for each morphometric character. The "multcomp" package was utilized to assess the habitat salinity- and source-induced body shape variation of *M. gulio* for each morphometric character, as described by Hothorn et al. (2008). Wilk's lambda was used to compare all individuals collected from the three salinity gradient habits and sources (wild and captive). The N:P ratio was calculated to determine whether *M. gulio* sample size was sufficient for consistent multivariate analysis results (Bujang et al., 2017). Furthermore, the Kaiser-Meier-Olkin (KMO) test and Bartlett's Test of Sphericity were also used to confirm the morphometric datasets' eligibility for multivariate analysis. The KMO test determines if the partial correlation between variables is high enough to determine sample adequacy tests (Yakubu et al., 2011). The KMO statistics, as reported by AnvariFar et al. (2011) and Yakubu et al. (2011), are bounded between 0 and 1. It is deemed acceptable for the KMO statistics to exceed 0.6. On the other hand, the Bartlett's Sphericity test hypothesized that the correlation matrix values are null and that statistically significant results ($P< 0.05$) are deemed appropriate.

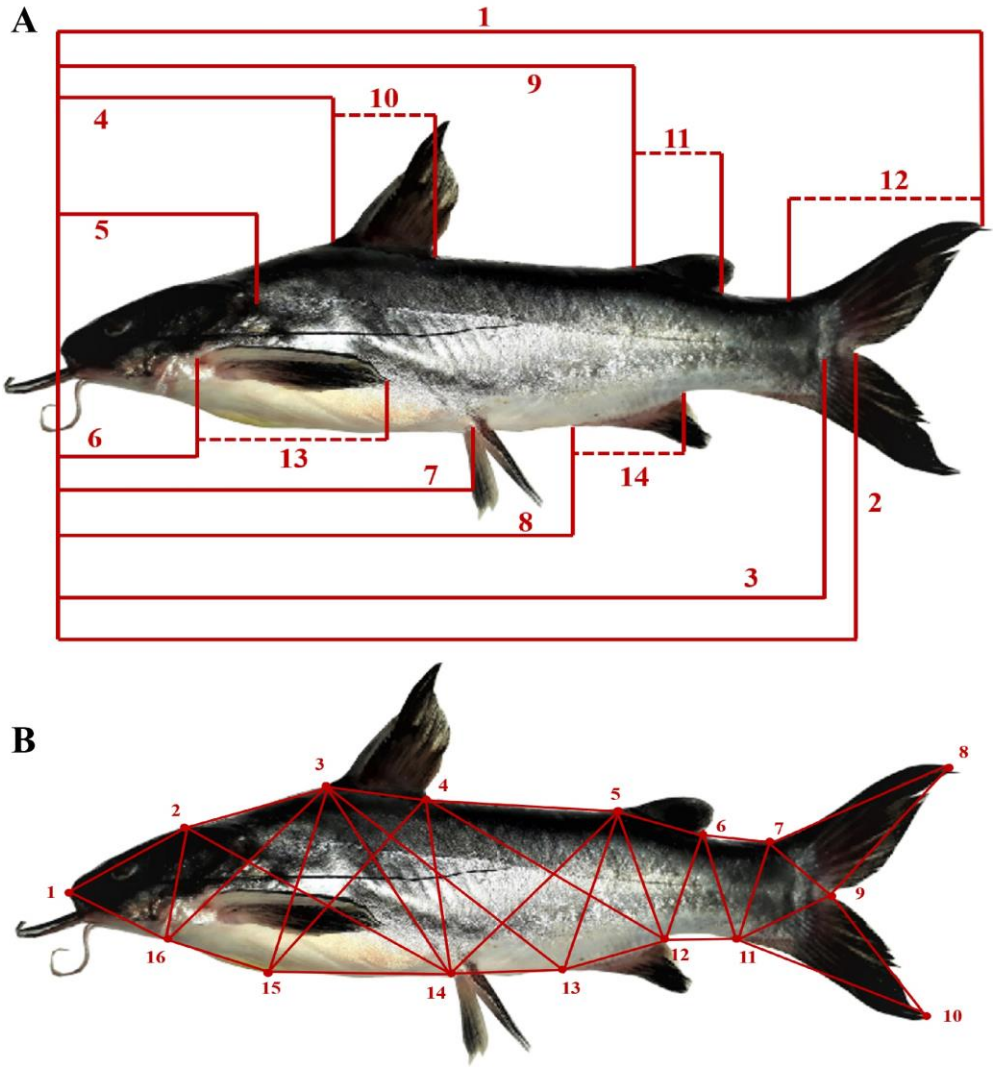

Figure 3 : Different landmark points for Truss network measurement. A) Linear distance measurement: 1 = Total length; 2 = Fork Length; 3 = Standard length; 4 = Pre-1st Dorsal fin distance; 5 = Head length; 6= Pre-pectoral distance; 7 = Pre-pelvic distance; 8= Pr Pre-anal distance; 9= Pre-2nd dorsal fin distance; 10 = 1st Dorsal fin base length; 11 = 2nd Dorsal fin length; 12 = Caudal fin length; 13 = Pectoral fin length; 14 = Anal fin length; B) Truss network landmark points of Mystus gulio: 1 = Snout; 2 = dorsal end of head; 3 = origin of 1st dorsal fin; 4 = end of 1st dorsal fin; 5= origin of 2nd dorsal fin; 6= end of 2nd dorsal fin; 7 = origin of upper caudal fin; 8 = end of upper caudal fin; 9 = middle end of caudal fin; 10 = end of lower caudal fin; 11 = origin of lower caudal fin; 12 = end of anal fin; 13 = origin of anal fin; 14 = origin of pelvic fin; 15 = highest lower body depth point; 16 = ventral end of head.

Table 1 : Descriptions of morphometric distances between landmarks points for truss network analysis of *Mystus gulio* populations collected from coastal areas of Bangladesh. (Landmark points are indicated in Figure 2 B)

SL No	Land Mark Points	Description
1	1-2	Distance from snout to dorsal end of head
2	2-3	Distance from dorsal end of head to origin of 1st dorsal fin
3	3-4	1st dorsal fin base length
4	4-5	Distance from end of 1st dorsal fin to origin of 2nd dorsal fin
5	5-6	2nd dorsal fin base length
6	6-7	Distance from end of 2nd dorsal fin to origin of upper caudal fin
7	7-8	Distance from origin of upper caudal fin to end of upper caudal fin
8	8-9	Distance from end of upper caudal fin to middle end of caudal fin
9	9-10	Distance from middle end of caudal fin to end of lower caudal fin
10	10-11	Distance from end of lower caudal fin to origin of lower caudal fin
11	11-12	Distance from origin of lower caudal fin to end of anal fin
12	12-13	Anal fin length
13	13-14	Distance from origin of anal fin to origin of pelvic fin
14	14-15	Distance from origin of pelvic fin to highest lower body depth point
15	15-16	Distance from highest lower body depth point to ventral end of head
16	16-1	Distance from ventral end of head to snout
17	2-16	Distance from dorsal end of head to ventral end of head
18	2-14	Distance from dorsal end of head to origin of pelvic fin
19	3-16	Distance from origin of 1st dorsal fin to ventral end of head
20	3-15	Distance from origin of 1st dorsal fin to highest lower body depth point
21	3-14	Distance from origin of 1st dorsal fin to origin of pelvic fin
22	3-13	Distance from origin of 1st dorsal fin to origin of anal fin
23	4-15	Distance from end of 1st dorsal fin to highest lower body depth point
24	4-14	Distance from end of 1st dorsal fin to origin of pelvic fin
25	4-12	Distance from end of 1st dorsal fin to end of anal fin
26	5-14	Distance from origin of 2nd dorsal fin to origin of pelvic fin
27	5-13	Distance from origin of 2nd dorsal fin to origin of anal fin
28	5-12	Distance from origin of 2nd dorsal fin to end of anal fin
29	6-12	Distance from end of 2nd dorsal fin to end of anal fin
30	6-11	Distance from end of 2nd dorsal fin to origin of lower caudal fin
31	7-11	Distance from origin of upper caudal fin to origin of lower caudal fin
32	7-9	Distance from origin of upper caudal fin to middle end of caudal fin
33	9-11	Distance from middle end of caudal fin to origin of lower caudal fin

Only the morphometric characters which showed significant variation ($P < 0.05$) were used to obtain a reliable result from the multivariate analysis. In this study, Canonical variates analysis (CVA), Euclidean cluster analysis (CA) and Principal component analysis (PCA) were performed to determine morphometric shape divergence resulting from habitat salinity and source-induced variation. The PCA was executed using the 'FactoMineR' package of R (Sebastien et al., 2008), version 4.0.5 (R development core team, 2021) to find the variation

due to salinity and source variation. The first and second PCs were used in all cases because they explained most differences. The "MASS" package of R was used to analyze the CVA (Venables and Ripley, 2002). The Linear Discriminant Function Analysis (LDFA) was executed to calculate the percentage of the correctly classified (PCC) population into their original groups of habitat salinity and sources and cross-validation using the classification matrix. Moreover, the morphometric distances between the individuals of various salinity habitats were calculated using cluster analysis, which used the Euclidean distance to quantify dissimilarity and the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) as a clustering algorithm (Veasey et al., 2001). The R package "dendextend" was used to perform cluster analysis (Galili, 2015). The 'ggplot2' package was used to generate all graphs (Wickham, 2009).

3.4 Geometric morphometrics

For the geometric morphometrics analysis, tpsDig2 software was used to superimpose 17 landmark points of each digital image (Figure 4) by following Rohlf's protocols (Rohlf et al., 2005a). The tpsUtil software was used to separate homologous points (fixed points) and points between fixed landmarks (placed in the curve portions). Relative warp (RW) scores were calculated from the landmark data using the tpsRelwv1.42 program (Rohlf, 2005b), which characterized the shape variance as departures from a consensus shape. The RWs are analogous to the principal components (Adam et al., 2004). The RWs were found to be comparable to the principal components observed in the PC analysis, as reported by Adams et al. (2004). Similar to the truss networking, the PCA was executed using the 'FactoMineR' package (Lê et al., 2008), version 4.0.5 (R development core team, 2021), and CVA was performed using the "MASS" package (Venables and Ripley, 2002). Finally, the MorphoJ software was used to graphically demonstrate the variations in the body form among various groups as recorded by these RW scores (Klingenberg, 2013).

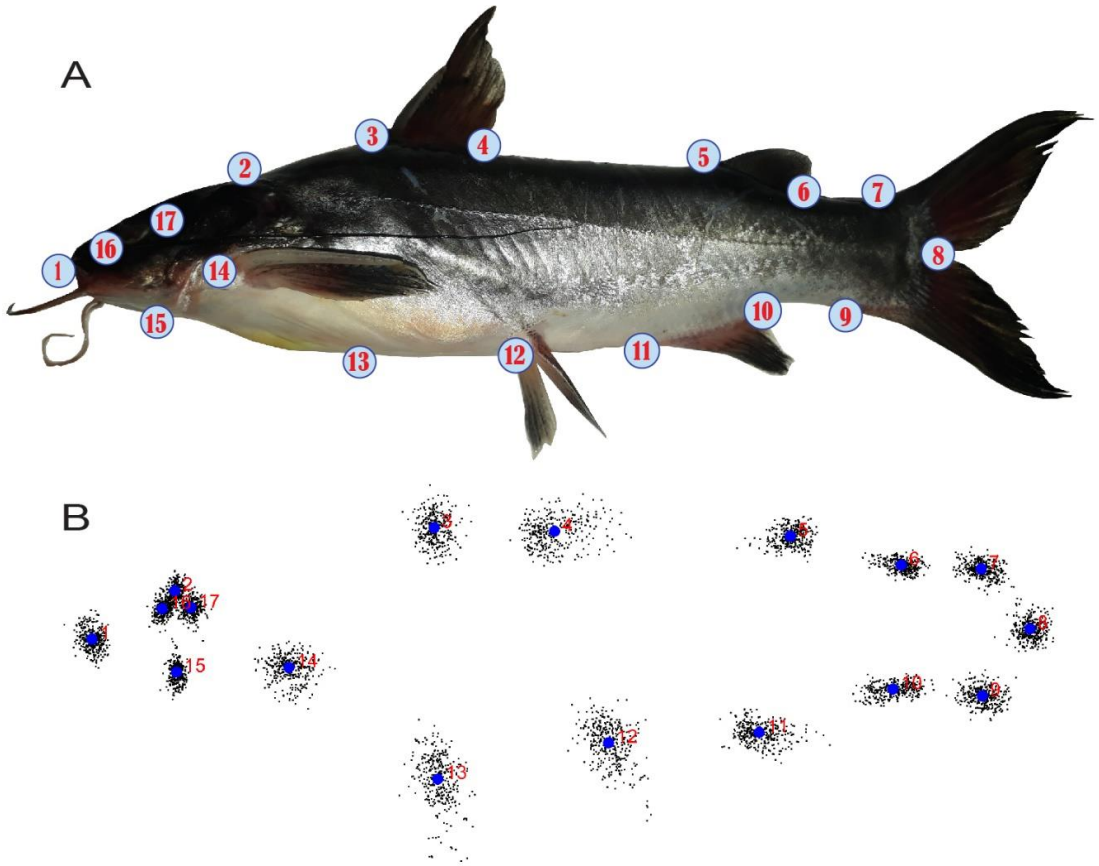

Figure 4 : Landmark points for geometric morphometric analysis of *M. gulio* collected from the coastal area of Bangladesh. A) 17 morphometric landmarks: 1 = Most anterior tip of upper jaw; 2 = End point at the midline of the dorsal supraoccipital crest; 3 = Dorsal fin anterior base; 4 = Dorsal fin posterior base; 5 = Adipose fin anterior base; 6 = Adipose fin posterior base; 7 = Origin of caudal fin on the dorsal midline; 8 = The caudal end of the hypural bone at the lateral midline; 9 = Origin of caudal fin on the ventral midline; 10 = Anal fin posterior insertion; 11 = Anal fin anterior insertion; 12 = Pelvic fin insertion into the body; 13 = The highest point at the ventral portion of the belly part; 14 = Pectoral fin insertion at ventral side; 15 = Most anteroventral point of coracoid; 16 = Most anterior point of the eye at midline; 17 = Most posterior point of the eye at midline; B) Procrustes superimposition plot of *M. gulio* landmark configurations.

CHAPTER IV

RESULTS

4. 1 Body Shape Plasticity of *M. Gulio* Collected from Different Salinity Gradient Habitats

4.1.1 Truss networking

For body shape plasticity in different salinity gradient habitats datasets, descriptive outcomes from the ANOVA test of different lengths and truss-networking distances are shown in Table 2. The ANOVA test demonstrated that 36 morphometric measurements (HL, PPCD, PPVD, P2DFD, 1DFBL, 2DFL, CFL, AFL, D1-2, D2-3, D3-4, D5-6, D6-7, D7-8, D8-9, D9-10, D10-11, D11-12, D13-14, D14-15, D15-16, D16-1, D2-16, D2-14, D3-16, D3-15, D3-14, D3-13, D4-15, D4-14, D5-14, D5-13, D5-12, D7-11, D7-9, D9-11) out of 47 were significant for the habitat salinity gradiant variations (Table 2).

Table 2 : Descriptive data from ANOVA test for each morphometric character of *Mystus gulio* for both different salinity gradient habitats and wild versus hatchery population.

Morphometric Parameters	Salinity					Fish Source			
	8-15 ppt (Mean±SD)	2-6 ppt	0 ppt	F Value	Level of Sign	Wild	Cultured	F Value	Level of Sign
FL	10.12±0.11^{a}	10.08±0.15^{a}	10.10±0.19^{a}	2.327	0.099NS	10.12±0.16	10.05±0.14	20.551	0.000***
PPVD	5.20±0.25^{a}	5.09±0.16^{b}	4.95±0.13c	63.816	0***	5.10±0.21	5.00±0.17	26.114	0.000***
P2DFD	6.97±0.14^{b}	6.98±0.14^{b}	6.89±0.15^{a}	16.937	0***	6.96±0.15	6.93±0.15	5.016	0.026*
2DFL	1.08±0.16^{a}	1.09±0.13^{a}	1.13±0.12^{b}	4.932	0.008**	1.09±0.14	1.12±0.12	6.280	0.013*
CFL	3.02±0.28^{b}	2.91±0.23^{a}	2.93±0.24^{a}	6.579	0.002**	2.98±0.25	2.87±0.23	21.218	0.000***
AFL	1.30±0.17^{b}	1.24±0.12^{a}	1.22±0.12^{a}	11.74	0***	1.26±0.14	1.23±0.12	6.394	0.012*
D1-2	1.82±0.15^{a}	1.88±0.14^{b}	1.92±0.17^{b}	14.052	0***	1.84±0.15	1.94±0.16	40.182	0.000***
D2-3	1.78±0.17^{b}	1.78±0.15^{b}	1.72±0.21^{a}	4.961	0.007**	1.79±0.16	1.71±0.19	23.709	0.000***
D5-6	1.09±0.18^{a}	1.14±0.13^{b}	1.18±0.14^{b}	9.955	0***	1.12±0.15	1.17±0.14	11.222	0.001**
D6-7	0.74±0.22^{a}	0.75±0.16^{a}	0.82±0.18^{b}	8.134	0***	0.74±0.19	0.82±0.17	20.668	0.000***
D7-8	3.07±0.23^{b}	2.96±0.20^{a}	2.94±0.30^{a}	9.299	0***	3.04±0.21	2.88±0.28	47.525	0.000***
D10-11	2.80±0.20^{b}	2.73±0.21^{a}	2.78±0.22^{ab}	4.791	0.009**	2.80±0.20	2.70±0.21	27.719	0.000***
D11-12	0.85±0.15^{a}	0.92±0.15^{b}	0.92±0.20^{b}	6.924	0.001**	0.87±0.15	0.96±0.19	24.534	0.000***
D13-14	1.44±0.13^{a}	1.52±0.15^{b}	1.57±0.20c	17.96	0***	1.50±0.15	1.55±0.20	7.194	0.008**
D14-15	1.53±0.38^{a}	1.62±0.39^{a}	1.74±0.39^{b}	8.774	0***	1.60±0.37	1.70±0.42	6.748	0.010*
D15-16	2.57±0.43^{a}	2.27±0.43^{b}	1.96±0.41c	64.509	0***	2.32±0.45	2.08±0.50	26.724	0.000***
D16-1	1.42±0.18^{a}	1.49±0.12^{b}	1.50±0.13^{b}	9.959	0***	1.46±0.15	1.50±0.13	5.529	0.019*
D2-16	1.53±0.10^{a}	1.58±0.09^{b}	1.63±0.19c	16.814	0***	1.55±0.09	1.64±0.18	49.265	0.000***
D2-14	4.07±0.27^{a}	3.93±0.17^{b}	3.70±0.29c	77.812	0***	3.94±0.23	3.78±0.32	38.754	0.000***
D3-16	2.82±0.15^{a}	2.88±0.12^{b}	2.86±0.17^{b}	4.6	0.011*	2.84±0.14	2.89±0.17	8.610	0.004**
D3-15	2.71±0.28^{a}	2.60±0.19^{b}	2.32±0.15^{b}	136.68	0***	2.56±0.26	2.46±0.24	17.109	0.000***
D3-14	3.03±0.26^{a}	2.97±0.15^{b}	2.71±0.16c	121.96	0***	2.92±0.23	2.83±0.22	17.754	0.000***
D3-13	3.76±0.13^{ab}	3.79±0.15^{b}	3.73±0.18^{a}	7.782	0***	3.76±0.14	3.76±0.18	0.026	0.871NS
D4-15	2.83±0.32^{b}	2.82±0.21^{b}	2.69±0.28^{a}	14.851	0***	2.76±0.28	2.79±0.26	1.451	0.229NS
D4-14	2.46±0.23^{b}	2.46±0.15^{b}	2.25±0.19^{a}	65.391	0***	2.38±0.22	2.38±0.21	0.176	0.675NS
D5-14	2.98±0.15^{b}	3.01±0.15^{b}	2.85±0.16^{a}	47.639	0***	2.96±0.17	2.92±0.17	8.363	0.004**
D5-13	2.02±0.08^{ab}	2.04±0.12^{b}	1.99±0.15^{a}	7.178	0.001**	2.01±0.11	2.04±0.14	5.983	0.015*
D6-12	1.28±0.05^{a}	1.27±0.08^{a}	1.28±0.10^{a}	0.671	0.512NS	1.26±0.07	1.29±0.10	11.511	0.001**

D6-11	1.42±0.14[a]	1.40±0.10[a]	1.41±0.11[a]	1.622	0.199NS	1.39±0.12	1.44±0.10	19.613	0.000***
D7-11	1.22±0.07[a]	1.24±0.07[a]	1.29±0.10[b]	28.566	0***	1.22±0.07	1.31±0.08	147.575	0.000***
D7-9	1.55±0.21[b]	1.42±0.16[a]	1.45±0.25[b]	13.589	0***	1.51±0.20	1.37±0.20	50.057	0.000***
D9-11	1.59±0.16[b]	1.53±0.15[a]	1.59±0.21[b]	6.774	0.001**	1.60±0.18	1.51±0.17	23.688	0.000***

Under these circumstances, the N:P ratio was 12.11 (436/36) revealing that the *M. gulio* sample size was adequate for stable outcomes of multivariate analyses (Bujang et al., 2017). Bartlett's Test of sphericity is statistically significant ($P < 0.01$), and the Kaiser-Meyer-Olkin (KMO) value for the whole matrix is 0.89. The outcomes of all these tests indicate that the morphometric data set is suitable for moving on with multivariate analysis .

Based on the outcomes of the univariate ANOVA model, the 36 significantly differed morphometric distance datasets were only used for different multivariate (PCA, LDFA , CVA) analyses. Initially, the contributions of these significantly different variables to principal components (PCs) were examined to ascertain which morphometric distances most effectively distinguished the body shape plasticity of the *M. gulio* population in response to the different habitat salinity. The PCA of 36 morphometric data extracted 13 factors with eigenvalues >1, explaining 82.04% of the variance in the original dataset. The first principal component (PC1) contributed 15.62% of the variation and was conquered mainly by the different body depth-related morphometric distances, such as D3-14, D3-15, D2-14, D4-14, and D5-14. The second principal component (PC2) was attributed to 12.61% of the variation and was subjugated mainly by the body length-related morphometric distances such as D7-9, D7-8, CFL, D9-11, D10-11 (Figure 5 A, B; Table 4). Similarly, the CVA analysis revealed that the first canonical variates (CV1) accounted for 78.33% of the variability, while the second canonical variates (CV2) explained 21.67% of the variation (Figure 5C). Both the PCA and CVA graphic plot also recognized that the multivariate spaces of these loading variables differentiate the populations in overlapping patterns for three salinity gradient habitats (Figure 5). Based on a visual analysis of the biplot graphs, it was observed that the populations with high and low salinity levels exhibited some degree of overlap with the freshwater populations. However, these two populations were still distinguishable from their freshwater counterparts. The distinctiveness among these groups was more prominently illustrated by the density plot obtained from the CVA analysis, as depicted in Figure 5D. The morphological divergence of these three salinity gradient populations of *M. gulio* was further investigated by the LDFA. The LDFA analysis, like the CVA, also revealed that

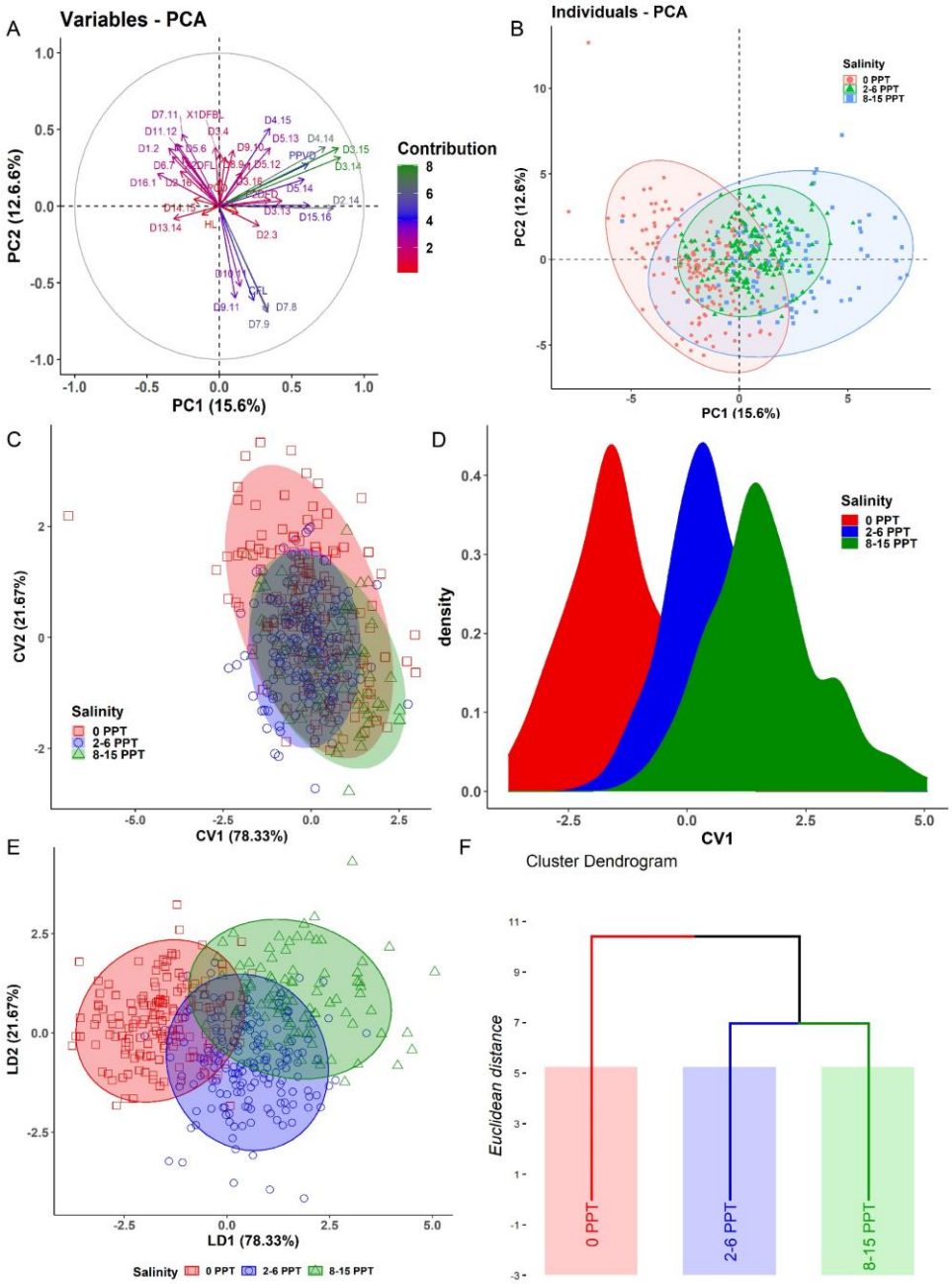

Figure 5 : Principal component analysis (PCA), Linear discriminant analysis (LDA), Canonical variates analysis (CVA), Cluster Analysis and Euclidean tree of truss networking data for depicting population decrimination due to salinity variation. (A-B) PCA biplots showed components with significant influnce in shape variation, (C-E) For three salinity gradient habitats, all plots showed variation with high degree of overlapped population in CVA plot and (F) Euclidean distance tree showed clear difference between populations.

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5E). The clustering pattern based on the biplots of LD1 and LD2 was also in complete

concurrence with the results attained by the PCA and CVA analysis (Figure 5). The LDFA analysis further showed that 86 of 100 (86%) high salinity individuals, 138 of 177 (78%) low salinity individuals, and 139 of 159 (87.4%) zero salinity individuals were correctly classified for their original group (Table 3). In the cross-validated group, percentages for correctly classified individuals for the high salinity population was 78%, low salinity population was 74% and the freshwater population was 82%, indicating a correct classification to their original population (Table 3). Along with CVA, PCA, and LDFA results, UPGMA clustering analysis based on Euclidean distances between groups of centroids revealed two principal clusters, one for zero salinity populations and another for two others closely related and overlapped populations of high salinity and low salinity populations (Figure 5F). From all these analyses (CVA, PCA, LDFA, and Cluster), it can be concluded that freshwater populations of *M. gulio* are more morphometrically diverge than the saline water populations.

Table 3 : Percentage classification of *Mystus gulio* individuals for their original group and cross-validation using classification matrix of the Discriminant function analysis (DFA) based different morphometric measurements due to salinity variation collected from different coastal places of Bangladesh.

Predicted Group Membership						
		Salinity	8-15 PPT	2-6 PPT	0 PPT	Total
Original	Count	8-15 PPT	86	10	4	100
		2-6 PPT	20	138	19	177
		0 PPT	6	14	139	159
	%	8-15 PPT	86.0	10.0	4.0	100.0
		2-6 PPT	11.3	78.0	10.7	100.0
		0 PPT	3.8	8.8	87.4	100.0
Cross-validated	Count	8-15 PPT	78	16	6	100
		2-6 PPT	24	131	22	177
		0 PPT	7	21	131	159
	%	8-15 PPT	78.0	16.0	6.0	100.0
		2-6 PPT	13.6	74.0	12.4	100.0
		0 PPT	4.4	13.2	82.4	100.0

Table 4 : The first five principal components with eigenvalues acquired by Principal component analysis (PCA) for different morphometric characters of *Mystus gulio* due to three different salinity gradient habitats

Principal Components											
Variable	PC1	PC2	PC3	PC4	PC5	Variable	PC1	PC2	PC3	PC4	PC5
Eigenvalues	5.62	4.54	2.83	2.48	2.36		5.62	4.54	2.83	2.48	2.36
% of variance	15.62	12.61	7.86	6.89	6.56		15.62	12.61	7.86	6.89	6.56
Cumulative%	15.62	28.23	36.09	42.99	49.54		15.62	28.23	36.09	42.99	49.54
Factor Loadings											
HL	-0.12	-0.06	-0.10	-0.11	0.19	D13-14	-0.31	-0.08	0.16	-0.07	-0.23
PPCD	0.01	0.18	-0.29	-0.04	0.21	D14-15	-0.18	0.05	-0.20	0.69	-0.33
PPVD	0.61	0.28	-0.43	0.01	0.20	D15-16	0.62	0.01	-0.03	-0.51	0.25
P2DFD	0.35	0.04	-0.38	0.07	-0.34	D16-1	-0.43	0.21	0.07	-0.20	0.34
X1DFBL	-0.03	0.39	-0.22	0.23	0.62	D2-16	-0.27	0.23	-0.20	0.22	-0.31
X2DFL	-0.26	0.32	0.60	0.12	0.25	D2-14	0.79	-0.01	-0.17	-0.04	0.19
CFL	0.24	-0.62	0.19	0.27	0.15	D3-16	0.16	0.22	0.06	0.39	-0.50
AFL	0.13	-0.04	0.18	0.09	0.17	D3-15	0.82	0.38	-0.01	0.03	-0.05
D1-2	-0.35	0.38	-0.26	0.10	-0.16	D3-14	0.84	0.32	-0.11	-0.09	0.03
D2-3	0.28	-0.13	0.21	0.16	0.00	D3-13	0.43	0.04	0.09	-0.23	-0.09
D3-4	0.00	0.34	-0.21	0.23	0.66	D4-15	0.35	0.51	-0.09	0.58	-0.09
D5-6	-0.29	0.41	0.62	0.13	0.12	D4-14	0.73	0.38	0.07	-0.07	-0.26
D6-7	-0.32	0.32	-0.43	-0.19	0.04	D5-14	0.59	0.17	0.26	-0.07	-0.30
D7-8	0.34	-0.68	0.15	0.30	0.20	D5-13	0.35	0.38	0.53	-0.05	-0.14
D8-9	0.04	0.32	0.09	0.42	0.19	D5-12	0.21	0.28	0.66	-0.07	0.17
D9-10	0.10	0.36	0.06	0.34	0.18	D7-11	-0.26	0.47	0.39	-0.02	-0.14
D10-11	0.14	-0.52	-0.02	0.51	0.18	D7-9	0.34	-0.69	0.18	-0.07	-0.03
D11-12	-0.31	0.40	-0.12	-0.32	-0.20	D9-11	0.11	-0.60	0.05	0.13	-0.03

4.1.2 Geometric morphometrics

The regression coefficient of procrustes distance (x-axis) and tangent distance (y-axis) was 0.85, which clearly indicated that the selected seventeen landmarks were valid and suitable for further analysis. Procrustes deformations grids and wireframe graphs for the first three PCAs exhibited the morphological body shape variation of *M. gulio* for salinity variation (Figure 6). The body shape plasticity from the three salinity gradient habitats was mainly visible in the width of the dorsal-ventral part, the snout shape, the tail shape, and the head shape (Figure 6B, C). The PCA of 17 landmark points for all individuals from different salinity sources showed high overlapping among themselves in the PC1 versus PC2 scatter plot (Figure 6A). The first seven principal components were responsible for 80.29% of the shape variation (PC1 28.64%, PC2 16.30%, PC3 11.80%, PC4 8.89%, PC5 6.26%, PC6

4.57%, PC7 3.83%). In the graph, PCA values indicated that individuals from zero ppt salinity and 2-6 salinity belong near zero to negative value and mostly positive value of the PC1 plot sides. In the case of the PC2 side, zero salinity and low salinity individuals were more widely distributed in both positive and negative values. In contrast, high-salinity individuals were mainly on the opposing side. CVA results revealed that there were three separate groups with slight overlapping patterns. CV1 (72.9%) and CV2 (19.8%)

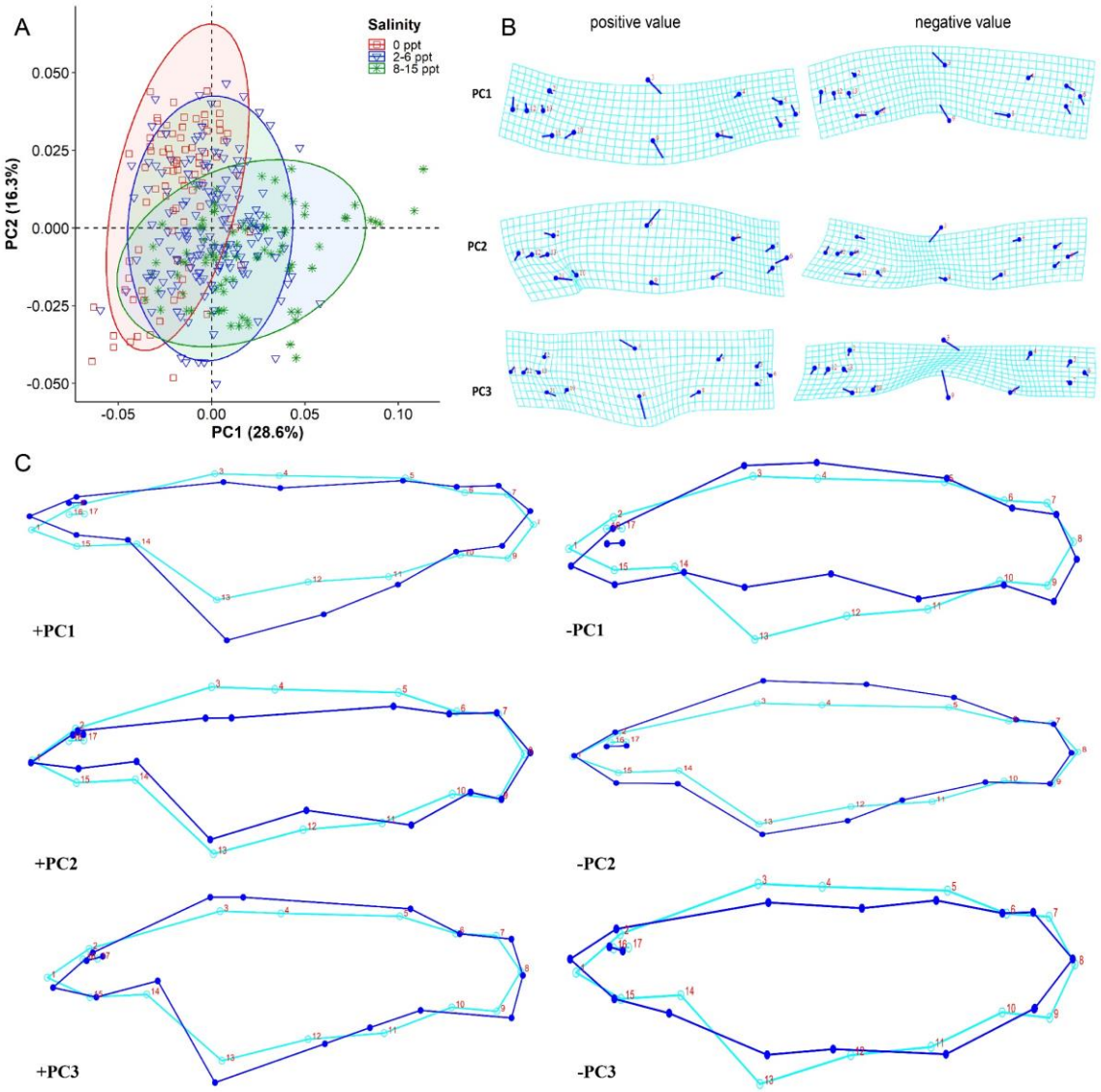

Figure 6 : Principal component analysis (PCA), thin-plate spline of Procrustes deformations and wireframe plots showing the morphological change described by each components for different salinity gradient habitats through geometric morphometrics of *M. gulio* : **(A)** PCA plot between PC1 and PC2 with percent contribution. **(B)** Procrustes deformations based on the first three principal components for both positive and negative value where each dot indicates the mean and line indicates the shape variation from the mean. **(C)** Shape variation in positive and negative wireframe plot for first three principal components due to salinity variation

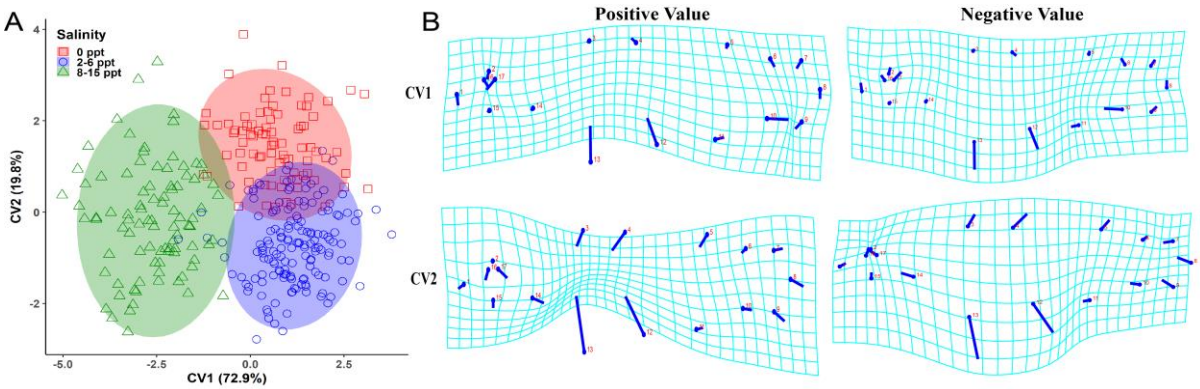

Figure 7 **:** Canonical variate analysis (CVA) and thin-plate spline of Procrustes deformations grid plots showing the morphological change described by each components for different salinity gradient habitats through geometric morphometrics of *M. gulio*.(A) CVA clearly showed the population shape variation due to salinity difference. (B) Procrustes deformations based on the first two canonical variates showed difference in body size from mean points for salinity variation.

cumulatively explained 92.71% of the population (Figure 7A). Zero salinity and low salinity individuals were mainly positioned at the positive value part of the CV1, while high salinity individuals were exhibited entirely in the negative value part. In the case of both CV1 and CV2, shape changes were reflected in the dorsal-ventral portion with compression and stretching. Slight shape changes also showed in the head and tail portions (Figure 7B).

4.2 Body Shape Plasticity between Wild versus Hatchery Population of *M. gulio*

4.2.1 Truss networking

The initial ANOVA testing showed that 29 morphometric measurements (FL, PPVD, P2DFD, 2DFL, CFL, AFL, D1-2, D2-3, D5-6, D6-7, D7-8, D10-11, D11-12, D13-14, D14-15, D15-16, D16-1, D2-16, D2-14, D3-16, D3-15, D3-14, D5-14, D5-13, D6-12, D6-11, D7-11, D7-9, D9-11) out of 47 were significant for two sources variation (wild and culture source) (Table 2). These significant data were used for multivariate (PCA, CVA, LDFA,

CA) analysis. For the source-induced shape variation dataset, the PCA of 29 morphometric data extracted 11 factors with eigenvalues > 1, explaining 82.05% of the variance. PC1 contributed 17.78% of the variation, and PC2 contributed 13.35% (Table 6). The highest significant variables

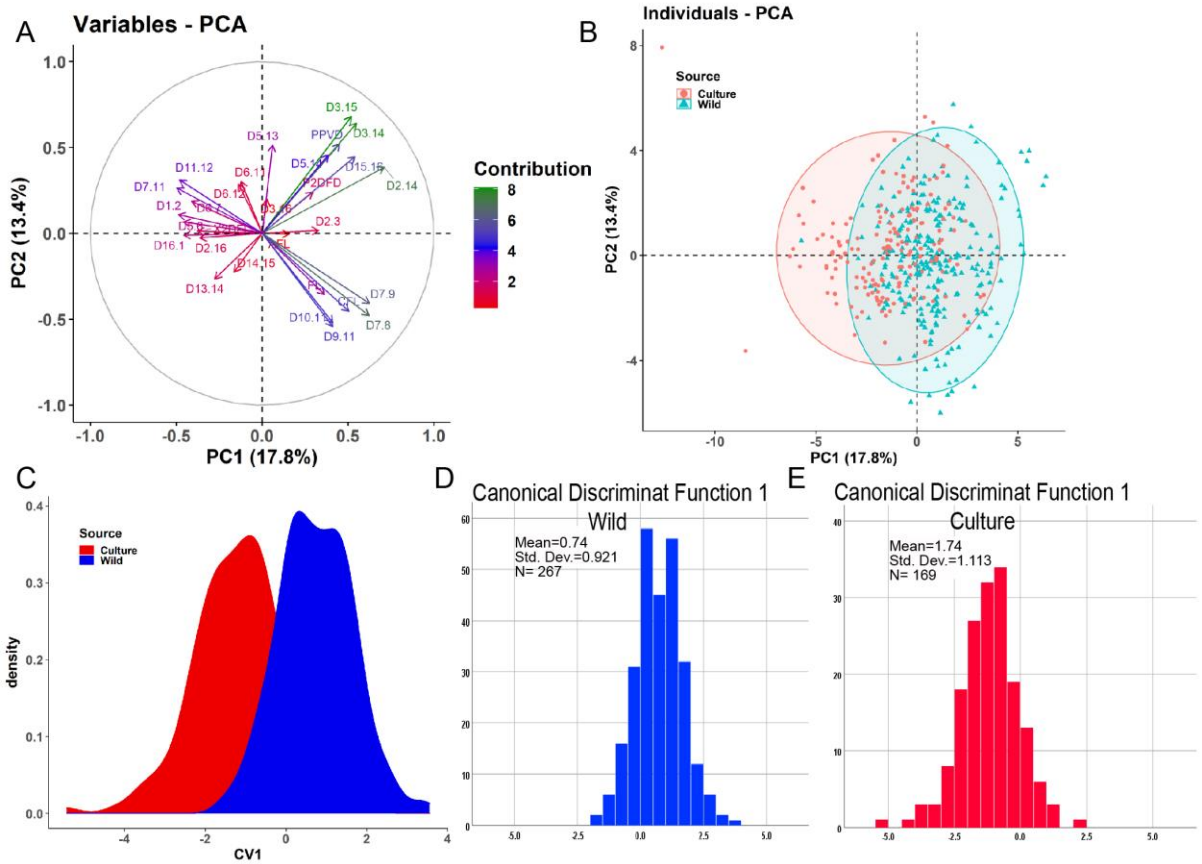

Figure 8 : Principal component analysis (PCA), Density biplot, and Canonical Discriminant Function of truss networking data for depicting population discrimination due to habitats variation. (A-B) PCA biplots showed components with significant influnce in shape variation, (C-E) For two sources difference, all plots showed variation with high degree of overlapping.

loadings on PC1 were D2-14, D7-9, D7-8, D3-14, D15-16, D3-15, CFL and on PC2 were D3-15, D3-14, PPVD, D5-13, D10-11, D9-11 (Table 5; Figure 8A). PCA biplot revealed that the multivariate spaces of wild and culture source samples had a high degree of overlapping pattern with somewhat discriminated shape (Figure 8B). Density plot with CV1 further confirmed the separation of these two groups (Figure 8C). Canonical discriminant function 1 plot and density plots for wild and culture sources also showed that populations had a high degree of overlapping (Figure 8D, E). LDFAshowed that 226 of 267 (84.6%)

wild source individuals and 138 of 169 (81.7%) culture source individuals were originally correctly classified for their original groups (Table 5). Cross-validation testing for source variations was 82% and 77.5% for the wild and culture source samples, respectively, indicating an almost correct classification for their original population. Based on CVA, PCA, and LDFA analysis, our results illustrated that populations from wild and culture sources were partially discriminated, although they frequently overlapped with each other.

Table 5: Percentage classification of *Mystus gulio* individuals for their original group and cross-validation using classification matrix of the DFA based on different morphometric measurements due to source variation collected from different coastal places of Bangladesh.

			Predicted Group Membership		
		Source	Wild	Cultured	Total
Original	Count	Wild	226	41	267
		Cultured	31	138	169
	%	Wild	84.6	15.4	100.0
		Cultured	18.3	81.7	100.0
Cross-validated	Count	Wild	219	48	267
		Cultured	38	131	169
	%	Wild	82.0	18.0	100.0
		Cultured	22.5	77.5	100.0

Table 6: The first five principal components with eigenvalues acquired by PCA for different morphometric characters of *Mystus gulio* due to source variation

Principal Components											
Variable	PC1	PC2	PC3	PC4	PC5	Variable	PC1	PC2	PC3	PC4	PC5
Eigenvalues	5.16	3.87	2.54	2.07	2.01		5.16	3.87	2.54	2.07	2.01
% of variance	17.78	13.35	8.77	7.13	6.92		17.78	13.35	8.77	7.13	6.92
Cumulative%	17.78	31.13	39.89	47.02	53.94		17.78	31.13	39.89	47.02	53.94
Factor Loadings											
FL	0.36	-0.36	-0.01	-0.10	0.32	D15.16	0.54	0.45	-0.08	-0.31	-0.33
PPVD	0.45	0.52	-0.33	0.25	-0.14	D16.1	-0.46	-0.01	0.07	-0.23	-0.21
P2DFD	0.30	0.24	-0.25	0.29	0.31	D2.16	-0.36	-0.03	-0.22	0.47	0.07
X2DFL	-0.38	0.01	0.58	0.21	-0.50	D2.14	0.71	0.38	-0.12	0.05	-0.14
CFL	0.50	-0.45	0.23	-0.10	0.08	D3.16	0.03	0.20	0.17	0.47	0.51
AFL	0.16	0.00	0.10	0.03	-0.26	D3.15	0.52	0.68	0.08	0.23	-0.04
D1.2	-0.49	0.11	-0.28	0.34	0.03	D3.14	0.55	0.64	-0.05	0.21	-0.17
D2.3	0.33	0.02	0.31	-0.01	0.18	D5.14	0.38	0.46	0.34	-0.01	0.27
D5.6	-0.46	0.07	0.63	0.26	-0.35	D5.13	0.06	0.51	0.60	-0.07	0.27
D6.7	-0.41	0.19	-0.49	-0.20	0.22	D6.12	-0.14	0.29	0.54	-0.07	0.35
D7.8	0.62	-0.48	0.15	-0.04	-0.07	D6.11	-0.12	0.30	-0.13	-0.56	0.36
D10.11	0.41	-0.52	0.06	0.31	-0.02	D7.11	-0.50	0.26	0.41	-0.03	0.27
D11.12	-0.48	0.31	-0.06	-0.29	0.26	D7.9	0.63	-0.41	0.19	-0.26	0.23
D13.14	-0.28	-0.26	0.07	-0.03	0.06	D9.11	0.41	-0.54	0.14	0.11	0.18
D14.15	-0.16	-0.23	-0.11	0.59	0.39						

4.2.2 Geometric morphometrics

In the PCA plot, PC1 explained 28.6% of the total variability, while PC2 contributed to 16.3% of the total variability (Figure 9A). The PCA biplot showed that the multivariate spaces of wild populations somewhat diverged from the domesticated hatchery source population, although individuals of both populations mostly overlapped. The CVA analysis depicted that CV1 explained 78.5% of the total variability and CV2 explained 21.5% of the

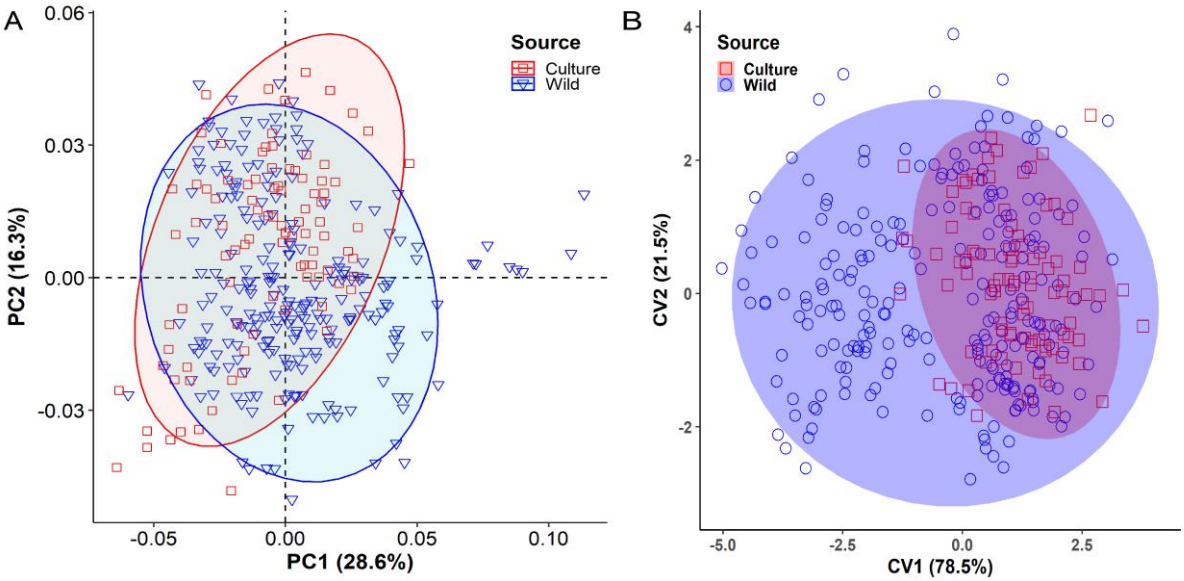

Figure 9 : Principal component analysis (PCA) and Canonical variate analysis (CVA) plots depicting population discrimination with most prominent components due to source (wild versus hatchery) variation through geometric morphometrics data of *M. gulio* population collected from coastal area of Bangladesh (A) PCA plot between PC1 and PC2 with percent contribution. (B) CVA plot between CV1 and CV2 with percent contribution for source variation.

total variability (Figure 9B). Culture source individuals mostly exhibited the positive value side of CV1, and wild source individuals belonged to both the positive and negative value side (Figure 9B). As like PCA, the CVA plot showed that multivariate spaces of both populations overlapped each other, although many individuals of wild sources are discriminated from the individuals of domesticated culture populations.

CHAPTER V

DISCUSSION

Bangladesh is known for its diverse coastal habitats ranging from high-salinity areas to low-salinity estuarine environments. As a result, the *M. gulio* in this region is exposed to a wide range of salinity levels, making it an ideal study system for investigating the effects of salinity on body shape. This study reports on the habitat salinity and source-induced body shape variation of the *M. gulio* along the southern coast of Bangladesh using traditional and geometric morphometrics. Although significant overlapping was observed, different multivariate analyses revealed discrimination among the individuals' body shapes of various populations of *M. gulio*.

5.1 Body Shape Plasticity of *M. gulio* Collected from Different Salinity Gradient Habitats

This study found significant body shape variation of *M. gulio* collected from different salinity gradient habitats. The observed body shape variation of different habitat salinity populations of *M. gulio* was in agreement with Ferdous (2013), who reported significant shape differences in the dorsoventral position of *M. scopoli*. There are a number of ways in which body shape may be linked to salinity niche. First, osmoregulatory capacity can be a primary driver of diversity in *M. gulio* body shape throughout ontogeny. Freshwater *M. gulio* are hyperosmotic. Freshwater fish must constantly produce vast amounts of dilute urine and reabsorb ions from well-developed glomeruli to fight water inflow across their skin and gills (Boeuf and Payan, 2001). Saltwater fish must be drunk to avoid dehydration (Bielmyer et al., 2005). Fusiform body forms in freshwater *M. gulio* are selectively beneficial over wider body shapes in saltwater species because less water diffuses passively into a fusiform body shape. Fish skin is permeable to water (Talbot et al., 1982); hence the diffusion rate should be proportional to the concentration gradient x surface area (Fick's Law). In freshwater settings, where water uptake is a barrier, positive selection should favour bodies with less surface area.

Different flow rates may be anticipated by salinity niche, which can substantially affect fish body shape variation (Blob et al., 2008). In many saltwater environments, periodic and persistent tidal fluctuations can generate higher flow rates than in freshwater bodies (Meyers & Belk, 2014). This constant water movement into and out of the marine environment may have influenced the optimal hydrodynamic conditions for saltwater fish. Another aspect that may impact the body structure of *Mystus gulio* is the surrounding structural elements, which may vary in salinity. Species in complex ecosystems with obstacles to locomotion generally have deep bodies and lower caudal fin aspect ratios. Species residing in more open environments are likely to adopt more streamlined body patterns (Langerhans and Reznick, 2010). *M. gulio*'s body shape and salinity niche may be linked to niche-specific feeding behaviour. It seems reasonable considering that feeding in different salinity niches has affected adaptive morphological divergence as in other fish species (e.g., three-spined sticklebacks; Ravinet et al., 2014). Saltwater species that eat suspended or buried food may have had a relaxed selection of mouth positioning and body structure, resulting in more variable morphologies. Another critical part that may have driven the evolution of *M. gulio*'s body shape is the prevalence of gape-limited predators. Fish often have more robust bodies in places with higher predation rates (Price et al., 2015). Predation pressure is generally higher in saline water than in freshwater. But as far know, no systematic examination has been done to identify the comparative predation pressure in different salinity sources. If *M. gulio* body shape variation were primarily caused by predation, then we would anticipate noticeably varied body shapes in "young" fish in response to the salinity niche because predation is exceptionally high at this stage (Herrel and Gibb, 2006).

Salinity altered the generations' phenotypic traits for three spine stickleback (Mazzarella et al., 2015). Body shape variation of mudskipper, *Scartelaos tenuis* also occurred due to the salinity variation in the Persian Gulf and Gulf of Makran (Ghanbarifardi et al., 2020). Along with fishes, crustaceans like shrimp *Xiphopenaeus kroyeri* also showed morphometric variation in the region of cephalothorax by the influence of salinity gradation (Bissaro et al., 2013). The salinity of waterbodies can significantly influence the morphological and physiological traits like growth rate, survival rate, and body shape of living aquatic organisms such as fish and mammals (Tran-Ngoc et al., 2017; Styga et al., 2019; Lisboa et al., 2015). Eagderi et al. (2019) also found salinity as a significant environmental factor that influences and regulates the body structure of swordtail (*X. helleri*) during morphogenesis.

Individuals are more phenotypically shaped by the environment when they are in the early growth stages (Pinheiro et al., 2005). During the process of fish population segregation, several authors have already considered the effects of environmental conditions (e.g., salinity) on the variation of morphometric characters (e.g., Cardin, 2000; Turan, 2000; Schroeder et al., 2022; Schroeder et al., 2023).

5.2 Body Shape Plasticity between Wild versus Hatchery Population of *M. gulio*

Due to source variation, both wild and cultured populations showed a high level of overlapping in PCA and CVA plots in truss network analysis. Similar phenomena were also shown in the geometric morphometric analysis. Although, previous studies found strong evidence that habitat or source can influence the shape of the organisms. Additionally, geological isolation can shape fish's reproductive patterns and growth (Heidari et al., 2013; Pollar et al., 2007). Apart from fishes, Asaduzzaman et al. (2020) reported that mud crab *Scylla* sp. populations belonging to different geographical areas were morphologically separate. But there also has evidence of phenotypic resemblance in the case of the same species from various cultured and wild sources. Sea bream individuals collected from different sources showed similar body shapes due to the similarity in feeding and stocking patterns (Coban et al., 2008). Although our analysis showed an evident overlapping, there is also significant discrimination between the two sources' populations. In contrast to wild populations, farmed fish are kept in a confined area where they are fed at regular intervals and have easy access to food. Therefore, the foraging behaviour of culture fish is distinct from that of wild fish (Arabaci et al., 2010). Environmental factors in the wild and captivity may contribute to the morphological divergence between wild and hatchery populations of *M. gulio*. In nature, fish must compete for territory, food, survival, and mates. Compared to culture fish, wild fish requires larger fins for quick movement and rapid swimming for feeding or fleeing predators (Basaran et al., 2007). The natural diet of fish is contingent upon the availability and accessibility of their preferred food. In contrast, cultured fish inhabit small areas with high stocking densities, periodic feeding rates, and readily available food. Therefore, cultured fish may require less movement to collect sufficient food than their wild counterparts. As documented in salmonids, crowding in high densities may also promote morphological change like fin nipping in some individuals, resulting in shortened fins (Abbott et al., 1985). Environmental factors may vary across sampling locations in the

wild and culture, resulting in significant interaction effects of populations and sampling locations on morphometric measures. Often, it is suggested that morphological trait variation of fish occurs due to the interaction of environmental and genetic factors (Poulet et al., 2004; Pinheiro et al., 2005). Individuals are more phenotypically shaped by the environment when they are in the early growth stages (Pinheiro et al., 2005). According to previous studies on fish morphometry, variation in morphology, like body shape changes, has been generated due to genetic and plastic responses that reinforce one other (e.g. Robinson and Wilson, 1996; Parsons, 1997). Both adaptive phenotypic changes and genetic divergence can influence the overall body shape of the fish population from a wide range of areas. Although, phenotypical differences may not always reflect genetic differences in populations (Ihssenet al., 1981, Tudela, 1999). Some other factors that can alter the body shape are feeding habits, lifestyle, swimming pattern (Cullen et al., 2007; Rincón et al., 2007) of the fish, water velocity (Imre et al., 2002), water depth (Rincón et al., 2007) and rearing temperature of the water (Marcil et al., 2006).

CHAPTER VI

CONCLUSION

Because the interconnections of populations of a species are critical for sustainable management and conservation, the application of the morphometric method can be a simple and cost-effective approach for characterizing fish stocks. The present study revealed that habitat salinity and captive rearing of wild populations persuaded body shape variation in different populations of *M. gulio*. Although traditional morphometrics (linear and truss-networking distances) and landmark-based geometric morphometrics provided almost consistent outcomes, geometric morphometrics showed more precise and visual observation of the body shape variation of *M. gulio*. The findings of the present study can be considered a first step towards exploring the stock structure of this species based on morphometric traits to build suitable conservation strategies and sustainable management of the long whiskers catfish fishery. Besides habitat and salinity, other external factors like temperature, wave, current, predation, and food habits can control and influence the observed morphometric variation of fishes, which needs further investigation. In addition, intensive molecular and genetic studies, and otolith chemistry could also be useful as complementary tools for further confirmation of the findings of this study.

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Appendix A (Table) Structure Matrix for pooled within-group correlations discriminating variable and standardized canonical discriminate function due to different salinity gradient habitats

Variables	Function		Variables	Function	
	1	2		1	2
D3-15	0.614*	-0.078	1DFBL	0.101*	0.054
D3-14	0.573*	-0.179	D5-14	0.313	-0.350*
D2-14	0.464*	0.008	D7-9	0.103	0.312*
PPVD	0.418*	0.086	D9-11	-0.054	0.240*
D15-16	0.416*	0.139	CFL	0.064	0.226*
D4-14	0.399*	-0.283	D10-11	-0.017	0.217*
D7-11	-0.278*	0.074	P2DFD	0.188	-0.203*
D13-14	-0.214*	-0.117	D11-12	-0.089	-0.201*
D2-16	-0.210*	-0.092	PPCD	0.036	0.201*
D4-15	0.195*	-0.106	D3-13	0.109	-0.188*
D1-2	-0.187*	-0.117	D3-16	-0.058	-0.184*
D5-6	-0.159*	-0.091	D7-8	0.130	0.179*
D14-15	-0.155*	-0.028	AFL	0.154	0.177*
D6-7	-0.146*	0.064	D5-13	0.110	-0.168*
2DFL	-0.114*	0.045	D16-1	-0.141	-0.168*
D2-3	0.111*	-0.070	HL	-0.080	-0.152*
D5-12	0.110*	0.056	D8-9	0.082	-0.113*
D3-4	0.109*	0.007	D3-15	0.614*	-0.078
D9-10	0.102*	-0.032			

Appendix B (Table) ANOVA testing of 8 different morphometric and 28 truss networks (Tests of Equality of Group Means) for different salinity gradient habitats

Variables	Wilks' Lambda	F	Variables	Wilks' Lambda	F
HL	0.979	4.595	D13-14	0.923	17.960
PPCD	0.980	4.510	D14-15	0.961	8.774
PPVD	0.772	63.816	D15-16	0.770	64.509
P2DFD	0.927	16.937	D16-1	0.956	9.959
1DFBL	0.982	4.000	D2-16	0.928	16.814
2DFL	0.978	4.932	D2-14	0.736	77.812
CFL	0.971	6.579	D3-16	0.979	4.600
AFL	0.949	11.740	D3-15	0.613	136.677
D1-2	0.939	14.052	D3-14	0.640	121.961
D2-3	0.978	4.961	D3-13	0.965	7.782
D3-4	0.980	4.332	D4-15	0.936	14.851
D5-6	0.956	9.955	D4-14	0.768	65.391
D6-7	0.964	8.134	D5-14	0.820	47.639
D7-8	0.959	9.299	D5-13	0.968	7.178
D8-9	0.983	3.705	D5-12	0.979	4.681
D9-10	0.982	3.861	D7-11	0.883	28.566
D10-11	0.978	4.791	D7-9	0.941	13.589
D11-12	0.969	6.924	D9-11	0.970	6.774

Appendix C (Table) Results of Wilks' lambda test for different salinity gradient habitats between *Mystus gulio* population

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	0.256	565.662	72	0.000
2	0.684	157.764	35	0.000

Appendix D (Table) Correlation between the assessed morphometric variables and linear discriminant functions of *Mystus gulio* for different salinity gradient habitats

Variables	DF1	DF2	Variables	DF1	DF2
HL	-0.163	-0.211	D13-14	0.078	0.049
PPCD	-0.092	0.341	D14-15	0.051	0.336
PPVD	0.242	-0.078	D15-16	-0.291	0.512
P2DFD	0.299	0.167	D16-1	-0.061	-0.256
1DFBL	-0.002	0.844	D2-16	-0.305	0.079
2DFL	0.180	0.386	D2-14	-0.053	0.203
CFL	-0.108	0.207	D3-16	-0.249	-0.542
AFL	0.042	0.027	D3-15	0.938	0.360
D1-2	-0.019	-0.154	D3-14	0.422	-0.535
D2-3	0.060	-0.116	D3-13	-0.446	-0.329
D3-4	0.353	-0.810	D4-15	-0.542	0.106
D5-6	-0.304	-0.375	D4-14	-0.170	-0.075
D6-7	-0.061	0.008	D5-14	0.176	-0.496
D7-8	0.180	-0.434	D5-13	-0.141	-0.084
D8-9	-0.019	0.070	D5-12	0.464	0.087
D9-10	0.143	0.132	D7-11	-0.420	0.559
D10-11	-0.133	0.127	D7-9	0.055	0.839
D11-12	0.172	-0.199	D9-11	0.018	-0.145

Appendix E (Table) Structure Matrix for pooled within-group correlations discriminating variable and standardized canonical discriminate function due to wild verses hatchery populations

Variables	Function 1	Variables	Function 1	Variables	Function 1
D7-11	-0.627	D2-3	0.251	D3-16	-0.151
D7-9	0.365	D9-11	0.251	D5-14	0.149
D2-16	-0.362	CFL	0.238	D13-14	-0.138
D7-8	0.356	D6-7	-0.235	D14-15	-0.134
D1-2	-0.327	FL	0.234	AFL	0.130
D2-14	0.321	D6-11	-0.229	2DFL	-0.129
D10-11	0.272	D3-14	0.217	D5-13	-0.126
D15-16	0.267	D3-15	0.213	D16-1	-0.121
PPVD	0.264	D6-12	-0.175	P2DFD	0.116
D11-12	-0.256	D5-6	-0.173		

Appendix F (Table) Results of Wilks' lambda test for wild verses hatchery populations of *Mystus gulio* population

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1	0.536	261.521	29	0.000

Appendix G (Table) ANOVA testing of 6 different morphometric and 23 truss networks (Tests of Equality of Group Means) for different salinity gradient habitats

Variables	Wilks' Lambda	F	Variables	Wilks' Lambda	F
FL	0.955	20.551	D15-16	0.942	26.724
PPVD	0.943	26.114	D16-1	0.987	5.529
P2DFD	0.989	5.016	D2-16	0.898	49.265
2DFL	0.986	6.280	D2-14	0.918	38.754
CFL	0.953	21.218	D3-16	0.981	8.610
AFL	0.985	6.394	D3-15	0.962	17.109
D1-2	0.915	40.182	D3-14	0.961	17.754
D2-3	0.948	23.709	D5-14	0.981	8.363
D5-6	0.975	11.222	D5-13	0.986	5.983
D6-7	0.955	20.668	D6-12	0.974	11.511
D7-8	0.901	47.525	D6-11	0.957	19.613
D10-11	0.940	27.719	D7-11	0.746	147.575
D11-12	0.946	24.534	D7-9	0.897	50.057
D13-14	0.984	7.194	D9-11	0.948	23.688
D14-15	0.985	6.748			

Appendix H (Table) Correlation between the assessed morphometric variables and linear discriminant functions of *Mystus gulio* for wild verses hatchery populations

Variables	DF1	Variables	DF1	Variables	DF1
FL	0.011	D7-8	0.480	D3-15	0.236
PPVD	0.640	D10-11	0.274	D3-14	0.015
P2DFD	0.042	D11-12	0.170	D5-14	0.183
2DFL	-0.056	D13-14	0.217	D5-13	-0.027
CFL	-0.324	D14-15	-0.374	D6-12	0.074
AFL	0.218	D15-16	-0.341	D6-11	-0.147
D1-2	0.310	D16-1	-0.185	D7-11	-0.651
D2-3	0.720	D2-16	-0.300	D7-9	0.363
D5-6	0.256	D2-14	-0.328	D9-11	-0.002
D6-7	0.221	D3-16	-0.527		

Appendix I (Table) Geometric Morphometric- Values of Principal Component Analysis (PCA) of *M. gulio* for different salinity gradient habitats: First ten principal components with cumulative values showed where the first five components consist of a total of 71.88%.

PCA Variables	Values	Cumulative Values	PCA Variables	Values	Cumulative Values
PC1	28.639	28.639	PC6	4.57	76.458
PC2	16.302	44.941	PC7	3.832	80.29
PC3	11.801	56.742	PC8	3.009	83.299
PC4	8.891	65.632	PC9	2.855	86.154
PC5	6.256	71.888	PC10	2.466	88.619