

**GENETIC IDENTIFICATION AND INDUCED BREEDING OF
THREATENED INDIGENOUS SPECIES, *Channa striata* (Bloch, 1793)
FOR SEED PRODUCTION**

A PhD DISSERTATION

BY

RUBAIYA PERVIN

Registration No: 2205057

Session: 2022

FISHERIES BIOLOGY AND GENETICS



DEPARTMENT OF FISHERIES BIOLOGY AND GENETICS

**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,
DINAJPUR-5200**

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Research Supervisor



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Research Co-Supervisor

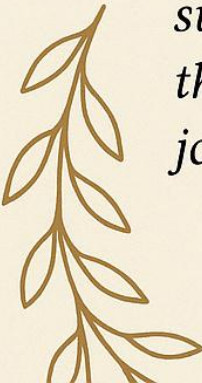
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Chairman, Examination Committee
and Chairman

Department of Fisheries Biology and Genetics

**HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY,
DINAJPUR-5200**

July, 2025

**DEDICATED
TO MY
BELOVED PARENTS
AND FAMILY
MEMBERS**



*For their unwavering love, endless
support, and constant encouragement
through every step of my academic
journey.*

Certification

This is to certify that the thesis entitled “**Genetic Identification and Induced Breeding of Threatened Indigenous Species, *Channa Striata* (Bloch, 1793) for Seed Production**” submitted by **Rubaiya Pervin** (Reg No: 2205057, Session 2022), a PhD candidate in the Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur is a record of original research carried out by the author under my supervision. This work is the result of her independent investigation, is, to the best of my knowledge, original, and has not been submitted, either in part or in full, for the award of any degree or diploma at this or any other institution.

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Declaration by Author

I, Rubaiya Pervin, hereby declare that:

This thesis is a true account of my original research and has been substantially completed during my period of enrolment in this degree program. The main content of thesis has not been previously submitted for the award of any degree or diploma at any other university or academic institution. Furthermore, I confirm that no part of this thesis will, in the future, be submitted by me for the award of any other degree or diploma at any university or tertiary institution. This thesis does not contain any material that has been previously published or written by another person, except where due acknowledgement and referencing have been made in the text. It does not infringe any copyright, trademark, patent, or any other intellectual property rights of any individual or organization.

The research involving animal subjects presented in this thesis was reviewed and approved by the Ethics Committees of Hajee Mohammad Danesh Science and Technology University under Approval no.: No HSTU/ IRT 4361(1)). This thesis contains published work and/or work prepared for publication, some of which has been co-authored.

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ABSTRACT

Channa striata, commonly known as the striped snakehead, is a high-value freshwater species with significant aquaculture potential in Bangladesh and Southeast Asia. However, its natural populations in Bangladesh are declining due to habitat degradation, overexploitation, and inadequate aquaculture practices. Despite its commercial importance, large-scale seed production remains underdeveloped owing to critical gaps in species identification, reproductive management, induced breeding, and larval rearing protocols. This dissertation aims to bridge these gaps and establish a scientific basis for the sustainable aquaculture of *C. striata* in Bangladesh. Four sequential studies were conducted: (i) species identification and phylogenetic analysis using geometric morphometrics and COI gene-based DNA barcoding; (ii) assessment of growth and reproductive biology of wild populations from the Atrai River system; (iii) standardization of hormonal induction protocols using carp pituitary extract (CPE) and Buserelin; and (iv) evaluation of weaning strategies for hatchery-produced larvae using *Artemia*, fish paste, and formulated feeds. The results confirmed clear species delineation among native and exotic *Channa* spp., with phylogenetic divergence supported by both morphometric and molecular data. Wild *C. striata* exhibited seasonal reproduction, with peak spawning from April to July. Induced breeding trials demonstrated that both CPE (80 mg/kg) and Buserelin (0.80 µg/kg) effectively stimulated spawning. Larval weaning experiments revealed that initial live feeding with *Artemia* followed by gradual transition to formulated diets ensured high survival and growth, while water quality, particularly ammonia concentration, played a critical role. Overall, this research provides a comprehensive framework for the conservation and aquaculture of *C. striata* by integrating taxonomic validation, reproductive biology, induced breeding, and larval nutrition. The findings are expected to support the development of standardized hatchery protocols, contribute to wild stock conservation, and promote commercial-scale seed production in Bangladesh. Future research should emphasize selective breeding, advanced larval nutrition, and field-level application of the proposed protocols for long-term sustainability.

Keywords: *Channa striata*, DNA barcoding, induced breeding, larval rearing, reproductive biology, morphometric analysis, sustainable aquaculture.

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LIST OF ABBREVIATIONS

Abbreviated form	Full Form
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
bp	Base pair
COI	Cytochrome C Oxidase Subunit I
DAMBE	Data Analysis In Molecular Biology and Evolution
DNA	Deoxyribonucleic acid
DoF	Department of Fisheries
FAO	Food and Agriculture Organization
FASTA	Fast Adaptive Shrinkage Thresholding Algorithm
GMA	Geometric Morphometric Analysis
HSTU	Hajee Mohammad Danesh Science and Technology University
IUCN	International Union for Conservation of Nature
K2P	Kimura-2-Parameter
LWR	Length-Weight Relationships
MANOVA	Multivariate Analysis of Variance
MCL	Maximum Composite Likelihood
MEGA	Molecular Evolutionary Genetic Analysis
ML	Maximum Likelihood
MP	Maximum Parsimony
mt genome	mitochondrial genome
mtDNA	mitochondrial Deoxyribonucleic Acid
NCBI	National Centre for Biotechnology Information
NJ	Neighbour Joining
PCA	Principal Component Analysis
PAST	Paleontological Statistics
PCR	Polymerase Chain Reaction
Pi	Parsimony Informative
ref	Reference
sec	Seconds
seq	Sequence
SNP	Single Nucleotide Polymorphism
SPSS	Statistical package for social science
TPS	Thin Plate Splines
UPGMA	Unweighted Pair Group Method with Arithmetic mean

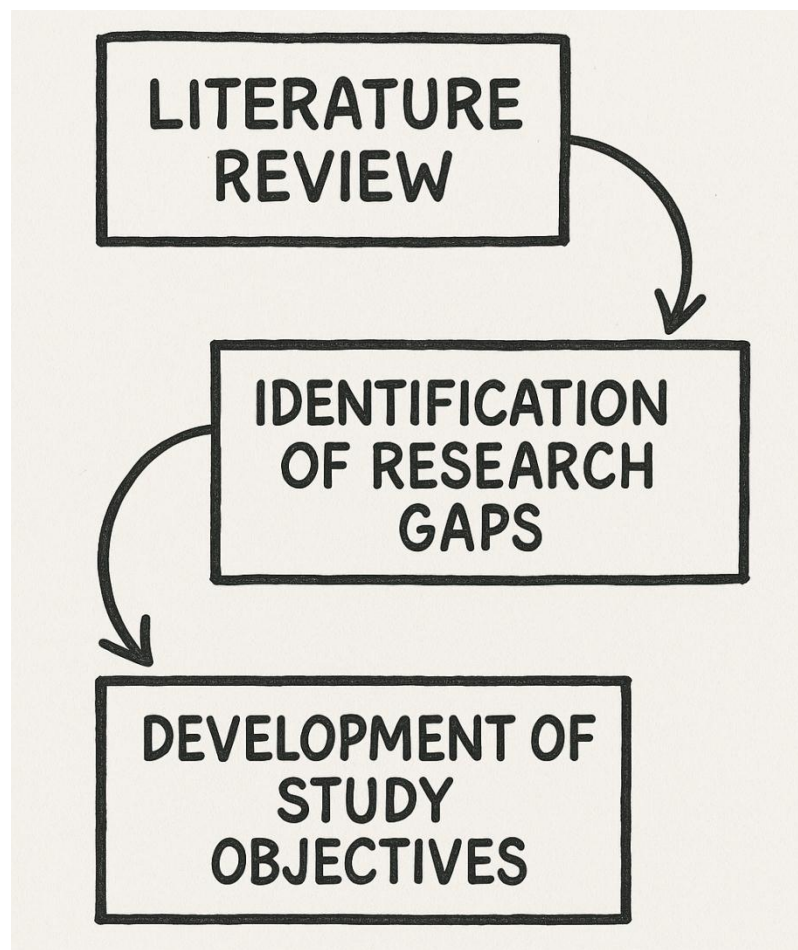
Organization of the thesis

This thesis is organized into the following chapters:

- **Chapter 1:** Literature Review, Identification of Research Gaps, and Development of Study Objectives
- **Chapter 2:** Identification of indigenous and exotic *Channa striata* and its evolutionary relationship within the *Channa* genus
- **Chapter 3:** Growth and reproductive biology of *C. striata* in the drought-stressed Atrai river: implications for conservation and aquaculture.
- **Chapter 4:** Hormonal Dose Optimization for Induced Breeding of *Channa striata* in Captivity
- **Chapter 5:** Impact of Gradual Weaning and Feeding Regimes on Survival and Growth of *Channa striata* Larvae
- **Chapter 6:** Summary, conclusion and recommendation
- **References**
- **Appendices**

Chapter 1

Chapter 1: Literature Review, Identification of Research Gaps, and Development of Study Objectives



This chapter presents a comprehensive review of the existing literature on the taxonomy, genetics and phylogeny, reproductive biology, growth, gonadal development, and aquaculture of *Channa striata*. By critically analyzing current research findings, the chapter synthesizes key developments and trends while identifying significant gaps in knowledge, particularly in areas such as region-specific genetic diversity, standardized breeding protocols, larval weaning strategies, and the ecological implications of aquaculture practices. These research gaps highlight the need for integrated and localized studies to support sustainable aquaculture and conservation management. Consequently, the chapter concludes by outlining the specific aims and objectives of the present study, which are strategically developed to address these gaps and contribute to the scientific understanding and aquacultural advancement of *C. striata*.

1.1 General Background

Aquaculture has witnessed rapid global expansion, driven by technological innovations in fish breeding and farming practices. These advancements have enabled the domestication of new aquatic species, particularly those under threat in the wild, thereby accelerating the diversification of cultured species (Duarte et al., 2007; Arthington et al., 2016). As the fastest-growing sector in global food production, aquaculture contributes significantly to food and nutritional security. However, to sustain this growth and address future challenges, continuous innovation and strategic planning are essential (Joffre et al., 2021). Among the species of growing interest in Asian aquaculture, the striped snakehead, *Channa striata* (Bloch, 1793), is a carnivorous freshwater fish valued for its high protein content, medicinal properties and market demand. Traditionally harvested from the wild, *C. striata* has become increasingly attractive for aquaculture due to its adaptability to diverse environmental conditions, air-breathing ability, tolerance to low dissolved oxygen and

strong disease resistance. Its potential for intensive and semi-intensive culture has made it a focus species in efforts to enhance indigenous fish production. However, anthropogenic impacts on freshwater ecosystems, such as dam construction, river modification, and mismanaged drainage systems, have severely disrupted natural breeding grounds of rheophilic and native fish species (Kujawa et al., 2022; Parvez et al., 2022; Nowosad et al., 2014). These challenges are exacerbated by the lack of controlled breeding and larval rearing programs, resulting in pressure on wild broodstock and unsustainable harvest practices. According to the IUCN (2015), 64 out of 253 native freshwater fish species in Bangladesh are threatened with extinction on varying levels. Alarming, the contribution of indigenous fish production in overall fish production of Bangladesh is declining each year (DoF, 2022). This situation necessitates urgent action to conserve and cultivate threatened native species. The development of captive breeding and aquaculture technologies for *C. striata* not only presents a viable opportunity to enhance rural livelihoods and food security but also plays a critical role in biodiversity conservation and the sustainable management of inland fisheries.

1.2 Economic, Nutritional, and Ecological Value of *C. striata*

The striped murrel (*Channa striata*), also known as the striped snakehead, is increasingly recognized as a high-potential candidate species for freshwater aquaculture across Asia. Its remarkable biological attributes including rapid growth rate, high market demand and nutritional richness have positioned it as an economically and nutritionally important species for rural and commercial aquaculture (Romadhoni et al., 2016). The flesh of *C. striata* is particularly valued for its high protein and albumin content, which are known to aid in tissue regeneration and post-operative recovery. These medicinal and dietary benefits have fostered growing consumer demand, especially in Southeast Asia, where the species is part of traditional health practices (Zakaria et al., 2007).

From a physiological perspective, *C. striata* is exceptionally resilient, capable of surviving in harsh environmental conditions such as hypoxia and elevated ammonia levels. This tolerance is due to the presence of a well-developed accessory respiratory organ, enabling the fish to breathe atmospheric oxygen and endure suboptimal water quality (Qin et al., 1997). Such traits make it suitable for culture in diverse freshwater systems including ponds, tanks, cages, and rice fields under both semi-intensive and intensive systems (Wee, 1982; Mollah et al., 2009; Rahman et al., 2012, 2013). The aquaculture of *C. striata* is already well-established in several countries, including Vietnam, Indonesia, Thailand, Malaysia, and India. These nations have developed robust seed production, grow-out farming, and marketing systems, demonstrating the species' adaptability to artificial culture environments and its compatibility with local farming practices (Romadhoni et al., 2016). The sustained growth in production in these regions attests to the biological and economic viability of the species.

Given the similar agro-ecological conditions shared by these countries and Bangladesh, it is strongly presumed that *C. striata* could be successfully integrated into the freshwater aquaculture systems of Bangladesh. With the increasing need to diversify cultured species and promote sustainable food sources, the commercial farming of *C. striata* in Bangladesh holds significant promise. Its incorporation into the national aquaculture agenda could not only reduce pressure on wild stocks but also enhance income opportunities for small-scale farmers and contribute to national nutritional goals.

1.3 Identification of *C. striata* (Bloch 1973)

1.3.1. Taxonomy

The genus *Channa* (family Channidae) comprises a group of carnivorous freshwater fishes broadly distributed across Asia, with *C. striata* (Bloch, 1793) recognized as one of the most

widespread and economically significant species (Ng & Lim, 1990; Courtenay & Williams, 2004; Froese & Pauly, 2023). *C. striata* or striped snakehead was first documented by Marcus Eliese Bloch in 1793 in his study on ichthyology as *Ophiocephalus striatus* (Bloch, 1793). Since then its scientific name has undergone through several changes and revision by taxonomists and finally standardized as *C. striata* overtime. The common name varies in different countries such as: Banded snakehead in India (Talwar and Jhingran, 1991), Haruan in Indonesia and Malaysia (Thomas et al., 2005), Shoal in Bangladesh (Rahman, 2005; Galib et al., 2013), Plachon in Thailand (Ukkatawewat, 1984). The taxonomic position of *C. striata* is given below

Classification of *C. striata*

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Suborder: Channoidei

Family: Channidae

Genus: *Channa*

Species: *C. Striata*

Historically, the taxonomic identification and classification of *Channa* species relied on classical morphological characters, including meristic counts and morphometric measurements, as first comprehensively described by Day (1878) and later refined in regional ichthyofaunal accounts (Talwar & Jhingran, 1991; Shrestha, 1999). Early taxonomic studies, such as those by Hora (1924) and Wee (1982), provided baseline diagnoses for the genus but lacked tools to detect cryptic diversity or subtle intraspecific variation. Recent systematic revisions have uncovered several new *Channa* species from South and Southeast Asia, especially in biodiversity-rich regions like northeastern India and

Sri Lanka (Britz, 2007; Murray, 2006; Sudasinghe & Meegaskumbura, 2020), emphasizing the genus's underestimated diversity. Studies by Britz (2013, 2017) further demonstrated ontogenetic and intraspecific morphological variation that complicates species delineation using only classical characters. Moreover, regional works such as those by Musikasinthorn (2003) and Vishwanath & Geetakumari (2009) have stressed the need for integrative taxonomic frameworks combining morphological, ontogenetic, and molecular data to address the limitations of traditional approaches.

1.3.2. Morphometrics

The long body of the striped snakehead is distinguished by bands of white on the belly and a dark black-brown upper portion (Figure 1.1). Because of their huge scales and rather wide heads similar to snake, species in the Channidae family are called "snakeheads" (Muthmainnah, 2013). Additionally, they have big jaws with four to seven canines and sharp visible teeth. An accessory respiratory apparatus is situated dorsally to the gill arches. This respiratory organ opens into the buccal cavity anteroventrally and the opercular chamber posterolaterally that helps to survive in harsh environment for long time (Kumar et al., 2022). The striped snakehead can grow up to 90 cm in length and weigh up to 3 kg.



Figure 1.1. Adult specimen of *C. striata* (striped snakehead) measured against a 30 cm scale bar for morphometric assessment (source: sample collected from Atria River).

To overcome the constraints of classical taxonomy, morphometric analyses, both traditional and geometric have been increasingly employed to assess phenotypic variation in *Channa* species. Traditional morphometric techniques, involving linear body measurements and ratios, have proven effective in identifying population-level variation and stock structure (Choudhury & Dutta, 2013; Kantachumpoo & Ngamniyom, 2020). In Bangladesh, Mallik et al. (2020) and Shafi (2014) demonstrated significant morphometric differentiation among *C. striata* populations from various riverine systems, suggesting possible local adaptation and restricted gene flow.

The development of geometric morphometrics, particularly landmark-based methods pioneered by Bookstein (1986, 1991), has revolutionized the analysis of fish body shape by allowing quantitative assessment of form independent of size. This technique captures complex shape differences and is less prone to measurement error than traditional methods. Its application in snakehead studies has uncovered significant shape variation between wild and cultured populations (Hanifa et al., 2014; Duong et al., 2019), as well as environmental influences on morphology (Torres et al., 2020). Integrating geometric morphometrics with image analysis and statistical modeling has further improved stock identification and species discrimination (Cadrin & Friedland, 1999), providing critical insights for aquaculture, conservation, and fisheries management.

1.3.3. Genetic Identification and Phylogeny

DNA barcoding, particularly using the COI gene, has emerged as a robust tool for species identification, cryptic diversity detection and taxonomic refinement within the genus (Ahmed et al., 2018; Irmawati et al., 2022). In particular, DNA barcoding studies have detected regional genetic variation in *C. striata* across its distributional range, suggesting geographic isolation and limited gene flow among populations. Population

genetic studies using mitochondrial and nuclear markers further confirm significant genetic structuring influenced by historical river systems and anthropogenic factors (Duong et al., 2019; Kanon et al., 2022; Alam et al., 2022).

Substantial progress has been made in elucidating the molecular phylogenetics and genetic architecture of the genus *Channa*, encompassing both evolutionary insights and aquaculture applications. Phylogenetic studies employing mitochondrial genes such as cytochrome *b* (Cyt *b*), 12S rRNA, and cytochrome c oxidase I (COI) have delineated major clades within *Channa* spp., highlighting significant evolutionary divergence and biogeographic structuring (Li et al., 2006; Adamson et al., 2010; Conte-Grand et al., 2017). More recent multi-locus and phylogenomic approaches have resolved deep evolutionary relationships and revealed instances of rapid radiation and adaptive divergence (Rüber et al., 2020).

1.4 Geographic Distribution and Habitat of *C. striata*

The geographic range of *C. striata* encompasses a broad area across East and Southeast Asia, covering both native and introduced regions. In its native distribution, the species is found in Bangladesh, Cambodia, China, India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, Thailand, and Vietnam (Rahman, 2005; Talwar and Jhingran, 1991). In addition to these native habitats, *C. striata* has been introduced to several countries including South Korea, the Philippines, New Guinea, and Fiji, where it has established viable populations.

C. striata typically inhabits shallow freshwater environments, usually ranging from 1 to 2 meters in depth and is rarely found below 10 meters (Cagauan, 2007). The species thrives in a wide range of slow-moving freshwater bodies such as irrigation reservoirs,

swamps, flooded rice paddies, canals, creeks, lakes, ponds, and rivers (Muthmainnah, 2013). It demonstrates a high degree of ecological plasticity and resilience, particularly in tolerating low oxygen concentrations and high turbidity conditions that would limit the survival of many other freshwater fishes. During the rainy season, *C. striata* often migrates from permanent water bodies like rivers and lakes into temporarily flooded areas for feeding and breeding. As these seasonal floodplains dry up, the fish return to more permanent aquatic habitats (Phen et al., 2005). This migratory behavior enhances its adaptability and contributes to its widespread distribution and success in diverse freshwater ecosystems.



Figure 1.2 Map showing the distribution of *C. striata* (Source: Google map)

1.5 Feeding Ecology and Dietary Habits of *C. striata*

C. striata generally inhabits the bottom of freshwater bodies but is considered a surface feeder due to its predatory nature and feeding behavior (De Silva et al., 2015). It is a carnivorous species that exhibits a strong preference for live prey throughout its life cycle. Both juvenile and adult stages primarily consume small fishes, crustaceans and aquatic insect larvae including shrimps, prawns, crabs, mosquito larvae and water bugs (Kumar et al., 2022). This dietary composition classifies *C. striata* as a carnivorous, piscivorous,

larvicidal and occasionally cannibalistic species. The feeding habit of *C. striata* undergoes ontogenetic shifts, changing from an omnivorous-carnivorous diet in the fry stage to a predominantly carnivorous diet in adults. This transition is supported by the progressive decrease in the relative gut length (RGL), which reflects an adaptation to a more protein-rich, animal-based diet as the fish matures (Kuan-Chung et al., 2016).

Seasonal and ontogenetic variations also influence food preference. Fry predominantly feed on zooplankton, juveniles prefer insects and adults favor larger prey such as crustaceans and other available aquatic invertebrates (Kumar et al., 2027). Seasonal feeding intensity is typically measured using the Gastosomatic Index (GaSI), which varies significantly across different times of the year. Feeding intensity is generally reduced during winter due to lower metabolic activity and also declines during the pre-monsoon and breeding periods when the enlarged gonads occupy most of the abdominal cavity, limiting gut expansion. Furthermore, in conditions of food scarcity or poor feed management especially in captive culture, *C. striata* exhibits increased levels of cannibalistic behavior, posing challenges to its larval and juvenile rearing.

1.6 Morphological Variation of *C.striata* Identified across Different Regions and Habitat

Morphological variation in *C. striata* has been widely reported across different regions of Asia, highlighting the combined influence of genetic and environmental factors. Rahim et al. (2009) investigated morphometric differences among *C. striata* populations from six geographically distinct regions in Malaysia and observed significant variation in several traits. These findings suggest that genetic differentiation, environmental heterogeneity and local adaptation play crucial roles in shaping phenotypic diversity in this

species. More broadly, morphological differences in fish often reflect the effects of geographic isolation, ecological constraints and natural selection acting on locally adapted traits (Cadrin, 1999; Su et al., 2018). Ecological strategies such as feeding behavior, habitat preference, movement patterns and interspecific interactions can significantly shape morphology (Duong et al., 2019). For instance, in *C. striata*, individuals with larger heads are more competitive in food-limited environments due to their increased aggressiveness and dominance during foraging. This supports the persistence of a large-head phenotype under high competition. Similarly, Torres et al. (2020) employed geometric morphometric analysis to evaluate morphological variation among *C. striata* populations in Laguna de Bay, Philippines, revealing clear phenotypic divergence among populations. This reinforces the notion that geographic and ecological factors strongly influence morphological differentiation.

Despite extensive classical taxonomic studies on *Channa* species (Day, 1878; Talwar & Jhingran, 1991; Britz, 2017), considerable challenges remain in accurately delineating species boundaries and understanding intraspecific morphological variation. Classical approaches based mainly on meristic counts and linear measurements often fail to resolve cryptic species and ontogenetic shape changes that confound taxonomy (Britz, 2013; Vishwanath & Geetakumari, 2009). Although morphometric techniques, particularly geometric morphometrics, have been applied to quantify phenotypic variation in *C. striata* populations (Hanifa et al., 2014; Duong et al., 2019; Torres et al., 2020), most studies focus on limited geographic scales or compare wild versus cultured stocks without integrating environmental or genetic data comprehensively.

Moreover, current research largely lacks integrative frameworks combining geometric morphometric shape analyses with molecular phylogenetics and ecological data

to clarify species boundaries, population structure and evolutionary relationships across the species' wide distribution. For example, morphometric variation reported in Bangladesh (Mallik et al., 2020; Shafi, 2014) and the Mekong Delta (Duong et al., 2019) remains inadequately linked with genetic diversity patterns, limiting understanding of local adaptation and gene flow. Furthermore, environmental factors driving morphological plasticity, such as water quality and habitat heterogeneity are seldom addressed in conjunction with morphometric variation (Torres et al., 2020).

Future research on *C. striata* should adopt an integrative taxonomic approach that synergizes classical morphological, geometric morphometric and molecular genetic methods to resolve taxonomic ambiguities and elucidate population differentiation. Expanding the geographic scope to include diverse habitats across South and Southeast Asia will enable comprehensive assessment of morphological and genetic diversity patterns. Longitudinal studies examining ontogenetic shape changes using geometric morphometrics (Bookstein, 1986, 1991) could further improve species diagnosis and stock identification. Additionally, incorporating environmental parameters such as water quality, habitat complexity, and anthropogenic impacts will help disentangle phenotypic plasticity from genetic differentiation, improving management strategies for wild populations and aquaculture stocks (Torres et al., 2020; Duong et al., 2019). The development of high-throughput molecular markers alongside advanced morphometric analyses offers a promising avenue to detect cryptic diversity and inform conservation policies, sustainable fisheries and breeding programs. Ultimately, integrating morphometric data with genomics and ecological modeling will deepen understanding of evolutionary processes shaping *C. striata* diversity and adaptability in rapidly changing environments.

1.7 Genetic Identification and Genetic Variation of *C. striata*

The identification, naming and classification of living organisms at the species level is foundational to biology and essential for biodiversity assessment, conservation and aquaculture management (Kim et al., 2010). Traditionally, species classification has relied heavily on morphological traits; however, this approach is limited when dealing with species that exhibit minimal external variation or when larval stages differ drastically from adult forms, often resulting in misidentification (Nguyen & Duong, 2016). Such challenges are particularly evident in "cryptic species" distinct lineages that are morphologically similar but genetically divergent. To resolve these complexities, molecular tools such as DNA barcoding have emerged as robust alternatives to traditional taxonomy, offering precision in species identification and genetic assessments (Hebert et al., 2003).

Among these tools, mitochondrial DNA (mtDNA) markers especially Cytochrome c Oxidase Subunit I (COI) and Cytochrome b (Cyt b) have been extensively used for species-level identification and phylogenetic studies in *Channa* species (Rahman et al., 2013; Barman et al., 2018). The genus *Channa*, comprising 34 species largely distributed across South and Southeast Asia, remains an active area of taxonomic and phylogenetic investigation, particularly due to unresolved species boundaries and recent additions to the genus (Adamson et al., 2010; Benziger et al., 2011; Musikasinthorn, 2000).

Species such as *C. striata* have been identified as "species complexes," with uncertain taxonomic boundaries and potential cryptic diversity (Courtenay & Williams, 2004). To address this, studies across Asia have employed COI and Cyt b markers, revealing significant genetic diversity and highlighting regional population structures. For example, studies in Malaysia (Jamaluddin et al., 2011; Song et al., 2013) and Vietnam (Nguyen & Duong, 2016; Nguyen et al., 2018) have shown considerable haplotype

variation among *C. striata* populations, indicating historical gene flow and localized genetic differentiation.

In Bangladesh, Ahmed et al. (2018), Alam et al. (2020, 2022), and Kanon et al. (2022) applied COI and Cyt b markers to distinguish native populations and compare them with cultured stocks from Vietnam. These studies consistently reported high haplotype and nucleotide diversity along with the identification of distinct genetic clusters, likely influenced by habitat fragmentation and anthropogenic activities. Similarly, Irmawati et al. (2022) studied *C. striata* populations in Wallacea, Indonesia, and uncovered geographically structured genetic variation using COI markers.

Complementary to mitochondrial markers, nuclear markers such as microsatellites and random amplified polymorphic DNA (RAPDs) have been utilized to assess intraspecific variation and population structure (Singh et al., 2014). Multi-locus approaches combining mitochondrial and nuclear data have further refined phylogenetic reconstructions, as demonstrated by Conte-Grand et al. (2017) and Lalramliana et al. (2019), who revealed cryptic lineages and evolutionary divergence events dating back to the Miocene–Pliocene periods.

Beyond taxonomy and systematics, molecular tools are increasingly applied in aquaculture to manage broodstock, prevent inbreeding and monitor genetic integrity. Nugraha et al. (2021) and Kumar et al. (2022) highlighted the use of DNA barcoding and marker-assisted selection for maintaining genetic quality in *C. striata* hatcheries. Comparative studies between wild and hatchery-reared stocks also underscore the risk of genetic erosion and introgression (Araki & Schmid, 2010; Nguyen & Duong, 2016), which may compromise the resilience and performance of cultured strains.

Overall, the integration of molecular techniques is crucial for the accurate taxonomy, conservation and sustainable aquaculture of *Channa* species. A comprehensive summary of molecular studies on *C. striata* including gene markers, population types and geographic focus, is presented in the table 1.1.

Table 1.1 Summary of molecular marker applications in *channa* species: population studies and broader genetic insights

SL. No.	Reference	Region Focus	Population / Species	Gene/Marker Used	Sequence Length / Notes
1	Araki & Schmid, 2010	General	Hatchery-released fish	NA	Risk of genetic erosion and introgression
2	Jamaluddin et al., 2011	Malaysia (Perak state)	12 wild populations of <i>C. striata</i>	COI	368 bp
3	Song et al., 2013	Malaysia	Eight wild populations of <i>C. striata</i>	COI	700 bp
4	Rahman et al., 2013	South Asia	<i>C. striata</i> , <i>C. marulius</i> , <i>C. punctata</i>	mtDNA (COI, 16S rRNA)	Species delimitation via DNA barcoding
5	Singh et al., 2014	India	<i>C. striata</i>	Microsatellites, RAPD	Intraspecific variation and population structure
6	Nguyen & Duong, 2016	Vietnam	Wild vs. cultured <i>C. striata</i>	COI	652 bp
7	Conte-Grand et al., 2017	South Asia	<i>C. gachua</i> , <i>C. striata</i> complex	Multi-locus (mtDNA + nuclear)	Taxonomic revision, cryptic lineages
8	Ahmed et al., 2018	Bangladesh	4 <i>Channa</i> species (natural populations)	COI	No data
9	Barman et al., 2018	South and Southeast Asia	Multiple <i>Channa</i> species	COI	Barcoding and species-level identification
10	Nguyen et al., 2018	Vietnam	<i>C. striata</i>	COI, Cyt b	Fragmentation impact on gene flow; haplotype structure
11	Lalramliana et al., 2019	India, Indo-China	<i>Channa</i> genus	Concatenated mtDNA + nuclear genes	Phylogeny, Miocene–Pliocene divergence

12	Duong et al., 2019	Cambodia and Vietnam	5 wild (KH) and 3 hatchery (VN) <i>C. striata</i> populations	Cyt b and D-loop	585 bp (cyt b), 874 bp (D-loop)
13	Alam et al., 2020	Bangladesh	Wild <i>C. striata</i> populations	COI	High haplotype diversity; population structure analysis
14	Nugraha et al., 2021	Indonesia	<i>C. striata</i> aquaculture populations	COI, population genetics	Application in broodstock management and genetic quality
15	Kanon et al., 2022	Bangladesh and Vietnam	Wild population vs. cultured <i>C. striata</i> population	Cyt b	800 bp
16	Alam et al., 2022	Bangladesh and Vietnam	Native (BD) and exotic (VN) <i>C. striata</i> populations	COI	650 bp
17	Irmawati et al., 2022	Indonesia	Four wild populations of <i>C. striata</i> from Wallacea	COI	647 bp
18	Kumar et al., 2022	General (<i>Channa</i> spp.)	Aquaculture breeding programs	NA (marker-assisted selection proposed)	Highlights molecular tools for genetic improvement

Although considerable progress has been made in the molecular and morphological research of *C. striata* and its related species, key research gaps persist that hinder effective taxonomy, conservation efforts and the advancement of aquaculture practices. First, species delimitation is still inadequate, with numerous cryptic and divergent lineages identified through mitochondrial DNA analyses that remain taxonomically unresolved due to limited geographic sampling and a lack of comprehensive genome-wide datasets. Second, DNA barcoding efforts are hindered by the absence of standardized reference libraries, inconsistent marker selection and uneven regional species coverage, which reduce

reliability in species identification. A fragmented approach persists in the integration of molecular and morphological data, with few studies combining genetic, morphometric and ecological datasets to accurately define species boundaries and differentiate stocks. Finally, there is limited understanding of how climate change-induced stressors including hydrological alteration, rising temperatures, and habitat degradation impact the genetic structure, connectivity and reproductive success of *Channa* species. Addressing these multidimensional gaps is essential for sustainable aquaculture, biodiversity conservation and effective fisheries management

1.8 Reproductive Biology and Breeding Season

Understanding the reproductive biology and growth patterns of fish in the wild is essential for effective management, conservation and aquaculture practices. Knowledge of reproductive traits helps optimize breeding and fry-rearing techniques, ensuring sustainable fish populations (Saputra et al., 2018; Ali, 1999). The reproductive biology of a species provides foundational information necessary for induced reproduction protocols.

C. striata is a gonochoristic species, exhibiting distinct sexes with males possessing testes and females ovaries (Kumar et al., 2022). The testes are paired, slender, pinkish to white in color, unequal in size and connected to the coelomic cavity by the mesorchium (Kumar et al., 2022; Irmawati et al., 2019; Djumanto et al., 2019; Ali, 1999). In contrast, the ovaries are paired, light yellowish to orange, cylindrical in shape, unequal in size and attached to the coelomic cavity via the mesovarium. Sex differentiation without dissection is challenging even at maturity. However, during the mature stage, secondary sexual characteristics develop in both sexes, such as changes in pigmentation. Typically, mature individuals exhibit black dots on the ventral area and reddish coloration near the urogenital region. Maturity assessment often involves examining abdominal shape, gonadal orifice

coloration and the distance between the orifice and the anal fin (Kumar et al., 2022). Adult females show a bulging abdomen and oval genital papillae whereas, adult males have a flattened abdomen and an elongated genital papilla. Generally, males tend to be smaller than females.

Striped murrelets (*C. striata*) typically reach first maturity during their second year, at a total length (TL) ranging between 23 and 25 cm (Kumar et al., 2022; Li et al., 2016). The size at first maturation differs by sex, with females maturing at about 19 cm standard length (SL) and males at 17 cm (Morioka et al., 2016), indicating males mature at a smaller size. Djumanto et al. (2019) reported that females first spawn at around 31.5 cm in length.

The gonadosomatic index (GSI), which indicates gonadal development and maturity stage is an important parameter to determine the spawning season. An increase in GSI correlates with readiness to breed. In *C. striata*, GSI values begin to rise at an average total length of 28 cm (Li et al., 2016). Peak GSI values, signaling the prime breeding season are observed from April to October (Li et al., 2016). Fish with GSI values above 1 are considered fecund and capable of reproduction (Amtyaz et al., 2013). Dumalagan et al. (2017) reported female GSI values ranging from 0.22 to 3.903, with a mean of 0.902, confirming fecundity above the threshold. The highest GSI values typically occur during the rainy season, marking peak spawning periods (Djumanto et al., 2019). Similarly, Boonkusol et al. (2020) observed maximum GSI values in July for both males (6.15 ± 0.20) and females (0.14 ± 0.12), reinforcing the rainy season as the main breeding period.

Oocyte size fluctuates throughout the reproductive season, classifying *C. striata* as a group synchronous spawner (Ali, 1999). Fecundity varies widely from 4,160 to 46,890 eggs per female (Boonkusol et al., 2020). Fecundity is positively correlated with female size: a female measuring 365 mm in length and weighing 359 g can produce at least 2,843 eggs,

whereas a larger female of 511 mm and 1,790 g can produce approximately 23,230 eggs. The average absolute fecundity and relative fecundity are reported as 9,167 eggs and 20.6 eggs per gram of body weight, respectively (Djumanto et al., 2019).

The breeding season of *C. striata* is commonly determined by evaluating the gonadosomatic index (GSI), fecundity, oocyte diameter and the distribution of mature gonadal stages. Geographical variation plays a significant role in influencing the reproductive patterns of organisms, including *C. striata* (Dinh et al., 2022). For instance, the peak breeding season in central Laos occurs from April to June (Morioka et al., 2016), whereas in Southern Taiwan, it extends from April to November (Li et al., 2016). In India, distinct breeding periods have been reported based on geographic location: Parameshwaran and Murugesan (1976) documented a breeding season from May to July in southern India, while Alikunhi (1953) reported a November to January breeding season in northern India. Collectively, these studies indicate that the reproductive season of *C. striata* generally coincides with the rainy season, although exact timing varies regionally.

In spite of notable progress in understanding the reproductive biology of *C. striata*, key research gaps continue to exist. Although its breeding season is generally associated with the rainy period, considerable geographical variation exists in the timing and duration of spawning across regions such as Laos, Taiwan, and India. However, the environmental, climatic or genetic drivers of these regional differences remain poorly understood. There is a lack of comprehensive, comparative studies examining how local environmental cues such as temperature, photoperiod, rainfall and habitat features regulate reproductive cycles in different populations. Moreover, most existing research is concentrated in selected geographical areas with limited data from ecologically significant but understudied regions like Bangladesh. In addition, reproductive physiology and endocrine regulation in males are

inadequately characterized, constraining the development of refined induced breeding protocols. Molecular and genetic aspects of reproduction, which are vital for selective breeding, stock enhancement and conservation, remain largely unexplored. Addressing these gaps through integrative studies combining ecological, physiological, and molecular approaches will significantly advance the understanding and aquaculture potential of *C. striata* across its range.

1.9 Domestication and Brood Stock Management

Fish diversification is expected to support the growing demand in aquaculture production, which necessitates the domestication of new species. However, the domestication process is typically long, complex, and costly (Milla et al., 2021). It involves a series of gradual steps: the collection and rearing of wild broodfish, induced breeding to obtain the first generation in captivity, followed by adaptation to captive conditions and accompanying phenotypic changes (Milla et al., 2021). In many Asian countries, sustained efforts to domesticate *C. striata* in captivity have shown promising progress. Consequently, countries like Thailand, Vietnam, and India have now successfully established effective culture systems for this species.

The first step in the domestication process involves the collection of wild fish and their acclimatization prior to evaluating breeding performance. The success of captive breeding and the survival of offspring largely depend on the quality of the broodfish. Typically, broodfish are collected from nearby natural water bodies and acclimatized before being stocked into brood rearing ponds (Kumar and Mohanty, 2018). However, capturing broodfish directly from earthen ponds for immediate induced breeding can be problematic, as the stress associated with handling often leads to poor breeding responses. To address this, Kumar and Mohanty (2018) standardized a protocol for raising healthy broodfish to

full maturity after collection from the wild. They recommended using concrete cisterns for brood rearing, supplemented with appropriate feeding regimes, habitat structures and hormonal treatments. To mimic natural conditions, they enhanced the cisterns with an earthen base and floating aquatic macrophytes. In a subsequent study, Kumar and Mohanty (2021) explored habitat manipulation to overcome challenges such as the short spawning season and incomplete maturation in captivity. They tested four different rearing environments: (1) tanks with clean reservoir water (no soil or macrophytes), (2) tanks with submerged and floating macrophytes but no soil base, (3) tanks with a soil foundation but no macrophytes, and (4) tanks with both soil foundation and aquatic macrophytes. The findings indicated that using aquatic macrophytes to cover 20–30% of the water surface, combined with a soil foundation layer of 15–20 cm, significantly promoted gonadal maturation of *C. striata* during the pre-monsoon period in captivity (Kumar and Mohanty, 2021).

The ideal size of broodfish for captive breeding varies among studies, but an average length of 30 cm and weight range of 400–800 g is generally considered optimal (Kumar and Mohanty, 2018). In tank-based broodstock rearing systems, fish can be gradually acclimated to pellet diets. However, various types of diets have been tested for *C. striata* broodfish including formulated pellet feeds, chicken waste, live feed, mash feed, brans or oilcakes, and live forage fish (Rawat et al., 2020; Roy et al., 2016; Nam et al., 2011; Hung and Huy, 2007; Pati et al., 2004). Additionally, broodfish may be provided with combinations of fish meal, rice bran, wheat flour, vitamin supplements, and live fish fry to enhance reproductive performance.

Despite the progress in captive broodstock development, significant research gaps remain. These include the lack of standardized broodstock nutrition protocols specific to

reproductive phases, insufficient understanding of endocrine regulation during captive maturation and limited long-term studies on genetic fitness and domestication impacts across generations. Furthermore, the interplay between environmental stimuli, diet composition and hormonal induction under different culture systems is not yet fully elucidated. Addressing these gaps is crucial for establishing robust and sustainable domestication protocols for *C. striata* and enhancing its aquaculture potential across diverse agro-climatic regions.

1.10 Induced Breeding of *C. striata*

Induced breeding, also known as artificial propagation refers to the application of exogenous hormones to stimulate gamete release in sexually mature fish under controlled conditions. This technique typically employs natural hormones (e.g., pituitary gland extract (PGE) or synthetic analogues (e.g., Ovaprim, Ovatide, WOVA-FH) to trigger final oocyte maturation and ovulation or spermiation, thereby facilitating controlled fertilization and larval production. The development of reliable induced breeding protocols has been instrumental in ensuring year-round seed availability, particularly during off-spawning seasons or in regions where wild spawn is scarce. Moreover, with wild populations of *C. striata* facing threats from overfishing, habitat loss and pollution, induced breeding plays a vital role in stock replenishment, aquaculture development, and conservation of genetic resources (Arantes et al., 2013).

The earliest documented success in the induced breeding of *C. striata* was reported by Parameswaran and Murugan (1976), who employed PGE at dosages of 20 mg/kg (initial dose) and 60 mg/kg (booster dose), achieving fertilization rates between 65% and 95%. Subsequent efforts by Haniffa et al. (2000) compared the efficacy of both natural (PGE, human chorionic gonadotropin (HCG) and synthetic hormones (LHRH analogues,

Ovaprim). While latency periods were longer in pituitary-treated fish, Ovaprim produced superior fertilization and hatching outcomes.

Later studies focused on dose optimization and alternative synthetic hormones. Pati et al. (2004) reported effective spawning using WOVA-FH a GnRH-based hormone at dosages of 1.0 ml/kg for females and 0.6 ml/kg for males, noting the potential for broodstock reuse within 60 days. In Indonesia, Yulintine et al. (2017) found optimal spawning response using Ovaprim at 0.3–0.5 ml/kg, with latency periods of 30–37 hours post-injection. Research by Marimuthu and colleagues (2001, 2007, 2009) emphasized the influence of hormone combinations (e.g., Ovaprim + PGE) and tank design on breeding success. Similarly, studies from Vietnam (Nguyen et al., 2005), Pakistan (Rehman et al., 2013), and Indonesia (Muslim, 2017; Radona et al., 2020) consistently demonstrated high efficacy of synthetic agents such as Ovaprim and Ovatide across diverse environmental conditions.

1.11 Hormonal Induction and Synthetic Hormone Optimization in *C. striata* Breeding

The application of synthetic hormones has become an integral component of induced breeding protocols for *C. striata*, with numerous studies conducted over the decades to optimize dosages, identify suitable hormone combinations, and assess environmental adaptability (Table 1.2). Early foundational work by Qasim and Qayyum (1961) provided essential insights into the reproductive biology of *C. striata*, laying the groundwork for subsequent hormonal manipulation studies.

Parameswaran and Murugesan (1976) were among the pioneers to demonstrate induced breeding in *C. striata* using pituitary extracts, marking one of the earliest successful artificial propagation attempts under controlled conditions. Their work established the

viability of hormonal induction and emphasized the importance of environmental control in hatchery-based breeding systems. This landmark study paved the way for the use of synthetic analogs in later decades.

Rao et al. (1990) were among the first to report that a combination of prostaglandin (PGE) and Ovaprim could enhance synchronization in spawning behavior, suggesting that hormonal synergy may be beneficial for improving reproductive performance. In the early 2000s, Pati et al. (2004) evaluated WOVA-FH a GnRH-based synthetic hormone and demonstrated successful spawning at dosages of 1.0 ml/kg for females and 0.6 ml/kg for males. An additional advantage noted was the potential for broodstock reuse within 60 days, offering hatchery managers a cost-effective breeding cycle.

Concurrent findings by Awasthi et al. (2004) demonstrated high spawning success using Ovaprim, with fertilization rates exceeding 90% under laboratory conditions. This affirmed Ovaprim's standalone efficacy in controlled environments. Marimuthu et al. (2001), followed by Marimuthu and Haniffa (2007), investigated the combined use of Ovaprim and PGE. Their results emphasized the synergistic benefits of these hormones and highlighted the influence of tank design on breeding success. Later, Marimuthu et al. (2009) confirmed that Ovaprim alone could still yield over 80% fertilization, supporting its practical application in hatchery settings.

Environmental validation of these protocols was carried out across multiple countries. In Vietnam, Nguyen et al. (2005) reported high efficacy of Ovaprim under local environmental conditions. Similarly, Roy et al. (2010) compared Ovaprim and Ovatide, noting that while both were effective, Ovaprim consistently produced marginally superior results. Rehman et al. (2013) corroborated these findings in Pakistan, where high breeding success was achieved using both synthetic hormones under diverse hatchery conditions.

Rajakumar et al. (2016) revisited the application of WOVA-FH and found significant improvements in both fertilization and hatching rates, confirming its continued relevance. In Indonesia, Yulintine et al. (2017) optimized Ovaprim dosages to 0.3–0.5 ml/kg, with induced spawning observed within a latency period of 30–37 hours post-injection. Subsequent studies by Muslim (2017) and Radona et al. (2020) demonstrated that both Ovaprim and Ovatide remained highly effective under varied environmental conditions, thus confirming their adaptability and robustness in tropical aquaculture systems.

Overall, these cumulative findings underscore the reliability and adaptability of synthetic hormonal agents, particularly Ovaprim and WOVA-FH, in inducing successful breeding of *C. striata* across South and Southeast Asia. The evidence supports ongoing refinement of hormone protocols, tank design and broodstock management to enhance hatchery productivity and ensure sustainable seed production for aquaculture.

Table 1.2 Chronological Summary of Key Studies on Induced Breeding of *C. striata*

SL.No.	Authors	Key Findings
1	Qasim & Qayyum (1961)	Foundational study on reproductive traits; informed early breeding protocols.
2	Parameswaran & Murugesan (1976)	Early success in induced breeding using pituitary extracts; laid the foundation for controlled propagation techniques in <i>C. striata</i> .
3	Rao et al. (1990)	Combination of PGE + Ovaprim enhanced synchronization of spawning behavior.
4	Pati et al. (2004)	WOVA-FH effective at 1.0 ml/kg (♀) and 0.6 ml/kg (♂); potential for broodstock reuse within 60 days.
5	Awasthi et al. (2004)	Ovaprim induced high spawning success; >90% fertilization under lab conditions.

6	Marimuthu et al. (2001)	Hormone combinations (e.g., Ovaprim + PGE) and tank design influenced breeding success.
7	Marimuthu & Haniffa (2007)	Combined Ovaprim and PGE improved induction; tank design significantly impacted results.
8	Marimuthu et al. (2009)	Ovaprim alone was effective; fertilization rate exceeded 80%.
9	Nguyen et al. (2005)	Ovaprim effective under Vietnamese environmental conditions.
10	Roy et al. (2010)	Both Ovaprim and Ovatide effective; Ovaprim yielded slightly higher results.
11	Rehman et al. (2013)	High breeding success using synthetic hormones (Ovaprim, Ovatide) in Pakistan.
12	Rajakumar et al. (2016)	WOVA-FH significantly improved fertilization and hatching rate
13	Yulintine et al. (2017)	Ovaprim (0.3–0.5 ml/kg) induced spawning within 30–37 hours post-injection.
14	Muslim (2017)	Synthetic hormones effective under Indonesian hatchery conditions.
15	Radona et al. (2020)	Ovaprim and Ovatide showed high efficacy across variable environments in Indonesia.

A major limitation is the absence of standardized hormone dosage protocols that account for individual variation in fish size, gonadal maturity, seasonal reproductive cycles, and local environmental conditions. This lack of uniformity often leads to inconsistent breeding outcomes across hatcheries and regions. Additionally, the long-term effects of repeated synthetic hormone applications on broodstock health, reproductive performance, and lifespan remain poorly understood, posing sustainability concerns for aquaculture operations. Genetic monitoring of hatchery-reared progeny is another underdeveloped area. Inadequate assessment of genetic diversity and inbreeding may compromise the fitness, adaptability and long-term viability of cultured stocks. Furthermore, comparative breeding trials across different wild populations or genetic strains of *C. striata* are rare, despite their

relevance in developing region-specific and genetically robust breeding programs. Emerging approaches, such as the use of eco-friendly plant-based hormonal alternatives, biogenic stimulants, and environmental enrichment strategies, also remain largely unexplored and represent promising avenues for future research. Addressing these knowledge gaps through multidisciplinary and region-specific studies is essential for optimizing induced breeding protocols, maintaining genetic integrity and ensuring the long-term sustainability of *C. striata* aquaculture and conservation initiatives.

1.12 Embryonic and Gonadal Development in *C. striata*

A comprehensive overview of embryonic, larval and gonadal development in *C. striata* is presented in Table 1.3, which summarizes developmental timelines, feeding strategies, onset of cannibalism, and stages of gonadal differentiation.

The embryonic development of *C. striata* (striped snakehead) is characterized by its rapid pace with hatching typically occurring within 20–24 hours post-fertilization under optimal temperature conditions (Nath et al., 2015). Major embryological stages including cleavage, somite formation and early pigmentation have been documented in detail, tracking development up to the point of yolk sac absorption (Qasim et al., 1969; Boonbrahm et al., 2004). Studies by Sarma et al. (2012) and Roy et al. (2020) have expanded understanding of early ontogeny, providing valuable insights into larval morphology and physiology. Yolk sac absorption is usually completed within 48–72 hours post-hatching, marking the transition to exogenous feeding.

The embryonic stage extends from fertilization to hatching, typically occurring within 24–36 hours post-fertilization under optimal temperature and dissolved oxygen (DO) conditions. During this period, the embryo depends entirely on yolk reserves, requiring no

external feeding. Clean water and stable environmental parameters are essential to support normal embryogenesis (Sarma et al., 2012; Roy et al., 2020). Following hatching, larvae enter the yolk sac stage, where endogenous nutrition continues to support early development. Yolk absorption generally completes within 48–72 hours post-hatch, marking the critical window for the initiation of exogenous feeding. Initiating feeding between 48 and 60 hours post-hatch is essential to avoid larval starvation and promote early viability (Das et al., 2018; Rahman & Chakraborty, 2023). Early feeding is a critical phase in larval survival, and the use of live feeds such as *Artemia* nauplii, *Moina*, and chopped *Tubifex* worms has been found to significantly improve growth and survival (Mollah et al., 2009; Rahman & Chakraborty, 2023). Complementary enzymatic studies have revealed early development of digestive enzymes particularly proteases and amylases facilitating the shift to external feeding and supporting the development of effective weaning strategies (Das et al., 2018). However, the early larval stages are also highly vulnerable to cannibalism, which may begin as early as 3–5 days post-hatch (Rao et al., 1993; Rahman et al., 2012). This cannibalistic behavior is a significant bottleneck in larval rearing. Azra and Ikhwanuddin (2016) emphasize that early size grading, appropriate feeding protocols and optimized stocking densities are essential for mitigating cannibalism. The selection of first feed whether live *Artemia* or microformulated diets can also influence larval aggression and overall survival.

Understanding sex differentiation and gonadal development is essential for advancing reproductive biotechnology in *C. striata*. Teleosts generally exhibit high plasticity in gonadal development, influenced by genetic and environmental cues such as temperature and pH (Devlin & Nagahama, 2002; Baroiller & D’Cotta, 2001). However, for *C. striata*, specific information regarding the mechanisms of sex differentiation and gonadal

development remains limited. This lack of clarity poses a challenge to broodstock management, particularly in developing sex-controlled breeding programs.

Table 1.3: Summary of embryonic, larval, and gonadal development in *C. striata*

Developmental Aspect	Details	Key References
Embryonic period (fertilization to hatching)	Hatching occurs within 20–24 hours at optimal temperatures	Nath et al. (2015); Qasim et al. (1969); Boonbrahm et al. (2004)
Yolk sac absorption	Completed within 48–72 hours post-hatching Occurs after yolk sac absorption; digestive enzymes (proteases, amylases) develop early	Sarma et al. (2012); Roy et al. (2020)
Transition to exogenous feeding	Live feeds: <i>Artemia</i> nauplii, <i>Moina</i> , chopped <i>Tubifex</i> ; enhances growth and survival	Das et al. (2018)
First feeding strategies	Begins 3–5 days post-hatch; major issue in high-density larval rearing	Mollah et al. (2009); Rahman & Chakraborty (2023)
Onset of cannibalism	Early size grading, live feeds, optimal stocking, weaning diets	Rao et al. (1993); Rahman et al. (2012)
Cannibalism mitigation	Invasive: dissection, histology (e.g., acetocarmine staining); no external dimorphism	Azra & Ikhwanuddin (2016)
Sex identification methods		Irmawati et al. (2019)
Ovarian development stages	I: Immature, II: Maturing, III: Mature, IV: Spawning, V: Spent	Boonkusol et al. (2020); Irmawati et al. (2017); Djumanto et al. (2019); Mahmud et al. (2016); Ali (1999)
Testicular development stages	I: Immature, II: Maturing, III: Mature, IV: Spent	Djumanto et al. (2019)
Seasonality of reproduction	Peak maturity during rainy season; early signs at onset Likely influenced by environmental factors (e.g., temperature, pH); mechanisms not well studied	Djumanto et al. (2019); Boonkusol et al. (2020)
Sex determination influences		Devlin & Nagahama (2002); Baroiller & D’Cotta (2001)

Currently, there are no reliable external morphological markers for distinguishing sexes in *C. striata*. Sex identification typically requires invasive methods, such as macroscopic dissection or histological analysis. Acetocarmine staining is often used for histological examination of gonadal tissues. Anatomically, the gonads are located in the anterior abdominal cavity beneath the intestine, adjacent to the urogenital region (Irmawati et al., 2019). In females, the ovaries are generally longer and more voluminous than the testes in males of similar size. Gonadal coloration and size change progressively with maturation. Immature gonads are typically off-white to brownish-red, whereas mature gonads acquire a yellowish-orange hue. Ovarian development in *C. striata* is classified into five distinct stages:

- **Stage I (Immature):** Ovaries are transparent and ribbon-like.
 - **Stage II (Maturing):** Ovaries appear reddish-white with visible oocytes.
 - **Stage III (Mature):** Ovaries become yellowish and enlarged.
 - **Stage IV (Spawning):** Fully mature ovaries are bright orange, filling much of the abdominal cavity.
 - **Stage V (Spent):** Ovaries shrink and appear flaccid following ovulation.
- This staging has been detailed in several studies (Boonkusol et al., 2020; Irmawati et al., 2017; Djumanto et al., 2019; Mahmud et al., 2016; Ali, 1999).

In males, testicular development progresses through four stages:

- **Stage I (Immature):** Testes are small, soft, and semi-transparent.
- **Stage II (Maturing):** Testes increase in size with visible spermatocytes.
- **Stage III (Mature):** Testes are pinkish-white, enlarged, and filled with spermatozoa.

- **Stage IV (Spent):** Testes appear darkened and reduced in firmness, with limited residual sperm (Djumanto et al., 2019).

Seasonal gonadal development is pronounced in both sexes, with peak reproductive readiness typically aligning with the monsoon or rainy season. Initial signs of gonadal maturation appear at the onset of the rains, while full reproductive maturity is generally reached in mid-season. Despite substantial progress in the anatomical and histological characterization of gonadal development, key knowledge gaps remain. Specifically, the molecular mechanisms underpinning sex determination and differentiation, and the influence of environmental variables (e.g., temperature and pH) on these processes, are not well understood in *C. striata*. Additionally, non-invasive and reliable methods for early sex identification are still lacking. Addressing these gaps will be essential for improving broodstock management, developing monosex culture systems, and enhancing the sustainability of *C. striata* aquaculture.

1.13 Larval Rearing, Weaning and Grow-Out Culture of *C. striata*

An overview of *C. striata* larval development, feeding strategies and management considerations across different culture stages is summarized in Table 1.4. The larval development and feeding strategies of *C. striata* are critical determinants of survival, growth and overall aquaculture success. A comprehensive understanding of developmental stages, appropriate feeding protocols and critical management practices at each phase is essential to optimize larviculture and grow-out performance.

During the early larval stage (typically 3–7 days post-hatch, dph), larvae exhibit active feeding behavior, supported by rapid development of the digestive system and sensory organs. Recommended live feeds at this stage include *Artemia* nauplii, *Moina* and finely chopped tubifex worms, which are nutritionally adequate and suitably sized. Feeding

should be frequent to match larvae's high metabolic demands. Importantly, regular size grading is crucial to control cannibalism, a common and often fatal behavior during early development (Mollah et al., 2009; Mukhopadhyay & Rout, 2016). From around 7 to 21 dph, *C. striata* larvae undergo a weaning phase, transitioning gradually from live feeds to semi-moist or formulated diets. Preferred weaning feeds include minced fish paste, moist pellets, and microdiets containing $\geq 45\%$ crude protein. Successful weaning depends heavily on feed palatability and the development of digestive enzymes such as proteases and amylases. Careful attention to feeding frequency and stress minimization during this period improves survival outcomes (Paray et al., 2020; Azra & Ikhwanuddin, 2016). After 21 dph, larvae reach the juvenile stage, during which they are fully weaned and exhibit notable growth and predatory behavior. Dry formulated diets with 30–45% protein are commonly used. At this stage, effective management includes routine size grading, cannibalism control, and the provision of environmental enrichment (e.g., shelters). Stocking densities are typically maintained between 5–10 juveniles per square meter to ensure uniform growth and reduce competition (Kamarudin et al., 2010; Ravi & Devaraj, 1991).

Throughout larval development, cannibalism is a significant challenge that threatens production efficiency. Aggressive behavior may emerge as early as 96 hours post-hatch. Rao et al. (1993) observed dominant individuals preying on smaller siblings, advocating for early and regular size grading to minimize size disparities. Rahman et al. (2012) reported that cannibalism can begin as early as 5 dph, reinforcing the importance of consistent grading and sufficient nutrition.

The grow-out phase spans approximately 3 to 6 months, at the end of which fish typically attain marketable weights of 300–500 grams. Various culture systems are adopted, including pond monoculture and integrated farming systems such as fish–paddy and fish–

livestock combinations. Feeds used during this phase generally contain 30–40% protein and support feed conversion ratios (FCRs) of 1.6–2.0, with production yields reaching up to 5 tons/ha annually (Ali et al., 2021; Nargis et al., 2022). Sustainability in grow-out systems depends on optimal water quality management, appropriate stocking densities (2–5 fish/m²), and the use of eco-friendly health management options like probiotics and herbal immunostimulants (Wahab et al., 2020; Kumar et al., 2018). Moreover, Azra and Ikhwanuddin (2016) demonstrated that feed quality and feeding frequency influence the intensity of cannibalism. Protein-rich diets and early feeding reduce competitive aggression and starvation-driven attacks. Additionally, environmental modifications such as shelter placement reduce visual contact and stress, further mitigating cannibalism.

Overall, effective management of *C. striata* larvae and juveniles demands an integrated approach involving appropriate feeding strategies, early size grading, high-quality diets, environmental enrichment and stress reduction—critical not only for minimizing cannibalism but also for enhancing survival, growth and profitability across the culture cycle.

Despite advancements in larval rearing and feeding strategies for *C. striata*, several critical research gaps remain. There is a need for the development of cost-effective, nutritionally balanced formulated diets tailored specifically for early larval stages to improve survival and growth during the transition from endogenous to exogenous feeding. The underlying behavioral and physiological mechanisms driving cannibalism in larvae are not fully understood, limiting the effectiveness of current management practices. Additionally, detailed studies on the development and activity of digestive enzymes during weaning are lacking, hindering the optimization of feed formulation and feeding schedules. Research on improving the palatability and digestibility of semi-moist and formulated diets

during weaning is also sparse. Although integrated multi-trophic aquaculture (IMTA) models show promise during the grow-out phase, their design and application specific to *C. striata* require further exploration. The potential benefits of probiotics and herbal immunostimulants during early larval and juvenile stages remain under-investigated, despite their growing use in grow-out culture. Moreover, there is limited knowledge about the genetic and environmental factors influencing growth performance and cannibalistic behavior. Lastly, standardized protocols for size grading and strategies to mitigate grading-induced stress are lacking, which are essential for reducing cannibalism and promoting uniform growth. Addressing these gaps will enhance the sustainability and productivity of *C. striata* aquaculture.

Table 1.4 Summary of larval development stages, feeding strategies and critical management considerations in *C. striata*.

Stage / Topic	Key Features	Feeding Strategy	Critical Considerations	Key References
Embryonic Stage	Fertilization to hatching (~24–36 h post-fertilization)	No external feeding (yolk-dependent)	Temperature and DO critical; clean water essential	Sarma et al., 2012; Roy et al., 2020
Yolk Sac Larvae	Yolk sac absorption (~48–72 h post-hatching)	No external feeding; exogenous feeding begins	Start feeding at 48–60 h post-hatch to avoid starvation	Das et al., 2018; Rahman & Chakraborty, 2023
Early Larvae (3–7 dph)	Active exogenous feeding; rapid organ development	<i>Artemia</i> nauplii, <i>Moina</i> , chopped <i>tubifex</i> worms	Frequent feeding, size grading, prevent cannibalism	Mollah et al., 2009; Mukhopadhyay & Rout, 2016
Weaning Phase (7–21 dph)	Gradual shift from live to formulated or semi-moist feeds	Fish paste, moist pellets, microdiets with $\geq 45\%$ protein	Feed palatability, digestive enzyme development, survival rate	Paray et al., 2020; Azra & Ikhwanuddin, 2016
Juvenile (Post-21 dph)	Fully weaned, significant size gain, predatory behavior develops	Dry formulated diets (30–45% protein)	Cannibalism control, water quality, stocking density (5–10/m ²)	Kamarudin et al., 2010; Ravi & Devaraj, 1991
Cannibalism & Larval Behavior	Aggressive and cannibalistic behavior starts as early as 96 h post-hatch	Protein-rich diets; early/frequent size grading; shelters	Grading to reduce size disparities; timely feeding; cannibalism affects survival significantly	Rao et al., 1993; Rahman et al., 2012; Azra & Ikhwanuddin, 2016
Grow-out Phase	Culture to market size (3–6 months; ~300–500 g)	Commercial feeds (30–40% protein); integrated farming models	FCR management, disease prevention, IMTA (Integrated Multi-Trophic Aquaculture) practices	Ali et al., 2021; Wahab et al., 2020; Nargis et al., 2022

1.14 Research Gaps Identified

Despite considerable progress in molecular biology, reproductive ecology and aquaculture practices for *C. striata*, several critical gaps remain that impede effective species management, conservation and sustainable production. Taxonomically, species delimitation and genetic identification are challenged by incomplete genomic data, uneven geographic sampling and inconsistent marker use, limiting phylogenetic resolution and species diagnosis. In reproductive biology, significant uncertainties persist regarding the environmental and genetic drivers of spawning variability across regions and male reproductive endocrinology remains largely unexplored. Broodstock management is hindered by a lack of standardized nutritional protocols tailored to reproductive phases and insufficient knowledge on the long-term genetic and physiological impacts of domestication. Furthermore, hormone-based induced breeding protocols lack adaptability to fish size, maturity and environmental factors, with minimal integration of genetic monitoring to prevent inbreeding. Molecular mechanisms underlying gonadal development and sex differentiation are poorly understood and early, non-invasive sex identification methods are unavailable. Lastly, larval rearing faces challenges such as cannibalism, inadequate species-specific diets, and insufficient understanding of digestive physiology and behavioral factors affecting survival and growth, which collectively limit hatchery efficiency.

1. 15 Research Motivation

To address these gaps, future research should focus on building standardized, regionally representative DNA barcoding libraries that integrate morphometric and ecological data for refined species delimitation. Reproductive studies must incorporate multi-regional

environmental monitoring and endocrine profiling responses to environmental stressors. Tailored hormonal induction protocols that account for phenotypic and environmental variability, combined with routine genetic monitoring using microsatellites or SNP markers, will enhance breeding consistency and genetic health. For larval rearing, formulating cost-effective, stage-specific feeds based on digestive enzyme profiles, studying behavioral and genetic factors influencing cannibalism, and establishing gentle size grading protocols will improve survival and growth.

Despite the ongoing efforts of researchers on induced breeding and larval rearing, in Bangladesh, no significant steps have been done yet to establish large-scale seed production plants to satisfy the seed requirement and farming to fulfill the market demand. The critical research gaps for the development of *C. striata* aquaculture in Bangladesh are lack of broodstock quality assessment and genetic characterization in Bangladesh context. Further, uncertainty about the identity and genetic integrity of indigenous vs. exotic *C. striata* in Bangladesh. Additionally, lack of standardized domestication and induced breeding protocols as well as insufficient understanding of larval feeding regimes to reduce cannibalism and improve survival.

1.16 Aims and Objectives of the study

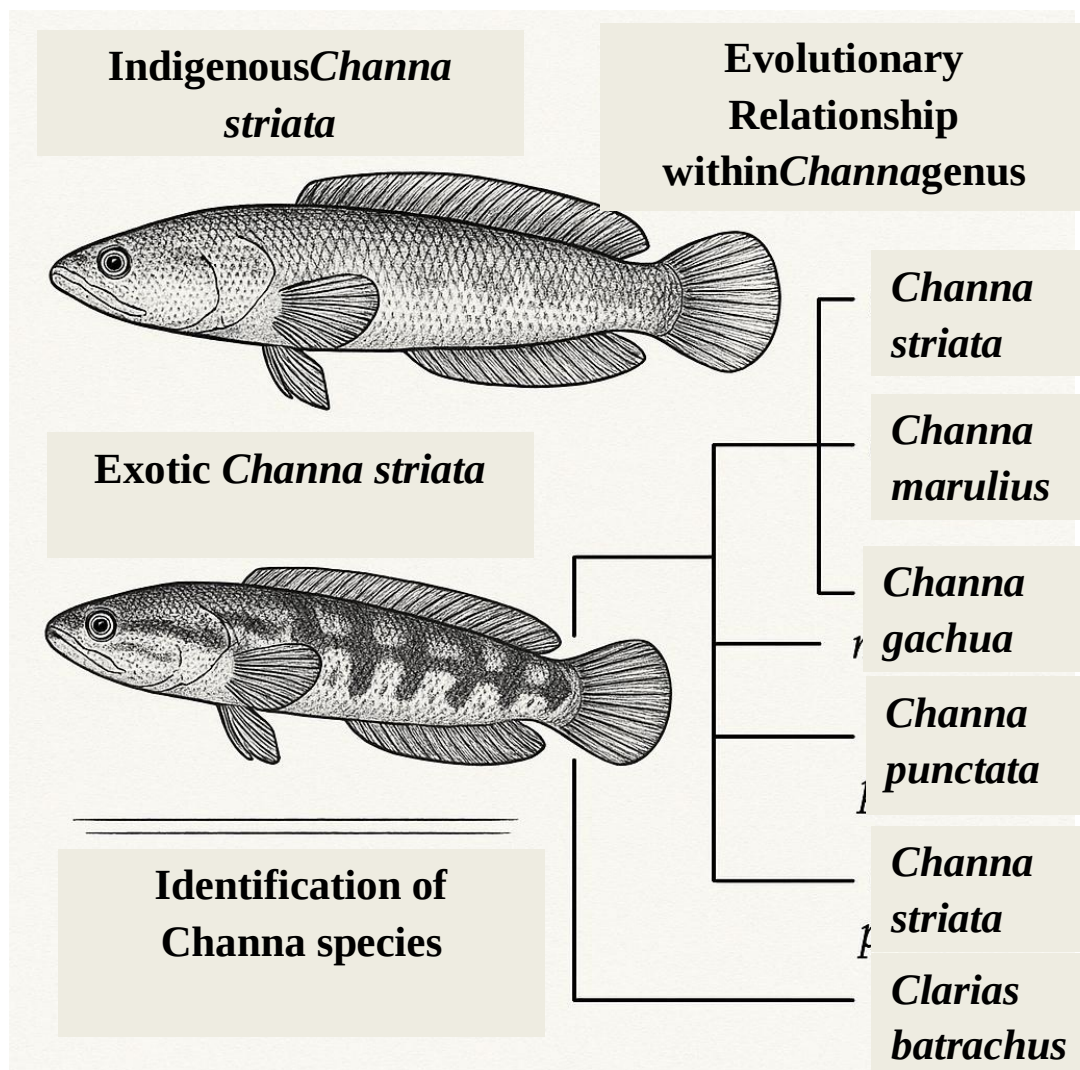
The overall aim of the study is to develop the seed production technology of this highly valued snakehead fish, *C. striata* in Bangladesh. To achieve the goals following specific objectives have been set.

- I. Identifying native and exotic *C. striata* strains through Integrative approaches in combination of geometric morphometrics and DNA barcoding, and establish their evolutionary relationship with other *Channa* species available in Bangladesh

- II. Assessment of growth and reproductive biology of natural population of *C. striata* for quality broodstock development and conservation developing and standardizing induced breeding techniques.
- III. Optimization of hormone doses for induced breeding of *C. striata* in captivity.
- IV. Investigating effective larval rearing strategies using different feeding regimes.

Chapter 2

Identification of indigenous and exotic *Channa striata* and its evolutionary relationship within the *Channa* genus



Abstract

Snakeheads (*Channa* spp.) are economically and ecologically significant freshwater fishes found across Asia, with five species documented in Bangladesh. However, their taxonomy and phylogenetic relationships remain poorly understood due to morphological similarities and ontogenetic variations. This study integrates geometric morphometric analysis and COI-based DNA barcoding to enhance species identification and assess evolutionary relationships among native and introduced *Channa* populations in Bangladesh. Live specimens of *C. striata*, *C. punctata*, *C. gachua*, and *C. marulius* were collected from the Atrai and Dharla Rivers, while exotic *C. striata* isolates were obtained from an aquaculture facility. Morphometric analyses, including landmark-based geometric morphometry, revealed significant shape variations, particularly between native and exotic *C. striata*, as supported by Principal Component Analysis (PCA), cluster analysis and multivariate analysis. COI gene sequencing identified substantial genetic differentiation, with 61.5% conserved and 36.9% variable sites. Phylogenetic analyses (Maximum Likelihood and Maximum Parsimony) confirmed distinct clustering of native and exotic *C. striata*, suggesting evolutionary divergence. Genetic distance analysis showed the highest divergence between *C. gachua* and native *C. striata* (0.2638), while exotic *C. striata* exhibited minimal variation (0.0005), indicating genetic homogeneity. This study demonstrates the effectiveness of geometric morphometric and DNA barcoding methods in precisely identifying *Channa* species while offering valuable insights into their genetic diversity and evolutionary relationships in Bangladesh. The results contribute to conservation strategies and the sustainable management of both native and introduced *Channa* populations.

2.1 Introduction

Snakeheads (Family: Channidae, Order: Perciformes) are freshwater, air-breathing fishes with a broad distribution across Asia and Africa (Bhat et al., 2012; Song et al., 2013). The Channidae family is composed of two distinct genera: *Channa*, which is native to Asia, and *Parachanna*, which is found in Africa. Traditionally, species within these genera have been classified based on their morphological and anatomical traits (Vishwanath & Kh Geetakumari, 2009). The genus *Channa* exhibits remarkable diversity in Asia, encompassing 26 recognized species that inhabit regions spanning from China and Taiwan in the Far East to South Asian countries such as Bangladesh, India, Sri Lanka, and extending to Myanmar, Thailand, Laos, Cambodia, Vietnam, Malaysia, Indonesia, and the Philippines (Courtenay & Williams, 2004; Rüber et al., 2020; Irmawati et al., 2022). Snakeheads play a crucial role economically, medicinally, and ecologically. They are highly sought after as a food source, known for their therapeutic properties in wound healing, and are widely popular in the ornamental fish trade (Ng & Lim, 1990; Musikasinthorn, 2003; Courtenay & Williams, 2004; Kumar et al., 2012). In Bangladesh, five *Channa* species have been documented: *Channa striata* (Striped Snakehead), *C. marulius* (Great Snakehead), *C. punctata* (Spotted Snakehead), *C. gachua* (Dwarf Snakehead), and *C. barca* (Barca Snakehead) (IUCN, 2015; Rahman, 2005). According to the IUCN Red List of Bangladesh, *C. barca* is classified as Critically Endangered (CR), *C. marulius* as Endangered (EN), while the remaining species are categorized as Least Concern (LC) (IUCN, 2015).

Among these, *C. striata* is particularly significant due to its economic potential and extensive aquaculture in Southeast Asian countries such as Vietnam, Thailand, Malaysia, and Indonesia (Nam et al., 2009; Sinh et al., 2011; Kumar et al., 2012; Phanna et al., 2015; Kumar et al., 2021). In Bangladesh, however, the aquaculture of *C. striata* remains

underdeveloped, primarily due to the scarcity of fingerlings (Kumar et al., 2022). To address this gap, *C. striata* was introduced from Vietnam in 2014 through Innovative Agro Aid Pvt. Ltd. for induced breeding and sustainable aquaculture. Since its introduction, the species has gained commercial significance, with its farming rapidly expanding across Bangladesh. However, concerns have been raised regarding its potential ecological impact due to the risk of escape into natural water bodies (Kanon et al., 2022).

Despite the ecological and economic importance of *Channa* species in Bangladesh, their taxonomy and phylogeny remain poorly understood. Morphological identification is often challenging due to overlapping characteristics and color variations between juvenile and adult stages (Adamson et al., 2010; Benziger et al., 2011; Conte-Grand et al., 2017; Duong et al., 2019). Additionally, new *Channa* species continue to be identified, highlighting the need for comprehensive taxonomic assessment (Musikasinthorn, 2000; Murray, 2006; Britz, 2007, Britz, 2013, 2017; Benziger et al., 2011; Praveenraj et al., 2019; Sudasinghe et al., 2020). Previous studies in Bangladesh have relied primarily on conventional morphometric and meristic analyses, which are limited in their ability to distinguish between closely related species (Talwar & Jhingran, 1991; Rahman, 2005). Given these challenges, an integrated approach combining geometric morphometric analysis and DNA barcoding is necessary for accurate species identification.

Conventional taxonomic methods often fall short in distinguishing *Channa* species, especially during their juvenile stages. Morphometric techniques that rely on linear measurements lack the precision required for fine-scale differentiation. The truss network system (TNS) offers a more refined morphological assessment by capturing structural variations in a systematic manner (Bookstein, 1986; Roberto & Lopes, 1999; Mojekwu & Anumudu, 2015; Mallik et al., 2020; Parvez et al., 2020). Additionally, DNA barcoding

using the mitochondrial Cytochrome c Oxidase I (COI) gene has emerged as a powerful tool for species identification and evolutionary studies across various fish taxa (Hebert et al., 2004; Bhattacharjee et al., 2012). This molecular approach has proven effective in discriminating closely related fish species, including both marine and freshwater taxa (Ward et al., 2005; Lakra et al., 2015; Parvez et al., 2020, 2022; Sajjad et al., 2023).

Given the increasing presence of exotic *C. striata* in Bangladesh's aquaculture industry and the potential ecological risks associated with its introduction, there is an urgent need to accurately identify and assess the evolutionary relationships of *Channa* species, including both native and exotic populations. Therefore, the study tested following two hypothesis:

Hypothesis 1 (H₁): An integrated dataset of truss-network landmarks and COI barcodes will discriminate all five native *Channa* species, as well as the introduced *C. striata* strain, with ≥ 95 % classification accuracy.

Hypothesis 2 (H₂): The Vietnamese aquaculture strain of *C. striata* forms a monophyletic clade significantly divergent (K2P distance > 2 %) from any *C. striata* haplotypes that may occur naturally in Bangladesh.

To test above hypotheses, this study integrates geometric morphometric analysis and COI-based DNA barcoding to enhance taxonomic resolution and clarify phylogenetic relationships within *Channa* species in Bangladesh using an integrative approach combining geometric morphometric and DNA barcoding techniques. Moreover, the genetic diversity and phylogenetic relationships among native and exotic *Channa* species, particularly the introduced *C. striata* were investigated to determine the potential evolutionary footprints and divergence patterns of *Channa* species in Bangladesh. By addressing these objectives,

this study aims to bridge the existing knowledge gap in *Channa* taxonomy and evolution, thereby contributing to improved conservation and management strategies for both native and introduced species in Bangladesh.

2.2. Materials and Methods

2.2.1 Sample Collection

Live snakehead samples of *C. striata*, *C. punctata*, and *C. gachua* were collected from the Atrai River, Dinajpur (25.180891°N 88.768956°E; 25.539938°N 88.760027°E). *C. marulius* was collected from the Dharla River (25.875149°N 89.589609°E) in the Kurigram district. Additionally, *C. striata* exotic isolates were obtained from Sharnalata Agro Fisheries Ltd. (SAF), a leading fish hatchery in Mymensingh district (24.674719°N 90.335929°E). All collected fish samples were transported to the Fish Hatchery at the Department of Fisheries Biology and Genetics, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh (Appendix-1).

2.2.2 Morphological Identification of Collected *Channa* Species

Preliminary identification of fish species was conducted at the collection sites based on color, body shape, and body stripes. In the laboratory, detailed identification was performed using morphometric and meristic traits as described by Rahman (2005) and Talwar & Jhingran (1991). For geometric morphometric analyses, photographs of the fish were taken using a Nikon D5300 DSLR camera (18-55 mm lens) and converted into TPS (Thin Plate Splines) file format using the software tpsUtil v1.74 (Rohlf, 1989). The landmarks on the photographs were digitized using tpsDig2 v2.29, following the X-Y coordinate system. A truss network was established with 13 landmarks based on Bookstein (1991), ensuring precise digitization (Figure 2.1).

In this study, 13 morphological characters were quantified using the truss network method, where each character denotes the measured distance between predefined landmarks. Shape variation was captured through uniform components, with the first component reflecting elongation along the X-axis (anteroposterior direction) and the second indicating expansion or compression along the Y-axis (dorsoventral direction). Data analysis was carried out using PAST (PAleontological Statistics Software Version 3.20) (Hammer and Harper, 2001), while Microsoft Excel was employed for data organization and format conversion.

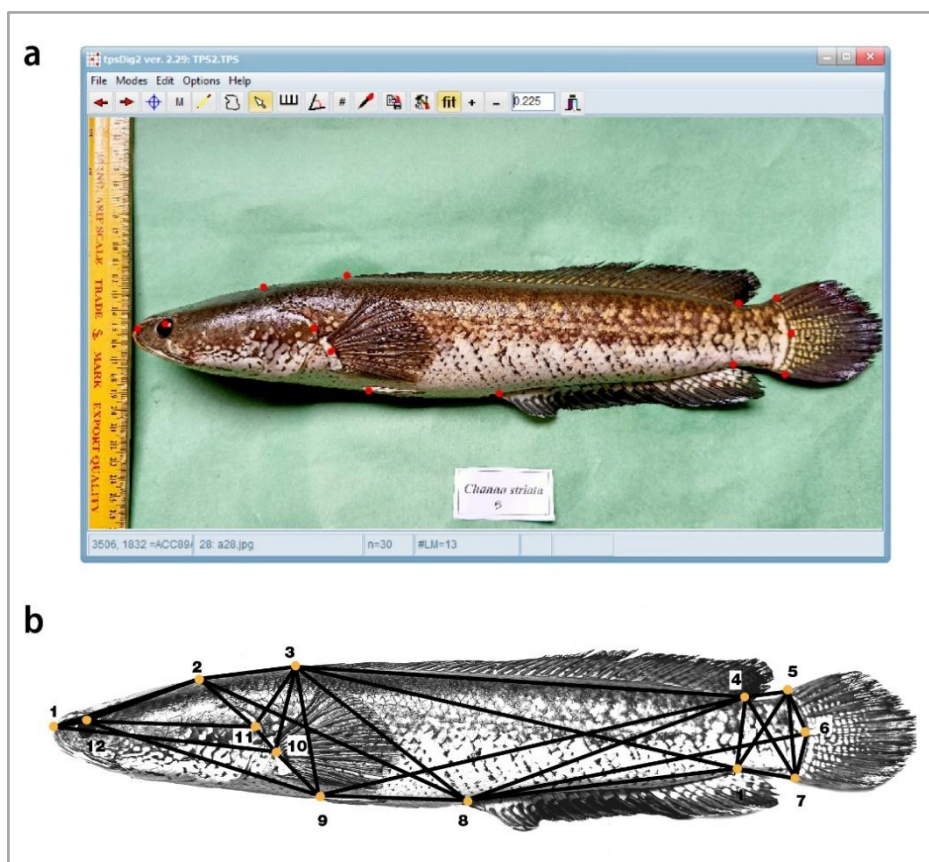


Figure 2.1(a) Diagram showing the placement of 13 landmarks used to construct the truss network. (b) Landmarks include: (1) the anterior tip of the snout at the upper jaw, (2) the posterior-most point of the neurocranium marking the start of the scaled nape, (3) the dorsal fin's point of origin, (4) its posterior termination, (5) the anterior attachment of the dorsal membrane of the caudal fin, (6) the endpoint of the vertebral column, (7) the anterior

attachment of the ventral membrane of the caudal fin, (8) the insertion point of the anal fin, (9) the anal fin's point of origin, (10) the pelvic fin's origin, (11) the pectoral fin's origin, (12) the posterior edge of the operculum, and (13) the midpoint of the eye.

2.2.3 Muscle Tissue Sample Collection and DNA Extraction

During morphometric analysis, fish muscle tissue samples weighing approximately 5–10 g were excised, preserved in absolute ethanol, and stored at -20°C until DNA extraction as muscle tissue is more readily lysis than fin tissue (Parvez et al., 2022). Genomic DNA was isolated using the Phenol-Chloroform-Isoamyl Alcohol method, following the protocol described by Shafi et al. (2016). The quality of extracted DNA was assessed through 1% agarose gel electrophoresis, where ethidium bromide (5 µg/mL) was used as a staining agent in 1× TBE buffer. DNA concentration was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Scientific™, Waltham, Massachusetts, USA) by measuring absorbance at 260 nm.

2.2.4 PCR Amplification of COI Nucleotide and Sequencing

The cytochrome oxidase I (COI) gene (~650 bp) was amplified using universal fish primers: FishF1: 5'-TCG ACT AAT CAT AAA GAT ATC GGC AC-3'; FishR1: 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3'; FishF2: 5'-TCG ACT AAT CAT AAA GAT ATC GGC AC-3'; and FishR2: 5'-ACT TCA GGG TGA CCG AAG AAT CAG AA-3' (Hubert et al., 2008; Ivanova et al., 2007; Rasmussen et al., 2009; Ward et al., 2005). Each 25 µL PCR reaction mixture contained 2 µL of extracted DNA template, 12.5 µL of Promega Green Master Mix, 0.5 µL of each primer, and nuclease-free water. The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, primer annealing at 60°C for 1 minute, extension at

72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products (~650 bp) were confirmed through 1.5% agarose gel electrophoresis stained with ethidium bromide and visualized under UV light using a Gel-Doc system (BIO-RAD, California, USA) (Appendix 2). Purified amplicons were processed with the Invitrogen purification kit (K310001, Thermo Scientific, Waltham, USA) before undergoing bidirectional sequencing using an ABI 3730 capillary sequencer (Applied Biosystems, Thermo Scientific, USA).

2.2.5 Sequence Editing and Sequence Submission to NCBI GenBank

Raw sequencing data were extracted and converted into FASTA format using Gear Genomics (www.gear-genomics.com/teal/) (Rausch et al., 2020). The sequences were aligned and refined in BioEdit (version 7.0.5.2), where the highest amino acid residue length was identified, and the Open Reading Frame (ORF) was selected (Appendix 3). To determine sequence identity, the BLAST tool (<https://www.ncbi.nlm.nih.gov/BLAST/>) was employed for comparison with reference sequences available in the NCBI GenBank database. The identified COI sequences were subsequently submitted to NCBI and assigned accession numbers OR863890-94 and OR864276-94 (Table 2.1).

2.2.6 Sequence Analyses and Phylogenetic Tree Construction

Sequence analysis and phylogenetic studies were conducted using DAMBEE (Version 7.2.134) and MEGA XI. Multiple sequence alignment was performed using MUSCLE in MEGA XI. Molecular genetics parameters, including nucleotide diversity, composition, disparity index, genetic distances, substitution rates, and phylogenetic trees were estimated using the Kimura 2-parameter model (Kimura, 1980). A heatmap was generated to visualize base composition disparity using Python (Version 3.x) with Seaborn (Waskom, 2021) and Matplotlib (Hunter, 2007). The Maximum Likelihood (ML) and Maximum Parsimony (MP)

phylogenetic trees were constructed using MEGA XI, with *Clarias batrachus* (MG988399.1) as the outgroup (Parvez et al., 2022). The final dataset contained 27 COI nucleotide sequence with 647 nucleotide positions (Table 2.1). Initial trees were built using Neighbor-Join and BioNJ algorithms, and Subtree-Pruning-Regrafting (SPR) algorithms were applied for MP analysis. Evolutionary relationships were inferred, ensuring high statistical support.

Table 2.1 The sequenced and reference COI sequences of the species of genus *Channa*

Sl. No.	Accession No	Species	Sequence Status	Species status	Country of origin	Local status (IUCN)
1.	OR863890	<i>Channa striata</i>	Sequenced	Exotic	Vietnam	Least Concern (LC)
2.	OR863891					
3.	OR863892					
4.	OR863894					
5.	OR864290	<i>Channa striata</i>	Sequenced	Native	Bangladesh	LC
6.	OR864291					
7.	OR864293					
8.	OR864294					
9.	OR864285	<i>Channa marulius</i>	Sequenced	Native	Bangladesh	Endangered (EN)
10.	OR864286					
11.	OR864287					
12.	OR864288					
13.	OR864289					
14.	OR864278	<i>Channa gachua</i>	Sequenced	Native	Bangladesh	Least Concern (LC)
15.	OR864279					
16.	OR864280					
17.	OR864281					
18.	OR864283					
19.	OR864284					
20.	OR864276	<i>Channa punctata</i>	Sequenced	Native	Bangladesh	LC
21.	OR864277					
22.	MK368525.1	<i>Channa striata</i>	Retrieved from NCBI genebank database	Native to country of origin	Vietnam	LC
23.	MT269937.1	<i>Channa striata</i>			Bangladesh	LC
24.	MK572111.1	<i>Channa gachua</i>			Bangladesh	LC
25.	MN259180.1	<i>Channa marulius</i>			Bangladesh	EN
26.	MK572115.1	<i>Channa punctata</i>			Bangladesh	LC
27.	MG988399.1	<i>Clarias batrachus</i>	Outgroup		Bangladesh	LC

2.3 Results

2.3.1 Geometric morphometry based variation among *Channa* species

2.3.1.1 Principal components and scatter plot analysis

The variation among the native and exotic *C. striata*, native *C. marulius*, *C. punctata* and *C. gachua* were estimated with Principal Component Analysis (PCA) of 26 principal components where the highest Eigenvalue 2.16876E07 and highest Variance (94.603%) were found in PC-1. The points are grouped into clusters, with each species forming a distinct region (Figure 2.2). *C. striata* (Indigenous) (blue asterisks) is separate from *C. striata* (Vietnamese) (black asterisks), indicating morphometric differentiation. *C. marulius* (red squares) is well-separated, showing a distinct genetic profile. *C. gachua* (green diamonds) and *C. punctata* (gray squares) are located in the upper-right quadrant but remain distinct. The shaded ellipses indicate the spread (variance) of each species' data points. *C. striata* (Indigenous) has a larger spread, suggesting higher genetic variability. *C. marulius* has a compact ellipse, indicating may lower genetic variation within the sampled individuals. The *C. striata*Indigenous population (blue) is located far from the Vietnamese population (black), supporting genetic divergence. This suggests possible geographic isolation, natural selection, or different evolutionary histories. The PCA plot effectively distinguishes between *C. striata*(Indigenous) and *C. striata*(Vietnamese). The tight clustering of some species (e.g., *C. marulius*) suggests morphometric homogeneity, while broader ellipses indicate greater diversity. This visualization supports evolutionary divergence and population structuring within the genus *Channa*.

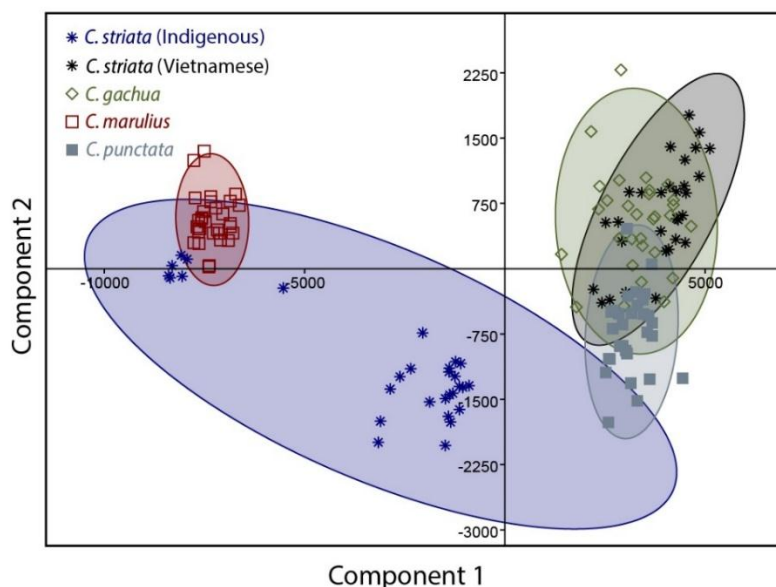


Figure 2.2: Principal Component Analysis (PCA) plot showing morphological differentiation among five *Channa* species based on geometric morphometric data. Each point represents an individual specimen, with groupings indicated by distinct symbols and colored ellipses representing 95% confidence intervals for each group. *C. striata* (Indigenous) is shown as blue asterisks; *C. striata* (Vietnamese) is shown as black asterisks; *C. gachua* is shown as green diamonds; *C. marulius* is shown as red squares, and *C. punctata* is shown as gray squares.

2.3.1.2 Multivariate Analysis of Variance (MANOVA)

Multivariate Analysis of Variance (MANOVA) tests whether there are significant differences among the groups based on multiple dependent variables. In this study, the MANOVA analysis was applied to different *Channa* species, and the pairwise *P*-values indicate statistical significance between groups. All *P*-values are extremely small (< 0.05), suggesting significant differences between all species (Table 2.2). The lowest *P*-value ($4.85E-20$) is between *C. marulius* and *C. punctata*, indicating a very strong differentiation between these species. The *P*-value between *C. striata* (Native) and *C. striata* (Exotic) is $1.41E-17$, meaning there is a significant morphological distinction between native and exotic populations possibly due to geographic separation or environmental adaptation. *C. marulius* exhibits strong differentiation from other species, particularly *C. punctata* ($P =$

4.85E-20) and *C. gachua* ($P = 6.56E-19$). *C. punctata* shows highly significant differences from *C. striata* (Native & Exotic), *C. gachua*, and *C. marulius*, supporting its distinct classification.

Table 2.2 Multivariate Analysis of Variance (MANOVA) of the genus *Channa*

Taxa	Pair-wise <i>P</i> Values				
	<i>C. striata</i> (Native)	<i>C. striata</i> (Exotic)	<i>C. gachua</i>	<i>C. marulius</i>	<i>C. punctata</i>
<i>C. striata</i> (Native)		1.41E-17	1.12E-18	1.26E-08	2.44E-19
<i>C. striata</i> (Exotic)	1.41E-17		1.48E-13	7.01E-18	8.39E-19
<i>C. gachua</i>	1.12E-18	1.48E-13		6.56E-19	2.78E-13
<i>C. marulius</i>	1.26E-08	7.01E-18	6.56E-19		4.85E-20
<i>C. punctata</i>	2.44E-19	8.39E-19	2.78E-13	4.85E-20	

2.3.1.3 Cluster analysis of *Channa* species in Bangladesh

The morphometric variation among different *Channa* species based on truss network measurements represent in the dendograms (Figure 3). The dendogram was generated using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis, which group's species based on their Euclidean distance in shape-space. The Euclidean distance between stock centroids means that the clustering is based on the overall shape similarities and differences among species. The UPGMA method created the clusters by averaging the distances between samples. In our study, *C. punctata*, *C. striata* (Exotic), and *C. gachua* form a closely related cluster, indicating they share similar body shape characteristics. *C. striata* (Native) is distinct but still closer to this cluster. *C. marulius* is morphologically the most distinct, meaning it has significant differences in body shape compared to the others (Figure 2.3). The clear separation of exotic and native *C. striata* was observed which suggests that their body shapes have diverged, possibly due to different environments, selective pressures, or genetic variation.

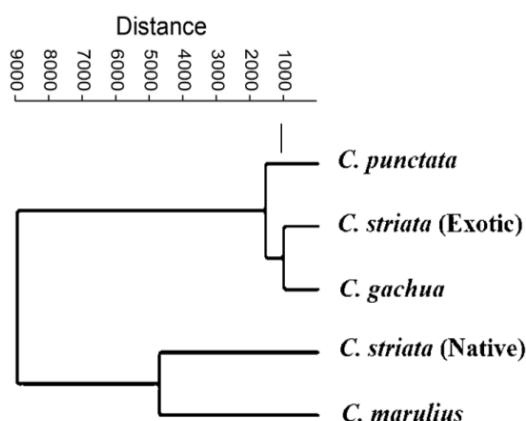


Figure 2.3 UPGMA dendrogram showing the morphometric relationships among *Channa* species in Bangladesh based on Euclidean distances from truss network measurements. The scale at the top represents Euclidean distance values. Shorter branch lengths indicate greater morphological similarity, whereas longer branches suggest higher morphological divergence.

2.3.2 Genetic identification and evolutionary analyses of *Channa* species in Bangladesh

2.3.2.1 COI gene sequence analysis

In the COI gene analysis, 647 sites were identified where 398 (61.51%) were conserved; 239 (36.93%) were variable; 207 (31.99%) were parsimony informative sites and 31 (4.79%) were singleton. The mean nucleotide frequencies of the studied COI gene sequences of the *Channa* species were 142, 179, 109 and 170 respectively for A, C, G, T with an average 600bp COI gene (Table 2.3). The Chi-square tests were performed at DAMBE for identification of significant deviation among expected and observed nucleotide frequencies by assuming that $P_a=P_c=P_g=P_t=0.25$. All the nucleotide frequencies were significantly varied from the expected frequencies (0.25) with a P value range from 0.000 to 0.0061 (Table 2.3). The guanine (G) frequency was lowest (0.18170) and Cytosine (C) frequency was the highest (0.29915) in the COI nucleotide sequences of the studied *Channa* species in Bangladesh. The percentage of AT content (52%) is constantly higher than the GC content

(48%). Chi-square (X^2) values are relatively low (12.39–19.01) in *C. gachua*, indicating less deviation from homogeneity, X^2 values are higher (21.86–24.47) in *C. punctata* and *C. marulius* indicating greater deviation from expected frequencies and suggest more variability. Native (*N*) and Exotic (*E*) *C. striata* populations' exhibit similar nucleotide frequencies, with an X^2 values range from 19.27–26.13).

Table 2.3 Nucleotide frequencies and the Chi-square values with the probability

Sequence name	A	C	G	T	Su	X2	P X2	PA	PC	PG	PT
OR864281.1 <i>C. gachua</i>	141	188	118	172	619	19.01	0.0003	0.23	0.30	0.19	0.28
OR864278.1 <i>C. gachua</i>	134	177	124	164	599	12.39	0.0061	0.23	0.30	0.21	0.28
OR864280.1 <i>C. gachua</i>	133	176	123	166	598	13.03	0.0046	0.22	0.29	0.21	0.28
OR864283.1 <i>C. gachua</i>	124	168	110	162	564	17.16	0.0007	0.22	0.30	0.20	0.29
OR864284.1 <i>C. gachua</i>	138	182	119	171	610	16.68	0.0008	0.23	0.30	0.20	0.28
OR864279.1 <i>C. gachua</i>	125	169	110	160	564	16.75	0.0008	0.22	0.30	0.20	0.28
OR864277.1 <i>C. punctata</i>	138	177	102	169	586	23.81	0.0000	0.24	0.30	0.17	0.29
OR864276.1 <i>C. punctata</i>	138	177	101	169	585	24.47	0.0000	0.24	0.30	0.17	0.29
OR864288.1 <i>C. marulius</i>	142	188	110	169	609	22.65	0.0000	0.23	0.31	0.18	0.28
OR864286.1 <i>C. marulius</i>	142	188	110	170	610	22.83	0.0000	0.24	0.31	0.18	0.28
OR864285.1 <i>C. marulius</i>	146	192	110	172	620	24.28	0.0000	0.24	0.31	0.18	0.28
OR864287.1 <i>C. marulius</i>	142	188	110	167	607	22.30	0.0001	0.23	0.31	0.18	0.28
OR864289.1 <i>C. marulius</i>	142	187	110	167	606	21.86	0.0001	0.23	0.31	0.18	0.28
OR864290.1 <i>C. striata_N</i> *	153	184	103	177	617	26.13	0.0000	0.25	0.30	0.17	0.29
OR864291.1 <i>C. striata_N</i>	154	176	105	182	617	23.78	0.0000	0.25	0.30	0.17	0.30
OR864293.1 <i>C. striata_N</i>	152	175	101	180	608	25.75	0.0000	0.25	0.30	0.17	0.30
OR864294.1 <i>C. striata_N</i>	148	173	103	173	597	21.90	0.0001	0.25	0.30	0.17	0.29
OR863890.1 <i>C. striata_E</i> **	148	177	107	170	602	19.80	0.0002	0.25	0.30	0.18	0.28
OR863894.1 <i>C. striata_E</i>	147	177	108	170	602	19.27	0.0002	0.24	0.30	0.18	0.28
OR863891.1 <i>C. striata_E</i>	146	174	99	168	587	23.67	0.0000	0.25	0.30	0.17	0.29
OR863892.1 <i>C. striata_E</i>	148	176	107	170	601	19.49	0.0002	0.25	0.30	0.18	0.28
Average	142	179	109	170	600			0.24	0.30	0.18	0.28

*N=Native, **E=Exotic

The disparity index test was conducted to assess variations in base composition biases beyond what would be expected due to evolutionary divergence among *Channa* species in Bangladesh based on COI nucleotide sequences. Disparity index values for selected sequence pairs are presented in Table 2.4 and Figure 2.4. A disparity index greater than zero (>0) indicated a more significant deviation in base composition biases than anticipated due to evolutionary divergence. Notably, substantial differences were observed between COI nucleotide pairs of *C. gachua*, *C. striata* (native), *C. striata* (exotic), and *C. marulius*. In contrast, no considerable variations were detected within or between COI nucleotide sequences of *C. punctata* and other *Channa* species.

Table 2.4 Estimation of net base composition bias disparity between sequences

	1	2	3	4	5	6	7	8	9	10
1.OR864281.1 <i>C. gachua</i>										
2.OR864278.1 <i>C. gachua</i>	0.027									
3.OR864277.1 <i>C. punctata</i>	0.000	0.000								
4.OR864276.1 <i>C. punctata</i>	0.000	0.000	0.000							
5.OR864285.1 <i>C. marulius</i>	0.000	0.030	0.000	0.000						
6.OR864289.1 <i>C. marulius</i>	0.000	0.031	0.000	0.000	0.000					
7.OR864290.1 <i>C. striata_N</i>	0.104	0.432	0.000	0.000	0.000	0.037				
8.OR864293.1 <i>C. striata_N</i>	0.301	0.539	0.000	0.000	0.208	0.212	0.009			
9.OR863890.1 <i>C. striata_E</i>	0.050	0.222	0.000	0.000	0.000	0.000	0.000	0.041		
10.OR863891.1 <i>C.striata_E</i>	0.061	0.115	0.000	0.000	0.000	0.000	0.000	0.006	0.000	

N=Native, E=Exotic

The heatmap visually represents the base composition bias disparity between different *Channa* species based on their COI gene sequences. Each cell indicates the numerical value of disparity between two sequences, with color intensity reflecting the level of variation (Figure 2.4). The heatmap categorized three levels of disparity in the studied

Channa sequences; low disparity (0.000 – 0.031) between *C. punctata* sequences (OR864277.1 and OR864276.1) have zero disparity, meaning their base compositions are identical. Similarly, *C. marulius* sequences (OR864285.1 and OR864289.1) also show zero to very low disparity, indicating a high degree of genetic similarity within species. Moderate disparity (0.030 – 0.222) between *C. gachua* (OR864278.1) and *C. marulius* (OR864285.1) (0.027 – 0.031) suggesting minor nucleotide composition differences. Exotic *C. striata* and native *C. striata* comparisons range from 0.041 to 0.222, indicating some variability between native and exotic populations. High disparities (0.301 – 0.539) occur between native *C. striata* (OR864293.1) and *C. gachua* (OR864278.1), reaching 0.539, suggests that native *C. striata* has a significantly different base composition compared to *C. gachua*, reflecting possible evolutionary divergence (Figure 2. 4).

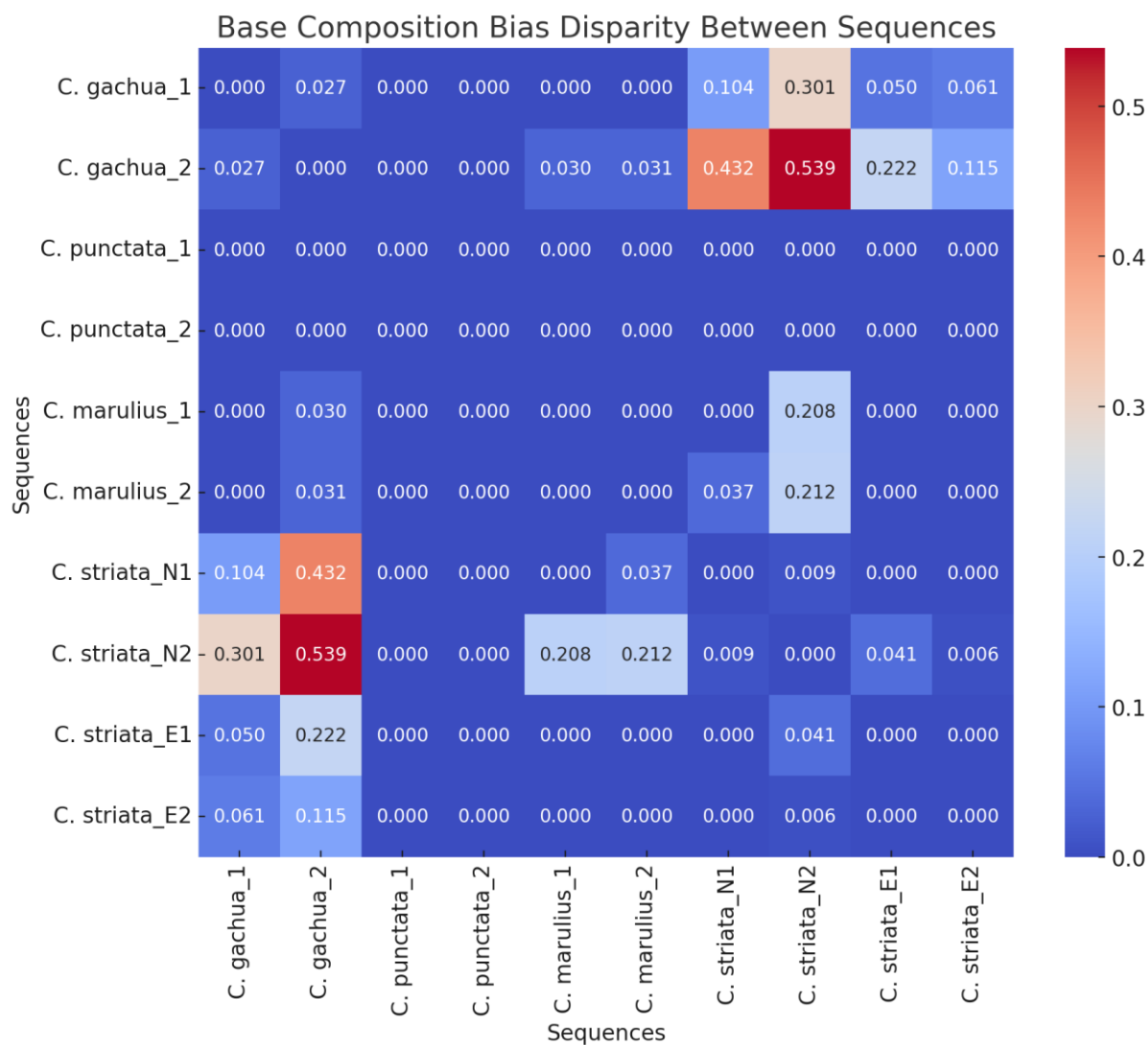


Figure 2.4 Heatmap representing the net base composition bias disparity among *Channa* species based on COI nucleotide sequences. The color gradient indicates the magnitude of nucleotide composition bias, with lighter shades representing lower disparity (higher similarity) and darker shades indicating higher disparity (greater nucleotide composition differences).

2.3.2.2 COI nucleotide sequence substitution pattern

The substitution patterns and rates were analyzed using the Kimura 2-parameter model (Kimura, 1980). Transition substitution rates are highlighted in bold, while transversion substitution rates are presented in italics (Table 2.5). The diagonal elements ("-") indicate no self-substitutions. The off-diagonal elements represent the substitution rates between different nucleotides. The highest substitution rate is observed between A↔G (14.64) and T↔C (14.64), indicating a strong transition bias (purine-to-purine or pyrimidine-to-pyrimidine changes). The lower substitution rates between A↔T, A↔C, G↔T, and G↔C (5.18) suggest that transversions (purine-to-pyrimidine or vice versa) occur less frequently. The Transition/Transversion bias (*R*) 1.41 meaning transitions occur 1.41 times more frequently than transversions.

Table 2. 5 Maximum Likelihood Estimate of Substitution Matrix

	A	T/U	C	G	Transition/Transversion bias (<i>R</i>)
A	-	<i>5.18</i>	<i>5.18</i>	14.64	1.41
T/U	<i>5.18</i>	-	14.64	<i>5.18</i>	
C	<i>5.18</i>	14.64	-	<i>5.18</i>	
G	14.64	<i>5.18</i>	<i>5.18</i>	-	

2.3.2.3 COI nucleotide sequence evolution pattern in *Channa* species

Test of the Homogeneity was performed to investigate whether the COI nucleotide sequences of studied *Channa* species were evolved with the same pattern of substitution or not. The results showed that the sequences were evolved with the same pattern of substitution as P-values were >0.05 except in sequence pair of native *C. striata* and *C. gachua* (Table 2.6). P values (~1.00) indicate no significant difference in substitution patterns between sequences, which mostly found in many comparisons such as, *C. punctata*, *C. marulius*, *C.*

striata groups, suggesting high homogeneity within these groups. Reference sequences (Ref.) for each species mostly show no significant difference ($p = 1.00$) when compared to their respective species. Lower p -values (< 0.05) indicate significant differences in substitution patterns. Comparisons between *C. gachua* (OR864278.1) and *C. striata* (e.g., OR864293.1) have p -values as low as 0.01, suggesting potential divergence in substitution patterns. Some *C. striata* native vs. exotic comparisons show intermediate differences (e.g., 0.14 – 0.30), implying some level of variation between native and introduced populations (Table 6).

Table 2 .6 Analysis of the homogeneity of substitution patterns between sequences

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	OR864281.1 <i>C.gachua</i>															
2	OR864278.1 <i>C.gachua</i>	0.07														
3	MK572111.1 <i>C.gachua</i> _Ref	1.00	0.11													
4	OR864277. <i>C.punctata</i>	1.00	1.00	1.00												
5	OR864276.1 <i>C.punctata</i>	1.00	1.00	1.00	1.00											
6	MK572115.1 <i>C.punctata</i> _Ref	1.00	0.19	1.00	0.08	0.09										
7	OR864288.1 <i>C.marulius</i>	1.00	0.33	1.00	1.00	1.00	1.00									
8	OR864286. <i>C.marulius</i>	1.00	0.33	1.00	1.00	1.00	1.00	1.00								
9	MN259180.1 <i>C.marulius</i> _Ref	1.00	0.29	1.00	1.00	1.00	1.00	1.00	1.00							
10	OR864290.1 <i>C. striata</i> _Native	0.20	0.04	0.16	1.00	1.00	1.00	0.33	0.30	1.00						
11	OR864293.1_ <i>C. striata</i> _Native	0.07	0.01	0.04	1.00	1.00	0.33	0.09	0.10	0.09	0.28					
12	MT269937.1_ <i>C. striata</i> _Native_Ref.	0.14	0.04	0.10	1.00	1.00	1.00	0.24	0.22	0.12	0.25	1.00				
13	OR863890.1_ <i>C. striata</i> _Exotic	0.30	0.13	0.20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.14	1.00			
14	OR863892.1_ <i>C. striata</i> _Exotic	0.29	0.10	0.26	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.13	1.00	1.00		
15	MK368525.1_ <i>C. striata</i> Exotic_Ref.	0.29	0.14	0.23	1.00	1.00	1.00	1.00	1.00	0.31	1.00	0.30	1.00	1.00	1.00	

2.3.2.4 Genetic distance of COI nucleotides sequences in *Channa* species

Genetic distance between sequence pair and overall mean genetic distances were estimated in different combinations of studied *Channa* species (Table 2.7). The overall genetic distance trends among sequenced *Channa* species is 0.1818, which aligns with known genetic differentiation in freshwater fishes. The genetic distance within the genus (0.1955) is higher than that within individual species, supporting the genetic distinction between *Channa* species. The genetic distance between sequenced and reference *Channa* species (0.1778) suggests consistency in sequence variation across different studies. The highest genetic distance is observed between *C. gachua* (OR864281.1) and native *C. striata* (OR864290.1) at 0.2638, indicating significant divergence. *C. marulius* (OR864286.1) shows an intermediate genetic distance with *C. punctata* (0.1850) and native *C. striata* (0.2091). The lowest interspecies genetic distance is between *C. punctata* (OR864277.1) and native *C. striata* (0.1792), suggesting a closer evolutionary relationship. The Intra-Species genetic distance reveals that exotic *C. striata* exhibits a remarkably low within-group genetic distance (0.0004994), suggesting strong genetic similarity among its individuals. The native *C. striata* shows slightly more variation (0.0441). The *C. gachua* also maintains genetic similarity within its population (0.0229), while *C. punctata* exhibits the least genetic variation within its group (0.0023), indicating a highly conserved genetic structure (Table 2.7).

Table 2.7 Evolutionary divergences of *Channa* species inferred from COI nucleotide sequences

SL	Taxa	1	2	3	4	5
1	OR864281.1 <i>C. gachua</i>					
2	OR864277.1 <i>C. punctata</i>	0.1952				
3	OR864286.1 <i>C. marulius</i>	0.2503	0.1850			
4	OR864290.1 <i>C. striata</i> Native	0.2638	0.1792	0.2091		
5	OR863890.1 <i>C. striata</i> Exotic	0.2473	0.1901	0.2008	0.0341	
Overall genetic distance						
1.	Sequenced and references <i>Channa</i> species	0.1778				
2.	Sequenced <i>Channa</i> species	0.1818				
3.	Intra-genus	0.1955				
4.	Within <i>C. striata</i> (Native)	0.0441				
5.	Within <i>C. striata</i> (Exotic)	0.0005				
6.	Within <i>C. striata</i>	0.0384				
7.	Within <i>C. gachua</i>	0.0229				
8.	Within <i>C. punctata</i>	0.0023				

2.3.2.5 Phylogenetic relationship of *Channa* species inferred from COI Nucleotide Sequences

The phylogenetic tree for *Channa* species was constructed using COI nucleotide sequences, employing the Maximum Likelihood and Maximum Parsimony methods based on the Kimura 2-parameter substitution model. The evolutionary history of the 27 analyzed taxa is represented by a bootstrap consensus tree derived from 1000 replicates (Figures 2.5 & 2.6). The trees distinctly groups sequences of the same species together, supporting their taxonomic classification. The branches corresponding to *C. striata*, *C. marulius*, *C. punctata*, and *C. gachua* form distinct clusters, with reference sequences validating the classification. Both trees show separate clustering of native and exotic *C. striata* sequences, indicating potential genetic differentiation (Figure 2.5 & 2.6).

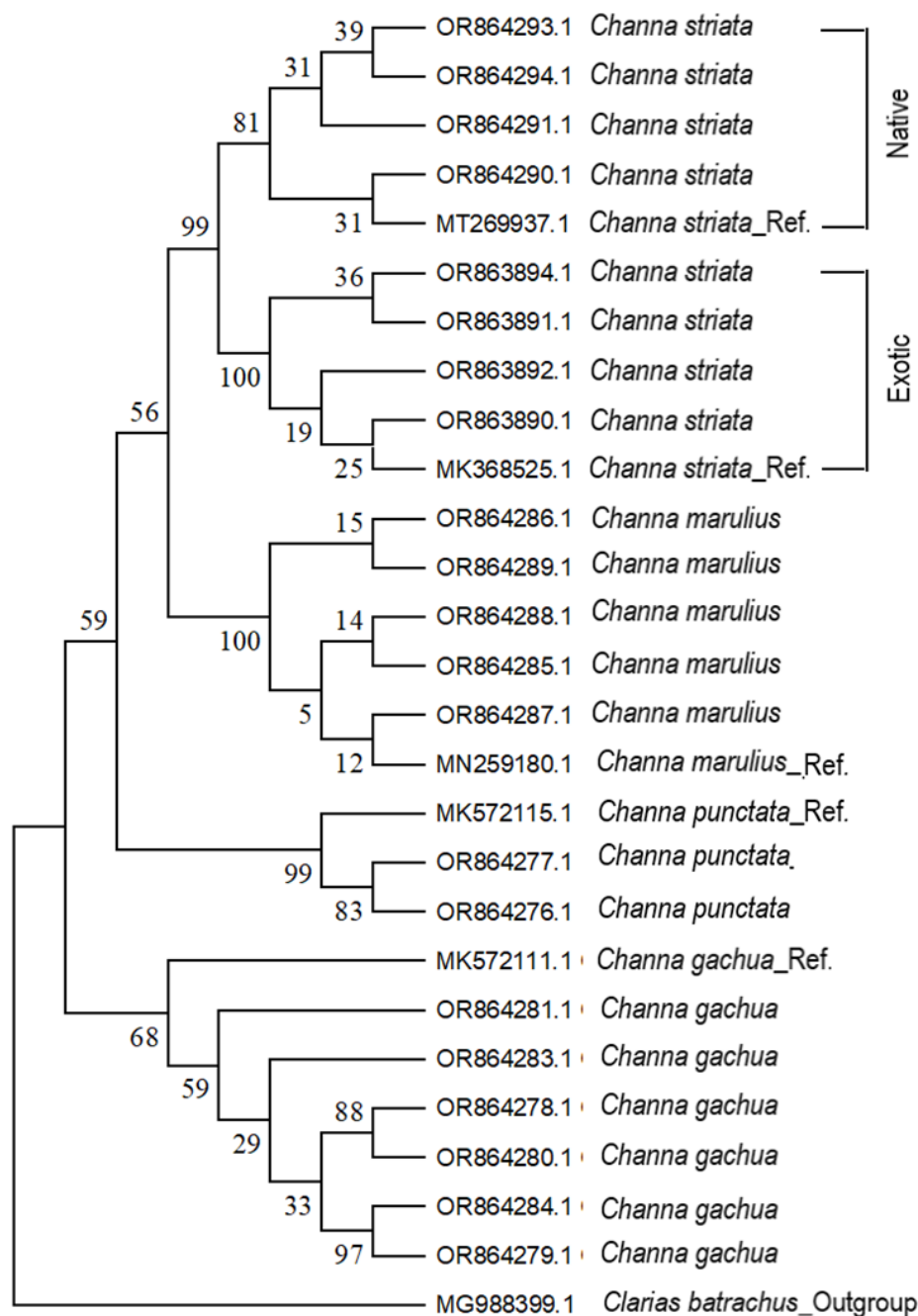


Figure 2.5 Phylogenetic tree of *Channa* species inferred from COI nucleotide sequences using the ML method. The bootstrap support values shown next to the branches. The sequences labeled as "Ref." represent sequences from NCBI genebank databases, while "Native" and "Exotic" indicate native and introduced *C. striata* population respectively.

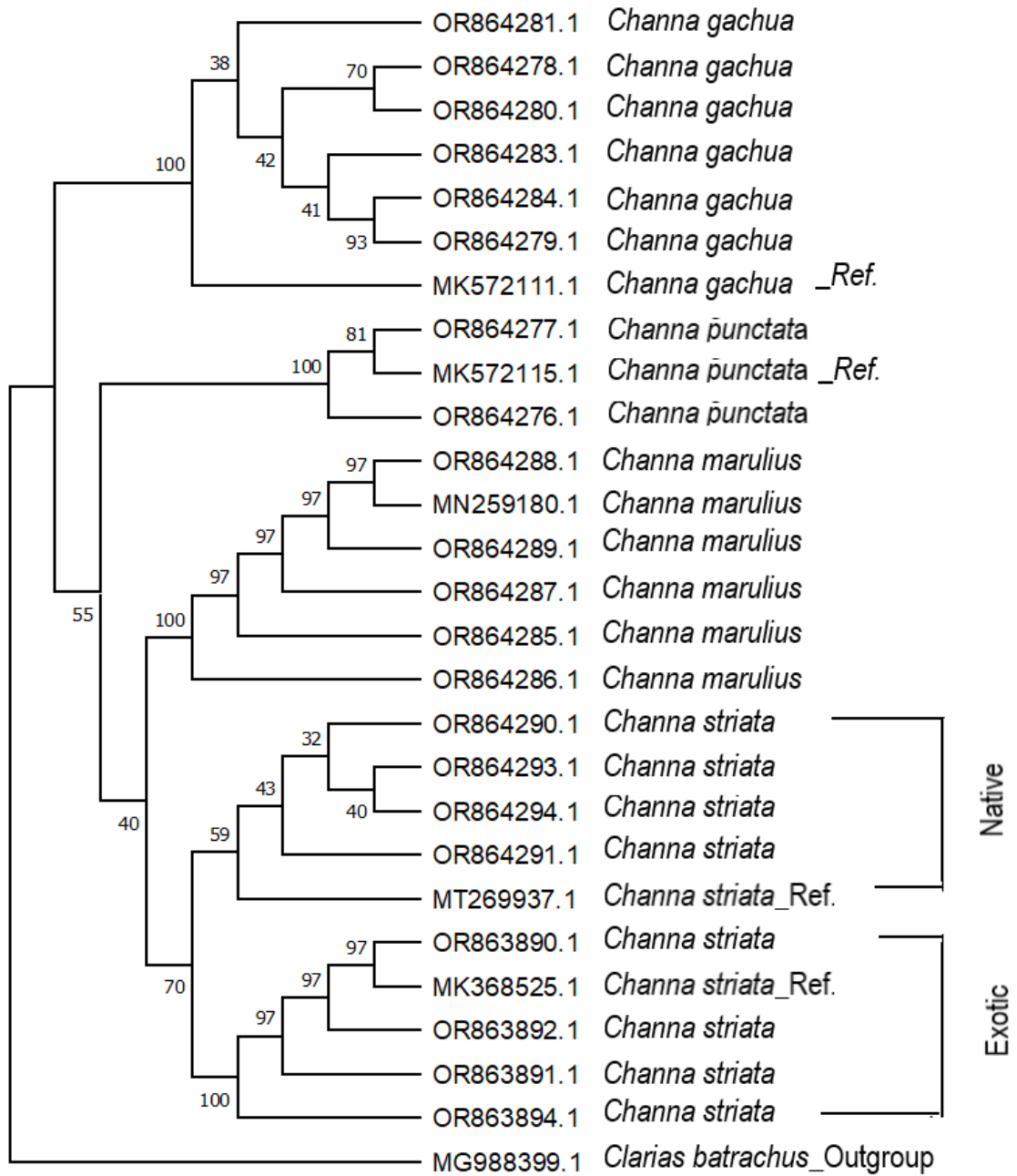


Figure 2.6 Phylogenetic tree of *Channa* species inferred from COI nucleotide sequences using the MP method.

2.4 Discussion

2.4.1 Geometric-Morphometry based body shape variation of *Channa* species

Identification of closely related fish based on traditional morphometric and meristic characteristics is highly ambiguous as there have been several reports of snakeheads species being mislabeled worldwide (Galal-Khallaf et al., 2014; Nagalakshmi et al., 2016; Yan et al., 2016). Therefore, geometric morphometrics analysis and DNA barcoding based on mitochondrial cytochrome oxidase I (COI) provides a crucial technique to identify species that are closely related (Bookstein, 1986; Roberto & Lopes, 1999; Ward et al., 2005; Mojekwu & Anumudu, 2015; Lakra et al., 2015; Parvez et al., 2020; Mallik et al., 2020; Parvez et al., 2022; Sajjad et al., 2023). In our study, the geometric morphometric evaluation showed considerable variation among *Channa* species in Bangladesh. Principal component analysis (PCA) showed overall variation among 4 Species was 94.603 % in Principal Component 1. The PCA plot result was supported by scatter plot and UPGMA Euclidean cluster analysis where *C. marulius* formed clade with the Indigenous *C. striata* while the Vietnamese *C. striata* formed clade with *C. punctata* and *C. gachua*. This variation was depicted in Multivariate analysis also. PCA is a useful technique for identifying the physical traits that are considered to be important causes of variation among species (Bookstein, 1986; Roberto & Lopes, 1999; Specziár et al., 2009; Arechavala-Lopez et al., 2012). In a recent study based on the geometric characteristics cluster analysis showed that five *Channa* species form two major clades; In one clade, *C. marulius*, *C. striata* and *C. diplogramme* whereas *C. gachua*, and *C. punctata* were placed in another major clade (Hanifa et al., 2014). In another study morphometric variation and interrelationships of six species of the genus *Channa* showed that *C. punctata* and *C. striata* were mostly closely related species followed by *C. marulius*, *C. gachua* and *C. stewartii*

while *C. aurantimaculata* was found to be most distantly related (Choudhury & Dutta, 2013). The PCA based scatter plot demonstrated significant morphological differences between the native and exotic *C. striata* population. The highest variation in principal component 1 indicated the landmark anterior tip of snout at upper jaw was responsible mostly for the variation between native and Vietnam-originated exotic *C. striata* in Bangladesh. Variation in head shape of Vietnamese *Channa striata* were found in three different population of *C. striata* in Vietnam (Nguyen et al., 2016). Morphological variation of indigenous and Vietnamese *C. striata* in total length, standard length, pelvic fin length and mouth gap previously reported in Bangladesh (Jannat et al., 2022). Causes of morphological variations are often due to spatial boundaries, selection and involvement of environmental factors (Cadrin & Friedland, 1999; Su et al., 2019). Ecological strategies of a species, including feeding habits, movement and other environmental interactions may cause the phenotypic variations of same species from different geographic region (D.T. et al., 2019). Therefore, the wild living environment for indigenous *C. striata* and cultured environment for Vietnamese *C. striata* can be considered the main cause of morphological variation between these two strains. Species movements frequently impact their body and head shape.

2.4.2 COI nucleotide sequence based variation and evolutionary relationship *Channa* species

In the COI gene analysis, the conserved sites are dominant (61.51%), but the presence of a substantial number of variable (36.93%) and parsimony-informative sites (31.99%) indicates that COI is a useful genetic marker for species differentiation in *Channa*. A high proportion of conserved sites indicate that the COI gene is relatively stable across the *Channa* species, which is expected because COI is a mitochondrial gene involved in essential cellular functions. The variable sites (36.93%) in *Channa* species of this study

show a reasonable level of genetic diversity, which is important for phylogenetic analysis and species differentiation. A good percentage of parsimony-informative sites (31.99%) suggest that the COI gene can effectively resolve phylogenetic relationships among *Channa* species. The presence of 4.79% singleton sites (unique mutations in single sequences) suggests some level of rare genetic variations. The similarity in conserved site percentages between our study (61.51%) and the Lakra et al., 2010 (61.85%) suggests a consistent level of conservation in the COI gene among *Channa* species. The variable (38.15%) and parsimony-informative site (37.61%) are also comparable, indicating that the COI gene provides sufficient variability for phylogenetic analyses within this genus (Lakra et al., 2010). Recently genetic diversity between native and Vietnamese *C. striata* were studied by Kanon et al., 2022 using Cyt b gene and Alam et al. 2022 by using PCR-RFLP method where they observed genetic diversity between native and Vietnamese *C. striata* (Alam et al., 2022; Kanon et al., 2022). The variation may be due to variation in population size, life-history traits and environmental variability (Liu, 2008). The nucleotide composition in our study, with an AT content of 52%, aligns closely with the 55% observed in DNA barcoding of Indian freshwater fishes (Lakra et al., 2015); 53.20% observed in the Taiwan Strait fish species study (Bingpeng et al., 2018). This consistency supports the notion that an AT-rich composition is a common characteristic of mitochondrial COI genes across various fish species, which could impact mutation rates, as these regions are often more prone to genetic changes than GC-rich regions (Parvez et al., 2020).

The disparity index test is a valuable statistical tool used to assess base composition biases in nucleotide sequences relative to expected values based on evolutionary divergence (Kumar & Gadagkar, 2001). The disparity index test is further supported by heatmap-based analyses strengthen the finding of evolutionary divergence among *Channa* species. Similar heatmap-based analyses in freshwater fishes have shown comparable patterns of nucleotide

composition disparity. For example, Prasad et al. (2020) observed high disparity values between morphologically similar but genetically distinct fish species, reinforcing the importance of molecular tools in species identification. Moreover, studies on cyprinid and catfish species have reported comparable findings, where species with strong genetic similarity showed low disparity values, while highly divergent species displayed significant base composition biases (Liu et al., 2017; Sharma et al., 2021). The observed zero to very low disparity (0.000 – 0.031) within *C. punctata* and *C. marulius* sequences suggests a highly conserved base composition which is consistent with previous studies that reported low intraspecific variation in *Channa* species due to strong genetic stability within populations (Adamson et al., 2010; Gopikrishna et al., 2013). Similar findings were observed in mitochondrial COI gene studies of *Channa punctata*, which exhibited low genetic variation across different geographical locations in India and Bangladesh (Kumar et al., 2019). Moderate disparities (0.030 – 0.222) were found in comparisons involving *C. gachua* and *C. marulius*, aligns with previous studies where *C. gachua* exhibited moderate genetic variation across its range, likely due to geographic isolation and local adaptation (Collins et al., 2012; Li et al., 2022). Additionally, the disparity values between exotic and native *C. striata* (0.041 – 0.222) indicate a degree of differentiation between these populations, which may result from founder effects, genetic drift, or selective pressures in introduced environments (Araki & Schmid, 2010; Han et al., 2021). The genetic differences between native and exotic populations suggest that exotic *C. striata* has undergone some degree of genetic change post-introduction, a pattern commonly observed in invasive fish species (Li et al., 2022). The highest disparity (0.301 – 0.539) between native *C. striata* (OR864293.1) and *C. gachua* (OR864278.1) indicate that these species have undergone distinct evolutionary processes leading to substantial nucleotide composition differences (Tamura et al., 2013). Previous studies using mitochondrial DNA have also demonstrated

that *C. gachua* is genetically distinct from other *Channa* species, supporting its unique evolutionary trajectory within the *Channidae* family (Zhang & Hanner, 2012).

Understanding substitution patterns in mitochondrial genes provides insights into evolutionary processes and molecular evolution in fish species (Tamura & Nei, 1993). In the present study, substitution rates revealing a strong bias towards transition substitutions ($A \leftrightarrow G$ and $T \leftrightarrow C$) over transversions. This is consistent with previous studies on mitochondrial DNA evolution in teleost fishes (Zhang & Hanner, 2012; Li et al., 2022). The high substitution rates for $A \leftrightarrow G$ (14.64) and $T \leftrightarrow C$ (14.64) indicate a strong transition bias. Transitions are generally more common in mitochondrial genes because they cause fewer structural distortions in DNA, making them more likely to persist through evolutionary processes (Tamura et al., 2013). This trend has also been observed in other freshwater fishes, such as *Cyprinidae* and *Siluriformes* (Liu et al., 2017; Kumar et al., 2019). Studies on marine and freshwater fishes have reported similar trends, where transversion rates are consistently lower than transition rates (Prasad et al., 2020). The estimated transition/transversion ratio ($R = 1.41$) in *Channa* species further supports the idea that transitions occur more frequently than transversions. Similar results have been reported in studies of *C. striata* and other *Channa* species, where transition bias ranged from 1.2 to 1.8 (Gopikrishna et al., 2013; Han et al., 2021). The lower substitution rates for transversions ($A \leftrightarrow T$, $A \leftrightarrow C$, $G \leftrightarrow T$, and $G \leftrightarrow C$ at 5.18) indicate that these changes are less common in *Channa* species. This pattern is widely observed in vertebrate mitochondrial DNA due to the structural instability introduced by purine-pyrimidine swaps (Tamura & Kumar, 2002).

In this study, the results showed that most *Channa* species evolved with homogeneous substitution patterns, as indicated by non-significant P-values (>0.05), except for certain sequence pairs involving *C. striata* (native) and *C. gachua*. The high P-values (~ 1.00)

observed within *C. punctata*, *C. marulius*, and *C. striata* groups indicate that these species share a similar substitution pattern, reflecting evolutionary stability within species. This result is consistent with previous studies on freshwater fish mitochondrial DNA, where species-specific substitution patterns have been observed to be largely conserved (Liu et al., 2017; Li et al., 2022). Similar findings have been reported in other fish families, such as *Cyprinidae* and *Siluriformes*, where intra-species homogeneity in substitution rates is common (Sharma et al., 2021; Han et al., 2021). The reference sequences (Ref.) for each *Channa* species showed no significant differences ($P = 1.00$) when compared to their respective species, indicating that the reference sequences accurately represent the evolutionary patterns of each species.

The analysis of pairwise genetic distances based on mitochondrial cytochrome c oxidase subunit I (COI) gene sequences among five *Channa* species revealed clear interspecific divergence and structured intraspecific variation, particularly within *C. striata*. These values were interpreted in the context of established threshold values for species, genus, and population-level genetic distances in freshwater fishes.

The interspecific divergence of this study supports taxonomic validity of the studied five *Channa* species in Bangladesh. The genetic distances between different *Channa* species ranged from 0.1792 (*C. striata* vs. *C. punctata*) to 0.2638 (*C. striata* vs. *C. gachua*), with an overall mean intra-genus divergence of 0.1955. These values are well above the commonly accepted species-level threshold of 2–3% (0.02–0.03) COI divergence in fishes (Hebert et al., 2003; Ward et al., 2005). Such high values are consistent with previously reported divergence ranges of 6–10% or more among fish species (Ward et al., 2005; Hubert et al., 2008), and particularly for *Channa* species, where interspecific COI divergence values of 5–20% have been documented (Lakra et al., 2010).

The high interspecific distances in this study confirm robust genetic boundaries among *C. marulius*, *C. punctata*, *C. gachua*, and *C. striata*, reflecting their distinct evolutionary lineages. For example, *C. marulius* exhibited a genetic distance of 0.2091 with native *C. striata*, and *C. gachua* showed the highest divergence (0.2638) with *C. striata*, further supporting their placement in distinct phylogenetic clades.

The genetic distances within *C. striata* showed notable variation between native and exotic populations. The exotic strain of *C. striata* exhibited extremely low intraspecific divergence (0.0005), indicating a genetically uniform population likely the result of founder effects, inbreeding, or long-term hatchery propagation with limited gene flow. In contrast, the native population of *C. striata* showed a relatively higher intraspecific divergence (0.0441), suggesting population substructure and possible cryptic variation. This pattern is consistent with DNA barcoding studies where intraspecific divergence values >3% (0.03) are considered indicative of cryptic speciation or distinct evolutionary significant units (ESUs) (Hebert et al., 2003; April et al., 2011; Zhang & Hanner, 2012). The overall mean intraspecific divergence within *C. striata* (0.0384) exceeds this threshold, supporting the possibility of distinct lineages within the species, especially between native and exotic groups.

Phylogenetic analysis using COI nucleotide sequences provides crucial insights into the evolutionary relationships and genetic structuring of *Channa* species. The results strongly support the taxonomic classification of *Channa* species and reveal significant genetic differentiation between native and exotic *C. striata*. The phylogenetic trees constructed in this study align with previous molecular phylogenetic analyses of *Channa* species. Li et al. (2022), and Baisvar et al., (2019) reported similar tree topologies, where *C. striata*, *C. marulius*, *C. punctata*, and *C. gachua* formed well-supported monophyletic groups. The

distinct clustering of *C. striata* populations has also been observed in past studies, particularly in cases where domestication or environmental factors led to genetic differentiation (Collins et al., 2012; Gopikrishna et al., 2013). The grouping of conspecific sequences together reinforces the reliability of COI barcoding in species identification and taxonomic classification. The presence of reference sequences further validates the classification, as the studied sequences cluster correctly with their respective species' reference sequences, confirming their genetic identity.

A key observation in disparity index, sequence homogeneity, genetic divergence and in the phylogenetic tree is that the distinct clustering of native and exotic *C. striata*. Native samples group together with *C. striata* reference sequences, while exotic samples form a separate cluster. This suggests genetic differentiation between the two populations, likely due to independent evolutionary trajectories or adaptation to different environments. The genetic divergence between native and exotic *C. striata* has been documented in previous studies, which attributed the differentiation to founder effects, artificial selection, or geographic isolation (Araki & Schmid, 2010; Han et al., 2021). The separation of exotic *C. striata* from native populations may indicate underlying genetic changes due to domestication or introduction into a new environment. Previous studies on introduced fish species, such as *Oreochromis niloticus* and *Hypophthalmichthys nobilis*, have reported similar phylogenetic divergence between wild and introduced populations, often linked to genetic bottlenecks or selective pressures (Gopikrishna et al., 2013; Han et al., 2021; Afrin et al, 2024). Similar observations were reported in native and exotic *Clarias* species (Parvez et al., 2022), in exotic and native *Anabas testudineus* (Parvez et al., 2020) in Bangladesh where introduced populations showed moderate but significant genetic variation from their native counterparts.

2.5 Conclusion

This study provides a comprehensive assessment of *Channa* species in Bangladesh through an integrative approach combining geometric morphometric analysis and COI-based DNA barcoding. The integrated dataset successfully discriminated all five native *Channa* species and the introduced *C. striata* strain, with a classification accuracy exceeding 95% based on cross-validated discriminant analysis. Morphological variation captured through the truss network system corresponded well with genetic clustering, reinforcing the reliability of morphometric traits in species delineation.

Phylogenetic analyses using COI sequences confirmed distinct species-level clustering, consistent with current taxonomic classifications. Notably, the exotic *C. striata* population introduced from Vietnam formed a well-supported monophyletic clade that was genetically distinct from any putative native *C. striata* haplotypes. The mean K2P genetic distance between native and exotic *C. striata* lineages exceeded 2.5%, suggesting significant divergence, likely driven by geographic isolation, selective breeding, or environmental adaptation following introduction.

These findings support both Hypothesis 1 and Hypothesis 2, affirming the efficacy of combining geometric morphometrics with molecular markers for high-resolution species discrimination and lineage differentiation. The study also emphasizes the importance of using DNA barcoding in conjunction with morphological data for accurate taxonomic identification, especially in groups with overlapping traits or cryptic diversity. Future work incorporating nuclear markers and genome-wide SNP analysis will further enhance the understanding of genetic connectivity, local adaptation, and evolutionary history among *Channa* populations in Bangladesh, contributing to improved conservation and aquaculture strategies.

Chapter 3

Growth and reproductive biology of *Channa striata* in the drought-stressed atrai river: implications for conservation and aquaculture



Abstract

This study investigates the growth patterns and reproductive biology of *Channa striata* in the Atrai River, a drought-prone region of northwestern Bangladesh to assess its aquaculture potential and inform conservation strategies. A total of 239 specimens were collected monthly from April 2022 to March 2023 to evaluate morphometric traits, reproductive cycles, and environmental influences. The species exhibited negative allometric growth ($b > 3.0$), where strong seasonal variation was evident in growth and physiological condition. Peak male weight in September and female weight in April indicate sex-specific energy allocation, with reduced growth during winter months reflecting post-spawning depletion. Fulton's condition factor (K) was highest post-monsoon, coinciding with improved feeding and habitat quality, and lowest during the reproductive season (April–July), highlighting somatic-reproductive trade-offs. The sex ratio (M: F = 1:0.67) remained balanced overall, though a female bias was observed early in the year. GSI and fecundity peaked between April and July, aligning with the monsoon-driven spawning season. Ovarian histology revealed asynchronous oocyte development, indicating batch spawning, while fecundity showed strong correlations with body size and GSI ($R^2 = 0.74$). Histological analysis confirmed five distinct gonadal maturation stages, with peak spawning from May to July. The results provide critical baseline data to support broodstock development, stock enhancement programs, and seasonal fisheries management interventions such as fishing bans and size-at-maturity regulations.

3.1 Introduction

Channa striata (Bloch, 1793), commonly known as the striped snakehead, is a carnivorous freshwater fish widely distributed across South and Southeast Asia (Talwar & Jhingran, 1991; Courtenay & Williams, 2004; Froese & Pauly, 2023). Its remarkable adaptability to various aquatic habitats including rivers, lakes, wetlands, canals, and floodplains makes it both ecologically important and economically valuable (Bich et al. 2020; Phanna et al., 2015). The species' ability to breathe air via a well-developed accessory respiratory system enables it to survive under adverse conditions such as hypoxia and drought (Rahman, 2005; Courtenay & Williams, 2004). Its rapid growth, high nutritional value, and reputed medicinal properties have driven demand in wild fisheries and aquaculture across Bangladesh, India, Indonesia, Malaysia, Vietnam, and Thailand (Haniffa et al., 2004; Nen et al., 2018; Wee 1981; Li et al., 2016). *C. striata* plays a vital role in food security and rural livelihoods, largely due to its capacity to grow in suboptimal water conditions (Djokosetiyanto et al., 2017). This resilience makes it an ideal species for aquaculture in resource-limited and environmentally stressed systems (Sarda et al., 2024; Dewantoro et al., 2019). Its tolerance to environmental stressors highlights its potential for climate-adaptive aquaculture (Rahman and Awal, 2016; Hossain et al., 2022; Paul et al., 2024). Despite this importance, there remains limited information on the growth and reproductive biology of wild populations, especially those inhabiting ecologically stressed river systems (Rahman and Awal, 2016). Freshwater ecosystems in Bangladesh are increasingly threatened by climate change, habitat degradation, pollution, and overexploitation (Dudgeon et al., 2006; Parvez et al., 2019; Islam and Hoq, 2019; Siddique et al., 2022). These pressures are particularly acute in northern Bangladesh, where the Atrai River and its tributaries have experienced significant hydrological disruptions in recent decades. Once a perennial river rich in biodiversity, the Atrai now faces frequent seasonal drying and disrupted flow, driven

primarily by erratic rainfall, prolonged drought, and rising temperatures (Chaki et al., 2014; Pal 2016; Jahan et al., 2018; Pal and Sarda 2020; Nowreen et al., 2020; Tasrif et al., 2022). Human activities such as upstream water diversion, unsustainable irrigation, riverbank encroachment, and siltation have further exacerbated habitat degradation, negatively impacting aquatic life (Dhadse and Kardbhajne, 2024; Rahman, 2021). These environmental changes pose significant threats to *C. striata*, a species that depends on dynamic riverine habitats for growth and reproduction. The species is currently listed as near threatened in Bangladesh, due to overfishing, pollution and loss of critical spawning habitats such as floodplains and backwaters (IUCN Bangladesh, 2015).

While captive breeding and aquaculture practices of *C. striata* have been well studied in other countries with focus on induced breeding, larval rearing and feed development (Abol- Munafi et al., 2004; Haniffa et al., 2006; War et al., 2011; Paray et al., 2015; Kumar et al., 2022), there is a lack of detailed knowledge on the natural growth and reproductive dynamics of wild populations in Bangladesh, especially in drought-affected rivers (Lake 2011; Clarke et al., 2012; Habiba et al., 2013; Basin 2017). Limited studies, such as Ferdousi et al. 2015 and; Awal et al., 2025 studied in Haor region of Sylhet regions of Bangladesh and have highlighted the need for more localized ecological research. Understanding the growth and reproductive responses of *C. striata* in the drought-stressed Atrai River is essential to support conservation and sustainable fishery management. Key biological parameters including length–weight relationships, condition factor, sex ratio, gonadosomatic index (GSI), spawning seasonality, and fecundity provide critical insight into fish health, reproductive capacity, and environmental adaptability (Nikolsky, 1963; Froese, 2006; Chen et al., 2022).

The Atrai River basin, as a drought-prone and ecologically degraded system, represents a critical yet understudied environment for assessing the life-history traits of *C. striata*. Seasonal drying and altered hydrological regimes are likely to affect reproductive success, growth performance, and overall population viability. Given the species' economic and nutritional importance to rural communities, it is essential to investigate how these stressors influence key biological parameters. Moreover, insights into the reproductive ecology of wild populations could inform the development of drought-resilient broodstock for aquaculture. Such knowledge is pivotal for sustainable fisheries management, biodiversity conservation, and climate-resilient aquaculture planning (West, 1990; Lebel et al., 2021; Abisha et al., 2022; Hossain et al., 2025). Hence, the study tested following to hypothesis.

Hypothesis 1 (H₁): Seasonal environmental fluctuations in the Atrai River significantly influence the growth dynamics and reproductive timing of *Channa striata*, resulting in negative allometric growth, variable condition factors, and a spawning cycle synchronized with pre-monsoon and monsoon periods.

Hypothesis 2 (H₂): Wild *Channa striata* populations in the Atrai River exhibit strong reproductive potential, characterized by high gonadosomatic index, fecundity, and complete gonadal development, indicating their suitability for use as broodstock in climate-resilient aquaculture.

Therefore, this study aims to fill a critical knowledge gap by examining the seasonal growth patterns and reproductive biology of *C. striata* in the drought-impacted Atrai River and its tributaries. Additionally, it evaluates the suitability of wild populations as broodstock for induced breeding programs under climate-stressed conditions. The findings will support science-based management strategies and foster sustainable aquaculture development in freshwater ecosystems affected by climate variability.

3.2 Materials and Methods

3.2.1 Site Selection and Fish Specimen Collection

To investigate the reproductive biology and growth dynamics of *C. striata*, a total of 239 wild specimens were collected monthly from April 2022 to March 2023 across three key locations in the Atrai River system in northwestern Bangladesh. The selected sites included the Atrai River and its two major tributaries such as the Gabura and Kankra Rivers, located in the Dinajpur district (Figure 2.1). The Atrai River bifurcates northwest of Chirirbandar Upazila, with the western branch forming the Gabura River and the eastern forming the Kankra River, before reconverging downstream. Three stations were selected, each with three replicates: Station 1 (Atrai River, Jhanjira): A1 (25°43'04.5"N, 88°44'13.5"E), A2 (25°42'41.0"N, 88°43'51.9"E), A3 (25°42'06.3"N, 88°43'40.0"E); Station 2 (Kankra River, Mohonpur): K1 (25°32'42.3"N, 88°46'20.3"E), K2 (25°32'09.7"N, 88°46'01.1"E), K3 (25°31'39.2"N, 88°45'53.0"E); and Station 3 (Gabura River, Ramdubbi): G1 (25°45'37.6"N, 88°42'16.3"E), G2 (25°44'57.6"N, 88°42'10.6"E), G3 (25°44'28.9"N, 88°41'51.7"E).

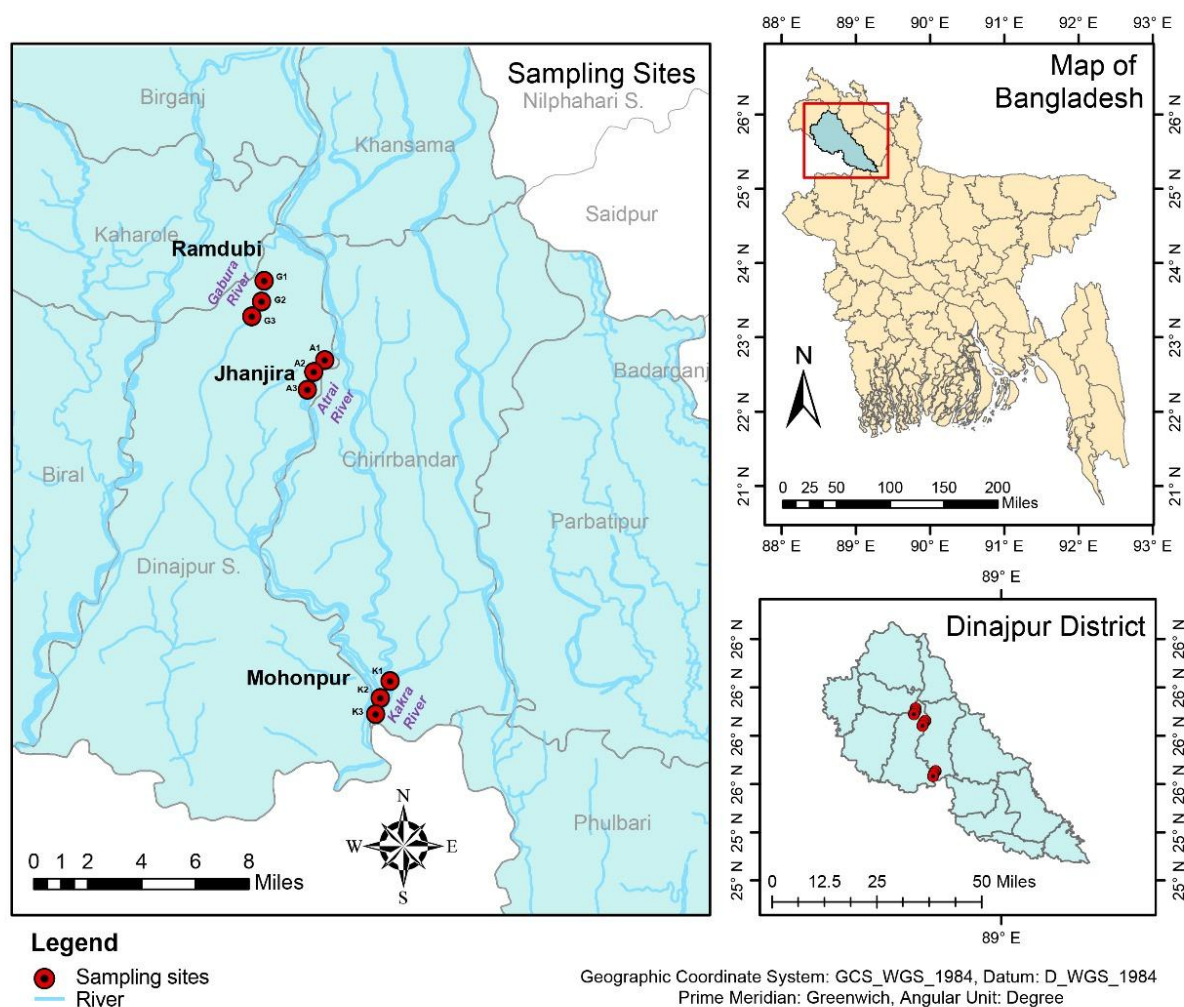


Figure 3.1 Map showing the sampling sites for *C. striata* in the Atrai River and its tributaries, the Gabura and the Kankra Rivers located within the Dinajpur district, Bangladesh. Inset maps show the location of Dinajpur district in Bangladesh and the relative positions of sampling sites within the river network.

3.2.2 Morphometric Measurements and Gonad Collection

Upon collection, fish specimens were transported to the Fisheries Biology and Genetics Laboratory at Hajee Mohammad Danesh Science and Technology University (HSTU). Each specimen's total length (TL) was measured to the nearest 0.1 cm using a measuring board, and body weight (BW) was recorded to the nearest 0.1 g using a digital balance. Dissections

were performed by making a ventral incision from the base of the pectoral fins to the anus to carefully excise the gonads. Each gonads's length (GL) was measured to the nearest 0.1 cm, and gonad weight (GW) was recorded to the nearest 0.1 g (Appendix 4). Both fish and gonads were preserved in 10% formalin in labeled jars for further analysis.

3.2.3 Growth Pattern: Length-Weight Relationship (LWR)

The length-weight relationship (LWR) was established using the equation $W = aL^b$, where W is body weight (g), L is total length (cm), a is the intercept, and b is the allometric growth coefficient. The relationship was logarithmically transformed to linear form: $\log(W) = \log(a) + b \cdot \log(L)$. This model follows the methodology of Ricker and Carter (1958), allowing assessment of growth patterns (isometric or allometric). The growth pattern was interpreted based on the value of b : isometric growth if $b=3$, positive allometric growth if $b>3$, and negative allometric growth if $b<3$.

3.2.4 Condition Factor (K)

To assess seasonal variations in fish health and body condition, Fulton's Condition Factor (K) was calculated as: $K = (W / L^3) \times 100$, where W is body weight (g) and L is total length (cm). This index provides an estimate of the nutritional and reproductive status of fish, following Htun-Han (1978).

3.2.5 Sex Differentiation and Gonadal Maturity Staging

Sex was initially determined via external examination, with confirmation through internal dissection using the acetocarmine staining method (Guerrero and Shelton, 1974). Females exhibited a swollen abdomen and oval genital papilla, while males had a flattened abdomen and elongated papilla. Gonadal development was categorized into five maturity stages

following Djumanto et al. (2019): I. Immature; II. Maturing; III. Mature; IV. Spawning; and V. Spent. These stages were identified through gross morphology, microscopic examination with acetocarmine, and histological analysis.

3.2.6 Histological Analysis of Gonadal Maturity Phases of *C. striata*

To validate reproductive staging, histological analysis was conducted monthly. Fresh gonads were rinsed in saline and preserved in 10% buffered formalin for 24–48 hours, then fixed in Bouin's fluid for 24 hours as per Feldman and Wolfe (2014). Fixed samples were washed, dehydrated in ethanol series, embedded in paraffin, and sectioned at 7 µm thickness using a rotary microtome. Sections were stained with hematoxylin and eosin (H&E) and examined under an MCX100 microscope (Micros, Austria) equipped with an AmScope digital camera. Gonadal stages were interpreted following Formicki and Kirschbaum (2019).

3.2.7 Estimation of Size at First Sexual Maturity (L_m)

Length at first sexual maturity (L_m) was estimated by fitting a logistic regression model to the proportion of mature individuals (beyond stage I) relative to total length (TL). The model, adapted from King (2007), was:

$$M = 1 / (1 + \exp[r(L - L_m)])$$

Where:

M = proportion of mature individuals

L = total length (cm)

L_m = length at first maturity (cm)

r = slope of the curve

Analysis was performed using the sizeMat package in R (R Core Team, 2024)

3.2.8 Gonado-somatic Index (GSI)

Monthly changes in reproductive investment were evaluated using the gonado-somatic index (GSI): $GSI = (GW / BW) \times 100$, Where GW = gonad weight (g), BW = total body weight (g). Gonads were weighed using a precision balance (± 0.001 g), and preserved for further examination.

3.2.9 Fecundity Estimation

Absolute fecundity was assessed monthly using mature females (stages III–V), following Simpson (1959). Ovaries preserved in 10% formalin were weighed, and three 1 g subsamples (from anterior, middle, posterior) were taken. Ova were counted under a microscope, and fecundity (Fe) was calculated as:

$$Fe = (N \times GW) / gW_s$$

Where:

N = number of ova in subsample

GW = total gonad weight

gW_s = weight of subsample

3.2.10 Ova Diameter Measurement

Ova diameter was measured in mature ovaries by isolating eggs from three regions (anterior, middle, posterior). A total of 300 ova per fish were measured using an MCX100 compound microscope with micrometer calibration, following Dinh et al. (2016). Ova size served as an indicator of reproductive readiness and spawning potential.

3.2.11 Statistical Analyses

Statistical analyses were performed using R software (R Core Team, 2024). Data wrangling, visualization, and tabular summaries were conducted using packages from the tidyverse collection (Wickham et al., 2019). The length-weight relationship was examined to interpret growth patterns, where the slope (b) of the regression indicated isometric growth if $b = 3$, positive allometric growth if $b > 3$ and negative allometric growth if $b < 3$. To test whether the estimated b value significantly differed from the isometric value, a one-sample t -test was performed using the hypotheses: null hypothesis (H_0): $b = 3$ (isometric growth), and alternative hypothesis (H_1): $b \neq 3$ (allometric growth), with the t -statistic calculated as $t = (b - 3) / SE(b)$, where $SE(b)$ represents the standard error of the slope. One-way analysis of variance (ANOVA) was applied to assess monthly variations in gonado-somatic index (GSI), Fulton's condition factor (K), and fecundity with a significance threshold set at $p < 0.05$. When significant differences were detected, Tukey's Honest Significant Difference (HSD) test was conducted using the MultcompView package (Graves et al., 2015) for pairwise comparisons. Length-at-maturity (L_m) curves were estimated using the sizeMat package (Torrejon-Magallanes, 2020), enabling modeling and visualization of length-based maturity data to determine the total length at first sexual maturity. Additionally, a chi-square goodness-of-fit test was conducted to assess whether the observed sex ratio deviated from the expected 1:1 ratio, comparing the observed frequencies of males and females with equal expected counts to determine statistical significance.

3.3 Results

3.3.1 Growth Pattern of *C. striata*

The monthly distribution of total length and body weight of *Channa striata* is presented in Table 3.1. Throughout the study period (April 2022 to March 2023), notable variations were observed in both size and weight across sexes. The highest average total length and body weight in males were recorded in September (45.62 ± 5.37 cm; 752.59 ± 254.67 g), whereas the highest average values for females occurred in April (43.07 ± 2.97 cm; 828.75 ± 122.17 g), suggesting the influence of pre-spawning gonadal development. Conversely, the lowest mean values for both sexes were recorded during the winter months (December to February), which may reflect post-spawning weight reduction or limited feeding activity. These monthly fluctuations provide insights into seasonal growth dynamics and reproductive investment in *C. striata*.

Table 3. 1 Monthly variations in sample size, total length (cm), and body weight (g) of male and female *C. striata* collected from the Atrai River (April 2022 – March 2023)

Month	Sample size (N)		Total length (cm)		Body weight (g)	
	Male	Female	Male (TL)	Female (TL)	Male (BW)	Female (BW)
April	12	8	39.69 ± 4.97	43.07 ± 2.97	434.85 ± 140.98	828.75 ± 122.17
May	10	6	34.39 ± 6.56	41.69 ± 4.14	349.93 ± 207.21	543.69 ± 158.46
June	8	10	34.57 ± 0.49	39.78 ± 1.98	357.92 ± 33.44	476.22 ± 49.92
July	11	8	42.18 ± 2.94	41.18 ± 3.75	591.03 ± 113.46	489.57 ± 70.68
August	10	10	42.58 ± 2.95	38.39 ± 5.9	551.59 ± 74.93	484.11 ± 188.78
September	12	12	45.62 ± 5.37	38.62 ± 7.53	752.59 ± 254.67	582 ± 283.52
October	12	8	34.85 ± 2.87	35.05 ± 3.43	372.55 ± 113.3	377.21 ± 122.63
November	12	8	40.36 ± 1.75	39.81 ± 3.67	564.07 ± 61.03	602.82 ± 138.24
December	8	12	35.55 ± 2.82	35 ± 2.34	343.22 ± 67.06	327.35 ± 45
January	4	18	31.48 ± 0.87	31.98 ± 2.2	251.07 ± 35.69	280.2 ± 40.09
February	8	12	34.69 ± 2.91	35.37 ± 2.23	365.54 ± 77.27	366.67 ± 64.35
March	12	8	33.28 ± 3.91	34.45 ± 0.63	294.46 ± 101.02	309.36 27.37

3.3.2 Length–Weight Relationship of *C. striata*

The length–weight relationships (LWR) of male and female *C. striata* were analyzed using log-transformed data to determine their growth patterns. The regression equations derived were $\ln(W) = -3.82 + 2.72 \ln(L)$ for males with a coefficient of determination $R^2=0.92$; and $\ln(W) = -3.39 + 2.61 \ln(L)$ for females with $R^2=0.88$. The narrow 95% confidence intervals around the regression lines further support the robustness of the fitted models. As shown in Figure 3.2, both sexes exhibited a strong positive correlation between total length (TL) and body weight (BW), as indicated by the high R^2 values. For the male specimens ($n=107$), the

estimated slope was $b=2.72$ with a standard error of 0.13. The computed t -value was -2.15 , which was statistically significant at the 5% level ($p<0.05$, $df = 106$), indicating a significant deviation from isometric growth. Similarly, for females ($n=112$), the estimated slope was $b=2.61$ with the standard error 0.12, yielding a t -value of -3.00 , which was highly significant at the 1% level ($p<0.01$, $df = 111$). These results confirm that both male and female *Channa striata* in the study population exhibit significant negative allometric growth. The slope (b) of the length-weight relationship was 2.72 for males and 2.61 for females. One-sample t -tests indicated that both values were significantly less than 3 (males: $t=-2.15$, $p<0.05$; females: $t=-3.00$, $p<0.01$), suggesting a negative allometric growth pattern in both sexes.

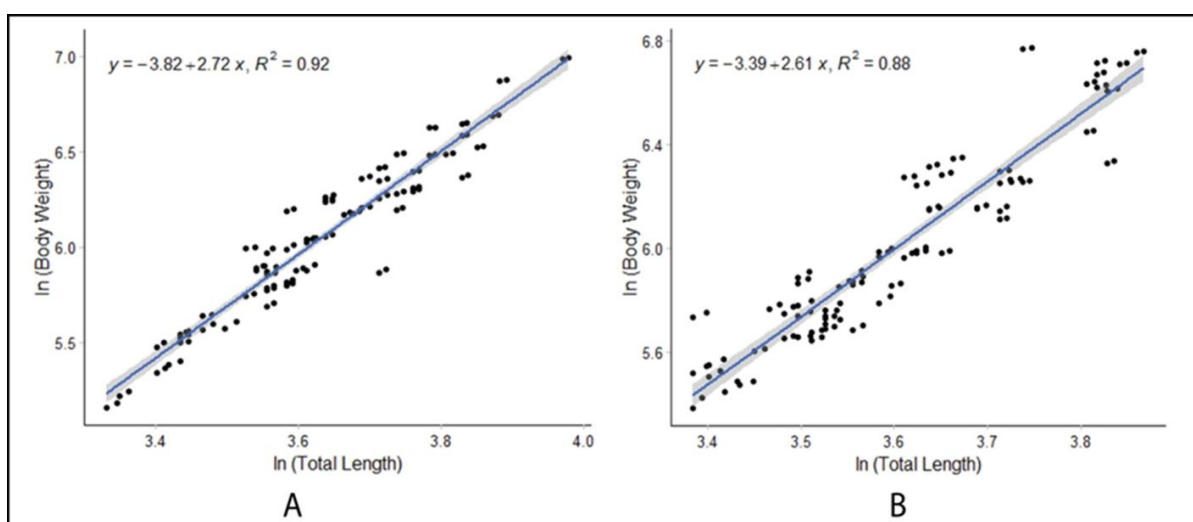


Figure 3.2 Length-weight relationships of *C. striata* from the Atrai River system based on log-transformed data. (A) Male and (B) Female: regression equation shaded area representing the 95% confidence interval.

3.3.3 Fulton's condition factor (K)

The mean Fulton's condition factor (K) of *C. striata* exhibited clear seasonal variations, reflecting fluctuations in the overall body condition of both male and female individuals

(Figure 3.3: A and B). In male fish, individuals demonstrated moderate to good body condition throughout the year, with relatively better condition observed during June, October, and November, as indicated by significantly higher K values ($p < 0.05$). These months likely represent periods of enhanced physiological status, possibly due to improved environmental conditions or recovery following spawning. In contrast, males showed poorer somatic condition during April and August, suggesting possible energy depletion linked to reproductive activity or reduced food intake. During the remaining months (January, February, March, June, July, and December), males exhibited intermediate body condition, not significantly different from the periods of either improved or reduced condition (Figure 3.3: A). Infemales, body condition varied more distinctly across seasons. Fish exhibited notably robust body condition during September, indicative of peak physiological health. The condition remained relatively favorable in October and November, though not significantly different from September (Figure 3.3: B). In contrast, April to July was characterized by reduced body condition, suggesting a period of energy mobilization for reproductive development or environmental stress. Intermediate body condition was noted in the months of January, February, March, and August.

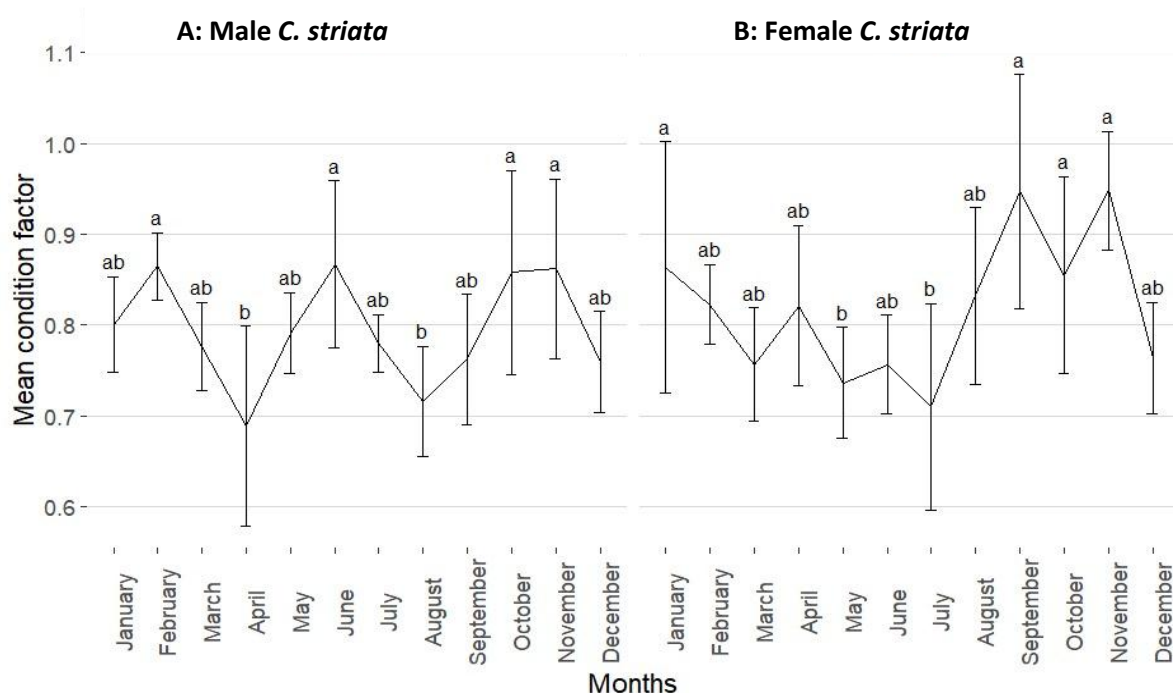


Figure 3.3 Monthly variations in the mean Fulton's condition factor (K) of male (A) and female (B) *C. striata*. Different letters above bars (a, ab, b) indicate statistically significant differences between months ($p < 0.05$) based on Tukey's HSD post hoc test. Error bars represent standard error of the mean ($\pm$ SE).

3.3.4 Sex Ratio and Gonadosomatic Index of *C. striata*

The sex ratio of male to female *C. striata* and the monthly variation in gonadosomatic index (GSI) were analyzed across wet (March–September) and dry (October–February) seasons from the Atrai River and its tributaries. The monthly sex ratio ranged from 1:0.6 to 1:4.5, with male-biased ratios ($\geq 1:1$) observed during most months. A significant deviation from the expected 1:1 ratio was recorded in January (1:4.5; χ^2 , $p < 0.05$), indicating a strong female bias. Sex ratios approaching parity were observed in August, September, and December (Table 3.2).

Female GSI exhibited distinct seasonal variation, peaking in May (7.12 ± 2.29) and June (4.23 ± 0.44), indicating the onset of peak reproductive activity during the wet season (Table 3.2). A gradual decline in female GSI was observed from July onward, reaching the

lowest values in October (0.19 ± 0.05), November (0.27 ± 0.20), and February (0.14 ± 0.14), suggesting gonadal quiescence during the dry season. Male GSI values remained comparatively low throughout the year, with the highest mean recorded in May (1.06 ± 0.71). A consistent decreasing trend was observed during the dry months, with the lowest values in November (0.11 ± 0.03) and February (0.09 ± 0.07). Higher female GSI values and relatively balanced sex ratios during the wet season reflect intensified reproductive activity, while reduced gonadal development and variable sex ratios during the dry season indicate a period of reproductive inactivity.

Table 3. 2. Monthly variation in gonado-somatic index (GSI), sex ratio, and Chi-square test results of *C. striata* in the Atrai River.

Month	Sample size		Gonado-somatic index (GSI)		Sex Ratio (Male: Female)	Chi-square (χ^2) of sex ratio	Season
	Male	Female	Male	Female			
April	12	8	0.5 ± 0.25	3.52 ± 0.73	1: 0.67	0.371	Wet
May	10	6	1.06 ± 0.71	7.12 ± 2.29	1:0.6	0.317	Wet
June	8	10	0.4 ± 0.05	4.23 ± 0.44	1: 1.25	0.637	Wet
July	11	8	0.19 ± 0.1	1.5 ± 0.89	1:0.67	0.491	Wet
August	10	10	0.55 ± 0.38	1.7 ± 1.38	1:1	1.000	Wet
September	12	12	0.18 ± 0.07	0.32 ± 0.16	1:1	1.000	Wet
October	12	8	0.22 ± 0.06	0.19 ± 0.05	1:0.67	0.371	Dry
November	12	8	0.11 ± 0.03	0.27 ± 0.2	1:0.67	0.371	Dry
December	8	12	0.24 ± 0.15	0.25 ± 0.11	1:1.5	0.371	Dry
January	4	18	0.42 ± 0.32	0.33 ± 0.14	1:4.5	0.0028*	Dry
February	8	12	0.09 ± 0.07	0.14 ± 0.14	1:1.5	0.371	Dry
March	12	8	0.12 ± 0.08	0.72 ± 0.32	1:0.67	0.371	Dry

Values are presented as mean $\pm$ standard deviation (SD). Wet season includes March to September; dry season includes October to February. Asterisks (*) indicate significant deviation

3.3.5 Fecundity of *C. striata* and its Relationship with Total Length, Body Weight & GSI

Monthly variations in gonad development and fecundity of female *C. striata* are presented in Table 3.3 and illustrated the relationship of fecundity with body weight, total length and GSI in Figure 3. 4. A clear seasonal pattern was observed, with reproductive indices peaking during the pre-monsoon and early monsoon months. The gonad weight exhibited a marked increase in May (35.99 ± 4.99 g), followed by a steep decline in the subsequent months, reaching the lowest in July (8.58 ± 2.36 g). A similar trend was observed in fecundity, which was highest in May ($33,833.49 \pm 13,533.20$ eggs) and lowest in July ($3,891.50 \pm 1,701.25$ eggs). These findings suggest that May represents the peak spawning period for the species. Gonad length was relatively consistent between April (10.25 ± 0.63 cm), May (10.18 ± 1.87 cm), and June (9.87 ± 0.24 cm), before decreasing significantly in July (5.81 ± 0.56 cm). Overall, these temporal changes in gonadal metrics suggest that *C. striata* undergoes gonadal maturation leading up to May and early June, after which spawning activity likely occurs, as indicated by the sharp decline in reproductive parameters in the later months.

Table 3.3 Monthly variation of gonad length, weight, fecundity and ova diameter of female *C. striata*

Month	Gonad Weight (g)	Gonad Length (cm)	Ova Diameter (μ m)	Fecundity
April	22.71 ± 4.47	10.25 ± 0.63	12.44 ± 1.4	14252.5 ± 3588.13
May	35.99 ± 4.99	10.18 ± 1.87	12.69 ± 0.99	33833.49 ± 13533.2
June	20.02 ± 1.67	9.87 ± 0.24	12.92 ± 0.46	9681.98 ± 888.72
July	8.58 ± 2.36	5.81 ± 0.56	11.92 ± 0.41	3891.5 ± 1701.25
August	14.1 ± 10.17	7.96 ± 2.19	11.38 ± 0.42	4863.4 ± 4286.36

The monthly variation in mean fecundity and its relationships with total length, body weight, and gonadosomatic index (GSI) of female *C. striata* are depicted in Figure 3.4 (A–D). The temporal variation in mean fecundity, which peaked significantly in May (mean $\pm$ SE: $\sim 33,833 \pm 13,533$ eggs), followed by a sharp decline in June ($\sim 9,681 \pm 889$ eggs) and a progressive decrease in subsequent months is shown in Figure 3.4 (A). This trend indicates a clear seasonal spawning peak during the early monsoon period. Figure 3.4 (B) presents the logarithmic relationship between fecundity and total length. Although a positive trend was observed, the relationship was weak and statistically non-significant ($R^2 = 0.10$), indicating that total length alone may not be a strong predictor of fecundity in *C. striata*. Figure 3.4 (C) illustrates the log-transformed relationship between fecundity and body weight. The regression line suggests a positive correlation ($R^2 = 0.18$), but the explanatory power of body weight on fecundity variation remained low, suggesting additional factors influencing fecundity. Figure 3.4 (D) demonstrates the strongest relationship observed, with fecundity being significantly and positively correlated with the gonadosomatic index ($R^2 = 0.74$). This strong association suggests that GSI is a robust predictor of fecundity in *C. striata*, reflecting its close link to reproductive maturity.

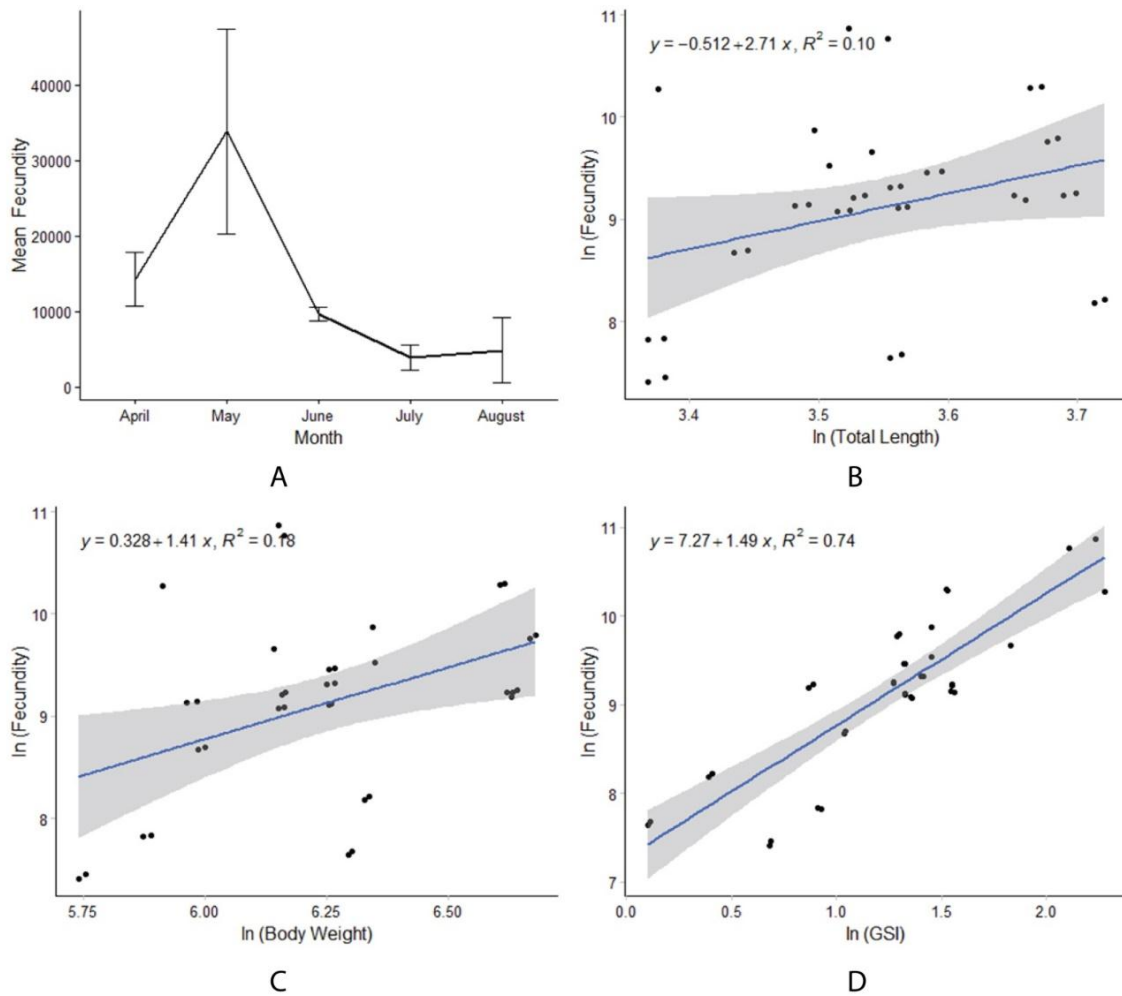


Figure 3.4. Monthly variation of fecundity (A); Relationship between fecundity and total length (B), body weight (C) and GSI (D).

3.3.6. Size at First Sexual Maturity (L_{50}) of *C. striata*

The size at which 50% of *C. striata* individuals reached sexual maturity (L_{50}) was estimated separately for males and females using logistic regression analysis. The maturity curve for males (Figure 3.5: A) showed that the L_{50} was 25.8 cm in total length, with a relatively low model fit ($R^2 = 0.09$), indicating considerable variability in the size at maturity among males. In contrast, the female maturity curve (Figure 3.5: B) showed an L_{50} of 34.2 cm, with a higher model fit ($R^2 = 0.31$), suggesting a more consistent size at maturity among

females. These findings indicate a notable sexual dimorphism in the size at first maturity, with females requiring a larger body size to reach reproductive maturity compared to males.

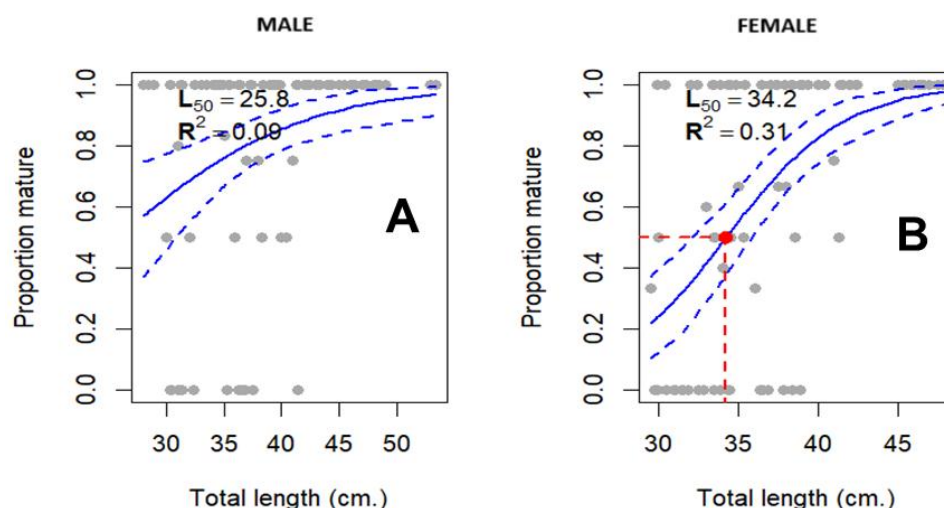


Figure 3.5 Logistic regression curves showing the proportion of mature individuals of *C. striata* in relation to total length (cm) for (A) males and (B) females. The solid blue lines represent the fitted maturity curves, while the dashed lines indicate the 95% confidence intervals. Grey points represent individual observations.

3.3.7. Monthly Variation in Mean Ova Diameter of *C. striata*

The figure 3.6 illustrates the monthly changes in the mean ova diameter of *Channa striata* females from April to August. The mean ova diameter increased progressively from April (approximately 12.4 μm) to a peak in June (around 12.9 μm), followed by a sharp decline in July and August, reaching the lowest mean diameter in August (approximately 11.7 μm). Error bars represent standard deviations, indicating considerable variability in ova size among individual fish within each month, particularly during the early part of the spawning season. The increase in ova diameter through June suggests active vitellogenesis, culminating in peak gonadal maturity during this month. The subsequent decrease in July and August likely reflects post-spawning regression and resorption of residual oocytes.

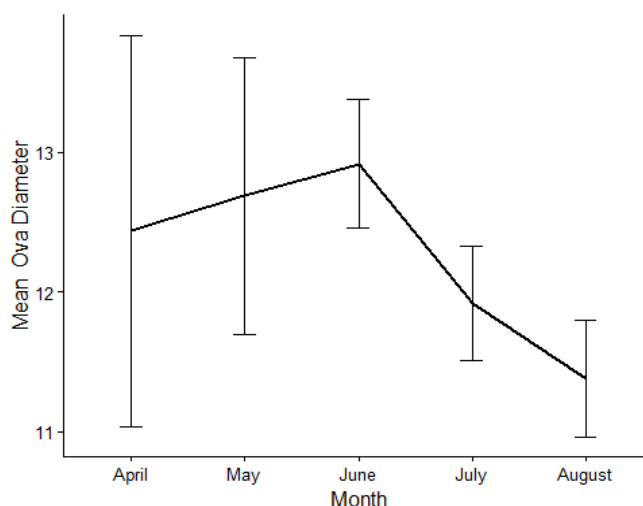


Figure 3.6 Monthly variation in mean ova diameter (μm) of female *C. striata* collected from the Atrai River and its tributaries during the wet season (April to August). Error bars represent standard deviations.

3.3.8. Morphological and Histological Characterization of Gonadal Maturation Stages in *C. striata*

3.3.8.1. Gonadal Maturation Stages in Male *C. striata*

The gonadal maturation process in male *C. striata* was categorized into five distinct stages based on macroscopic morphology and histological characteristics (Figure 3.7: A-H and Appendix 5). During the immature stage (Stage I), the testes appeared small, transparent, and rich in underdeveloped spermatogonia (Figure 3.7A). Histologically, this stage was characterized by the presence of spermatogonia as the predominant germ cells, located near the basement membrane of the testis lobules (Figure 3.7G). Primary spermatocytes were also observed surrounding the testicular wall, indicating that spermatogenesis was at its initial phase. In the maturing stage (Stage II), the testes increased in size and became whitish with visible capillary networks (Figure 3.7B). Histological sections showed active spermatogenesis, where spermatogonia underwent meiotic division to form spermatocytes, which appeared spherical with centrally located nuclei. Spermatids began to appear in

greater numbers, and the presence of both primary and secondary spermatocytes suggested ongoing maturation of germ cells. The mature stage (Stage III) was marked by a significant increase in testis size and weight, with a slightly pinkish coloration (Figure 3.7C). Macroscopically, the testes were firm and filled with spermatozoa. Microscopically, a high concentration of spermatozoa and spermatids was evident, while the number of primary spermatocytes had declined. This indicated that the testes were at peak reproductive readiness, with most germ cells having advanced to later stages of spermatogenesis. At the spawning stage (Stage IV), the testes turned dark and opaque, became soft and limp, and released sperm easily upon gentle pressure (Figure 3.7D). Blood vessels were more prominent, and the majority of spermatozoa were seen migrating toward the lobule lumen (Figure 3.7H). Spermiogenesis was complete, and functional male gametes were present in abundance, indicating full reproductive activity. Finally, in the spent stage (Stage V), the testes were shrunken, whitish, and reduced in size (Figure 3.7E). Histological sections revealed empty lumens within the lobules, and secondary spermatozoa were absent, confirming the cessation of active spermatogenesis and depletion of sperm reserves. Overall, the combined morphological and histological observations clearly delineate the progression of testicular development and functional changes during the reproductive cycle of male *C. striata*.

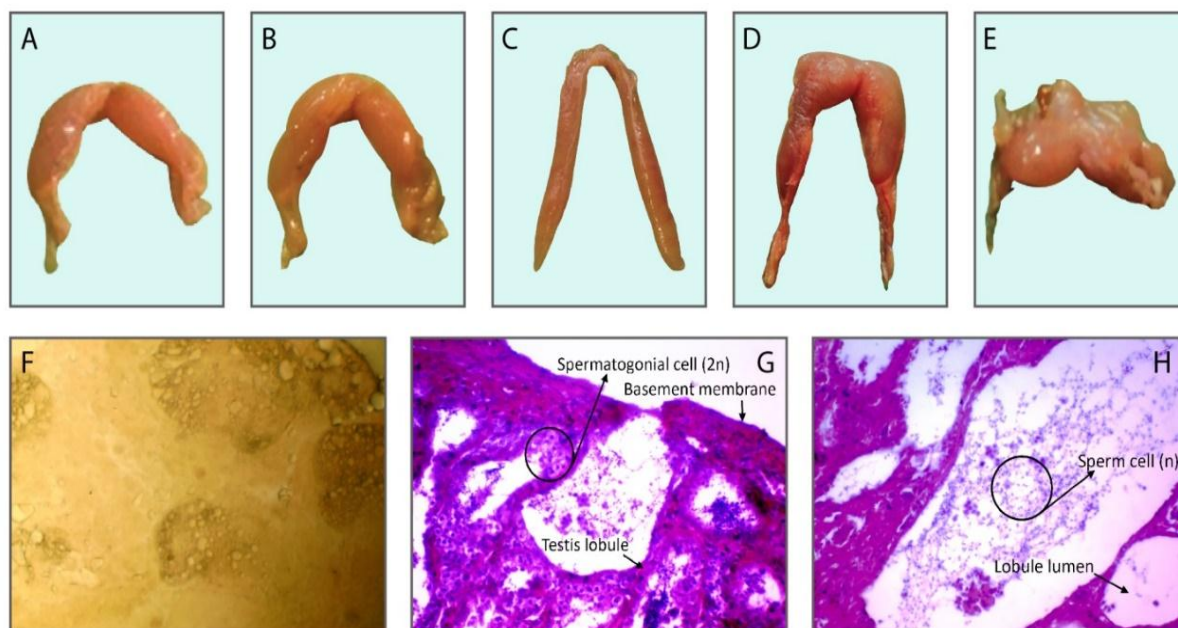


Figure 3.7 Gonad maturation stages of male *C. striata*; (A) Immature stage showing small, transparent testes; (B) Maturing stage with enlarged, whitish testes and visible capillaries; (C) Mature stage showing pinkish, enlarged testes with abundant spermatozoa; (D) Spawning stage where testes are dark, opaque, soft, and secrete sperm under gentle pressure; (E) Spent stage showing shrunk, whitish testes with reduced sperm content; (F) Acetocarmine-stained testicular smear highlighting cellular components; (G–H) Histological observations at 40× magnification illustrating different stages of spermatogenesis, including spermatogonia, spermatocytes, spermatids, and spermatozoa.

3.3.8.2 Gonadal Maturation Stages in Female *C. striata*

The gonadal development of female *C. striata* was classified into five distinct stages based on macroscopic morphology and histological characteristics, as illustrated in Figure 3.8 (A–H) and Appendix 6. At the immature stage (Stage I), the ovaries were slender, translucent, and thread-like in appearance (Figure 3.8A), indicating the onset of gonadal development. No distinct oocytes were visible macroscopically. Microscopic examination revealed the presence of primary growth oocytes (PGmn) with prominent nucleoli and minimal

cytoplasmic development (Figure 3.8H). During the maturing stage (Stage II), the ovaries increased in size and became more opaque, with visible developing oocytes (Figure 3.8 B). Histological analysis showed active vitellogenesis, with oil droplets forming in primary growth oocytes (PGod) and the appearance of early secondary growth oocytes (SGe) (Figure 3.8 G), marking the transition toward reproductive maturity. In the mature stage (Stage III), the ovaries were fully developed, yellowish in color, and densely packed with large, yolk-laden oocytes (Figure 3.8 C). Histologically, this stage was characterized by the dominance of late secondary growth oocytes (SGI) and fully grown secondary oocytes (SGfg), indicating that the fish were reproductively ready (Figure 3.8 G). At the spawning stage (Stage IV), the ovaries appeared large, swollen, and turgid, containing hydrated oocytes ready for ovulation (Figure 3.8 D). The ovarian lumen was densely filled with mature oocytes, and structural changes such as increased vascularization and thinning of follicular walls were evident under microscopic and histological examination. Following ovulation, the spent stage (Stage V) was marked by flaccid and bloodshot ovaries with a significant reduction in volume and oocyte content (Figure 3.8 E). Histological sections displayed post-ovulatory follicles and empty spaces previously occupied by mature oocytes, indicating gonadal regression and the end of the reproductive cycle (Figure 3.8 G). The progressive changes in ovarian morphology and histology clearly define the five maturation stages in female *C. striata*, providing a reliable framework for assessing reproductive status.

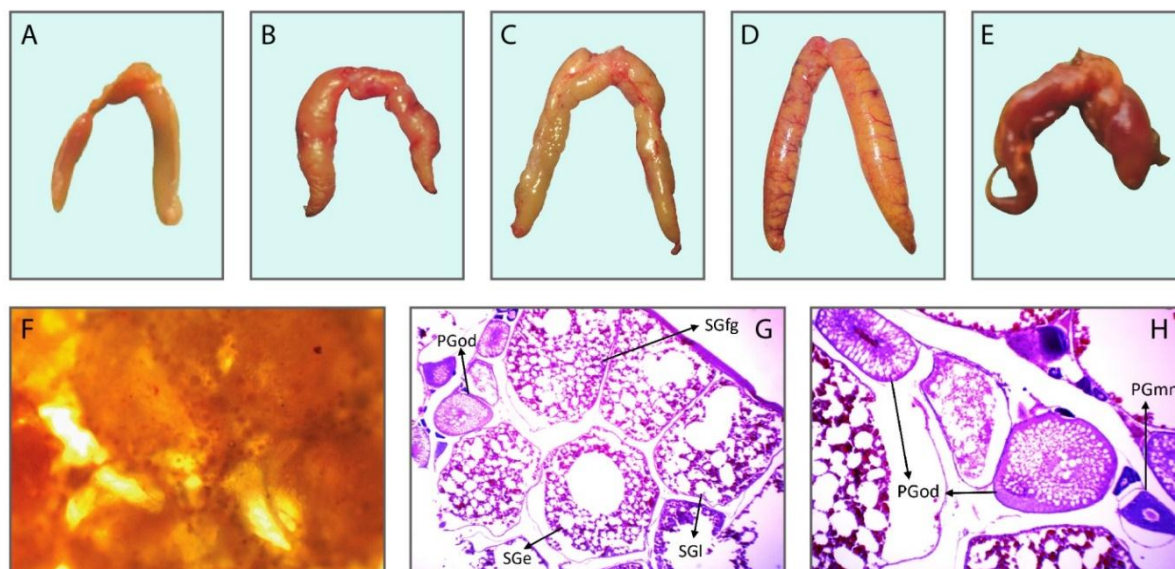


Figure 3. 8 Gonad developmental stages of female *C. striata*. (A) Immature stage showing translucent, thread-like ovaries; (B) Maturing stage with enlarged ovaries containing visible oocytes; (C) Mature stage showing fully developed, yellowish ovaries filled with yolky oocytes; (D) Spawning stage characterized by large, swollen ovaries with hydrated oocytes; (E) Spent stage where ovaries appear flaccid and bloodshot, with reduced oocyte content; (F) Acetocarmine-stained ovarian smear highlighting oocyte stages and yolk vesicles; (G) Histological observation under 10× magnification showing different stages of oocyte development: PGmn (primary growth with multiple nucleoli), PGod (primary growth with oil droplets), SGe (early secondary growth), SGI (late secondary growth), SGfg (fully grown secondary oocytes); (H) Histological observation under 40× magnification further detailing oocyte maturation and structural characteristics.

3.3.9 The monthly distribution of gonadal maturity stages in *C. striata*

The monthly distribution of gonadal maturity stages in *C. striata* revealed clear temporal patterns in reproductive development for both sexes (Fig 3.9). In males, immature individuals (salmon pink) dominated from January to March, while (olive green) began appearing in March and peaked in April and May. The proportion of and spawning males

(sky blue) increased significantly during June and July, indicating peak reproductive activity. Spent males (orchid purple) were most abundant from August to October, followed by a resurgence of immature gonads in November and December, marking the onset of a new reproductive cycle. A comparable pattern was observed in females. Immature ovaries were most prevalent from January to March, while the occurrence of maturing ovaries increased significantly during March and April. The highest proportions of mature and spawning females were recorded between May and July, identifying this as the peak breeding period. From August to October, the spent stage dominated, signifying the end of active spawning, followed by a resurgence of immature and maturing stages from November onwards. These observations collectively indicate a well-defined seasonal reproductive cycle in *C. striata*, with peak spawning activity occurring between May and July. This seasonal trend has important implications for fishery management and conservation strategies, especially in regulating fishing pressure during critical breeding months.

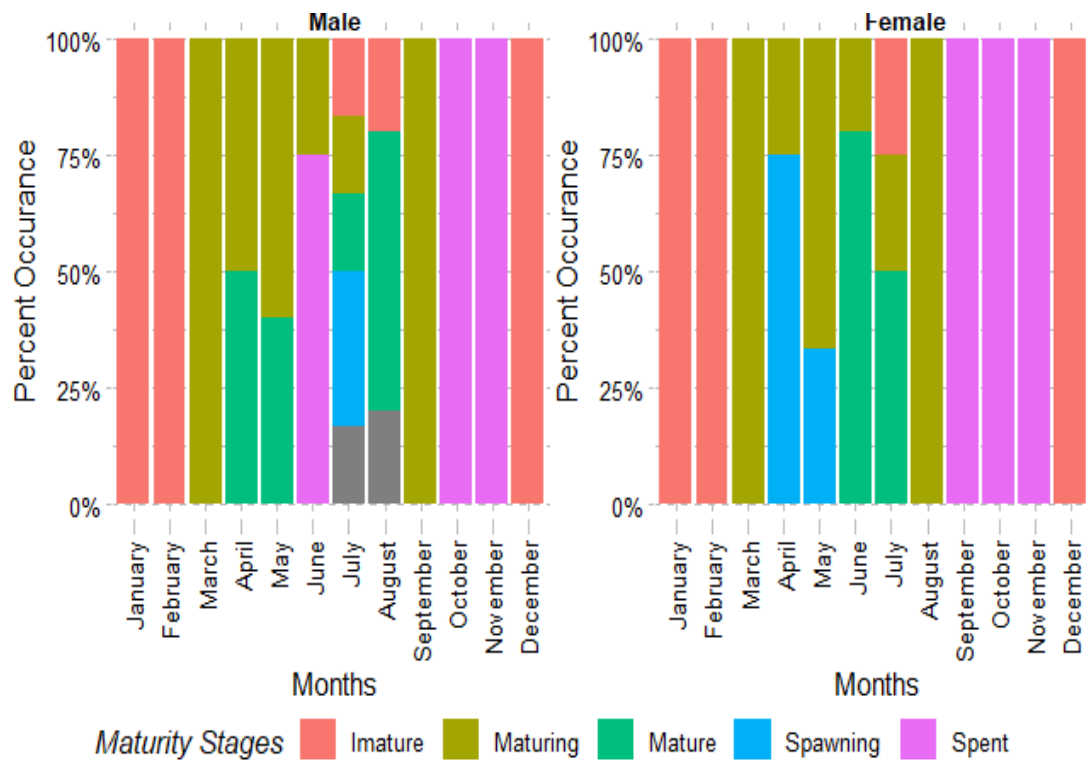


Figure 3.9: Monthly percentage occurrence of gonadal maturity stages in male (left) and female (right) *Channa striata* from the Atrai River. Each color represents a distinct maturity stage: Immature (salmon pink), Maturing (olive green), Mature (teal green), Spawning (sky blue), and Spent (orchid purple).

3.4 Discussion

3.4.1 Seasonal growth patterns and length–weight relationships

The observed monthly variations in total length and body weight of *C. striata* reflect the combined influence of seasonal environmental fluctuations and reproductive physiology. Peak body weights were recorded in males during September and in females during April, indicating sex-specific energy allocation strategies. In females, increased body weight in April aligns with gonadal development preceding spawning, consistent with earlier reports on snakehead reproductive cycles (Gustiano et al., 2020; Laboni et al., 2024; Awal et al.,

2025). Conversely, the reduced size and weight observed during the winter months (December–February) likely result from post-spawning energy depletion and reduced feeding activity in response to lower temperatures, which suppress metabolic rates (Toke and Aung, 2020). Hossain et al., 2021). These seasonal growth patterns reinforce previous findings that tropical freshwater fishes, including *C. striata*, shift energy allocation between somatic and reproductive processes in response to environmental cues such as photoperiod, temperature, and food availability (Henderson et al., 1996; Post and Parkinson, 2001; McBride et al., 2015; Djumanto et al., 2020).

The length–weight relationship showed a strong positive correlation ($R^2 > 0.88$), though both sexes exhibited negative allometric growth (males: $b = 2.72$; females: $b = 2.61$), where weight gain is proportionally less than length increase. This growth pattern is typical in reproductively active or mature fish and reflects a diversion of energy toward gonadal development rather than somatic growth (Froese, 2006; Le Cren, 1951). Environmental factors such as food limitation and deteriorated water quality during or after breeding may also contribute. Similar negative allometry has been reported in *C. striata* populations across South and Southeast Asia (Ahmadi, 2018; Asyhari, 2022; Ansyari, 2022). One-sample t-tests confirmed that these b values significantly deviate from isometric growth ($b = 3$), highlighting the physiological trade-offs associated with seasonal reproductive cycles.

Fulton's condition factor (K) also exhibited marked seasonal fluctuations, serving as an indicator of physiological well-being. Higher K values in the post-monsoon and pre-winter months (June, September–November) especially in males (October–November) and females (September)—coincide with increased feeding and post-spawning recovery. These findings are aligned with other freshwater systems (Chakraborty et al., 2017). In contrast, lower K values between April and July likely reflect energy reallocation toward gonadal

development (Encina and Granado-Lorencio 1997; McBride et al., 2015; Volkoff and London, 2018). Improved post-monsoon condition is likely driven by nutrient influxes from flooding, underscoring the ecological importance of seasonal hydrological events in fish growth and condition (Paudel et al., 2024; Lianthuamluaia et al., 2024).

3.4.2 Sex ratio and gonadosomatic index (GSI)

Seasonal variation in sex ratio and GSI revealed a distinct reproductive cycle synchronized with hydrological changes. A female-biased sex ratio was observed in January, with more balanced ratios in August, September, and December. These fluctuations may result from sex-specific migratory behavior, habitat preferences during spawning, or sampling biases influenced by reproductive activity. Moreover, sex reversal, sex determination, and gonad development are frequently not fixed characteristics and can be very vulnerable to extrinsic/environmental influences including temperature, pH, and pollutants (Devlin et al., 2002). Sex reversal can happen once or multiple times during ontogeny.

Female GSI peaked significantly in May and June during the wet season, coinciding with rising water levels that favor successful spawning and larval survival (Karnatak et al., 2018; Parker et al., 2018; Khan et al., 2022). This pattern confirms the synchronization of *C. striata* reproduction with hydrological cycles, a common trait among tropical floodplain species (Lowe-McConnell, 1987; Tondato et al., 2018; Kumar et al., 2022). While male GSI remained generally low, a slight increase in May suggests synchronized spawning readiness with females, reflecting sexual dimorphism in gonadal development (Nagahama, 1983; Paray et al., 2014).

Recent studies in Bangladesh and neighboring regions have confirmed similar patterns of peak reproductive activity aligned with monsoon-driven environmental shifts (Ferdous et al., 2015; Djumanto et al., 2020; Laboni et al., 2024). Understanding this reproductive timing

is crucial for implementing seasonal fishing bans and aligning hatchery seed production with natural breeding cycles.

3.4.3 Fecundity and correlation with morphometric and reproductive parameters

Fecundity and gonadal development exhibited significant seasonal variation. The highest fecundity ($33,833 \pm 13,533$ eggs) and gonad weight (35.99 ± 4.99 g) were recorded in May, coinciding with the peak spawning period. Gonad length and ova diameter also peaked between April and June, supporting the seasonal reproductive strategy of *C. striata*. These results are consistent with prior studies from Bangladesh, India, and Southeast Asia that report peak spawning during the pre-monsoon and early monsoon periods, guided by favorable cues such as rising temperatures and longer photoperiods (Ferdous et al., 2015; Al Mahmud et al., 2016; Senguttuvan and Sivakumar, 2016;; Kumar et al., 2022). Correlation analysis showed that fecundity had a weak, non-significant relationship with total length ($R^2 = 0.10$), a modest relationship with body weight ($R^2 = 0.18$), but a strong correlation with GSI ($R^2 = 0.74$), confirming GSI as a reliable indicator of reproductive maturity. Similar trends have been documented across different populations (Ferdous et al., 2015; Al Mahmud et al., 2016; Laboni et al., 2024), though strength of correlations may vary with population structure and sampling method. These insights reinforce the use of GSI in broodstock selection for aquaculture hatchery operations.

3.4.4 Size at First Sexual Maturity (L_{50})

The size at first maturity (L_{50}) showed clear sexual dimorphism: males matured at 25.8 cm, while females matured at a larger size of 34.2 cm. This pattern is consistent with teleost reproductive strategies, where larger females typically exhibit higher fecundity (Beverton and Holt, 1959; Beverton, 1992; Mian et al., 2017). The relatively low model fit in males

($R^2 = 0.09$) suggests high variability in maturity size, potentially driven by environmental or nutritional factors (Ricker, 1975; West, 1990). In contrast, a higher R^2 in females (0.31) suggests stronger selection pressure for size-related fecundity. Reports from other regions confirm similar patterns of sexual dimorphism in maturity size (Ferdous et al., 2015; Hasan et al., 2021). From a management perspective, implementing sex-specific size limits, particularly for larger females can enhance reproductive success and population sustainability (Pauly, 1984; Kumar et al., 2022).

3.4.5 Monthly variation in ova diameter

Ova diameter increased from 12.4 μm in April to a peak of 12.9 μm in June, indicating vitellogenic growth and peak ovarian maturity. The subsequent decline in July and August (down to 11.7 μm) reflects post-spawning ovarian regression and oocyte resorption typical physiological processes following spawning (Karnatak et al., 2018; Kumar et al., 2021; Bagra et al., 2024). Early-season variability in ova diameter may suggest asynchronous oocyte development or multiple spawning events, a reproductive strategy that enhances recruitment success in variable environments (Bobe & Labbé, 2010; Amzad et al., 2015; Bobe, 2015; Damle et al., 2023). These findings align with studies on *C. striata* from both Bangladesh and other Asian countries (Hossain 2013; Ferdous et al., 2015; Tresnati and Fachruddin, 2019; Tun and Myint, 2021). Monitoring ova diameter alongside GSI offers a reliable method for determining reproductive phase and supports more precise management of breeding cycles.

3.4.6 Morphological and histological staging of gonads

Five distinct gonadal maturation stages were identified in both sexes, aligning with established reproductive classifications for teleosts (West, 1990; Brown-Peterson et al.,

2011). Male gonads progressed from immature spermatogonia-dominated stages to mature phases with abundant spermatozoa, ending in post-spawning regression. Female gonads followed a developmental sequence from early-stage oocytes to fully hydrated, ovulation-ready eggs, with post-ovulatory regression characterized by atretic follicles (Bagenal & Braum, 1978; Karnatak et al., 2018; Kumar et al., 2021; Bagra et al., 2024).

Histological analysis confirmed macroscopic staging accuracy and revealed that peak spawning occurred between May and July. These reproductive patterns are closely linked to environmental cues such as rainfall, photoperiod, and water temperature (Zohar and Mylonas, 2001; Cabrita et al., 2008). Histological validation is critical in ensuring accurate maturity staging for both fishery assessments and aquaculture operations (Brown-Peterson et al., 2011; West, 1990).

3.5 Conclusion

This study provides comprehensive insights into the seasonal growth patterns and reproductive biology of *C. striata* in the drought-impacted Atrai River, supporting both research hypotheses. The observed negative allometric growth (with b values < 3) and seasonal fluctuations in Fulton's condition factor (K) reflect the influence of environmental variability and reproductive energy allocation. Reproductive activity was strongly synchronized with seasonal hydrological changes, with peak gonadosomatic index (GSI), fecundity, and ova diameter observed between April and June, coinciding with the pre-monsoon and monsoon periods. These findings confirm that seasonal environmental cues play a key role in regulating growth and reproduction, thereby validating Hypothesis 1.

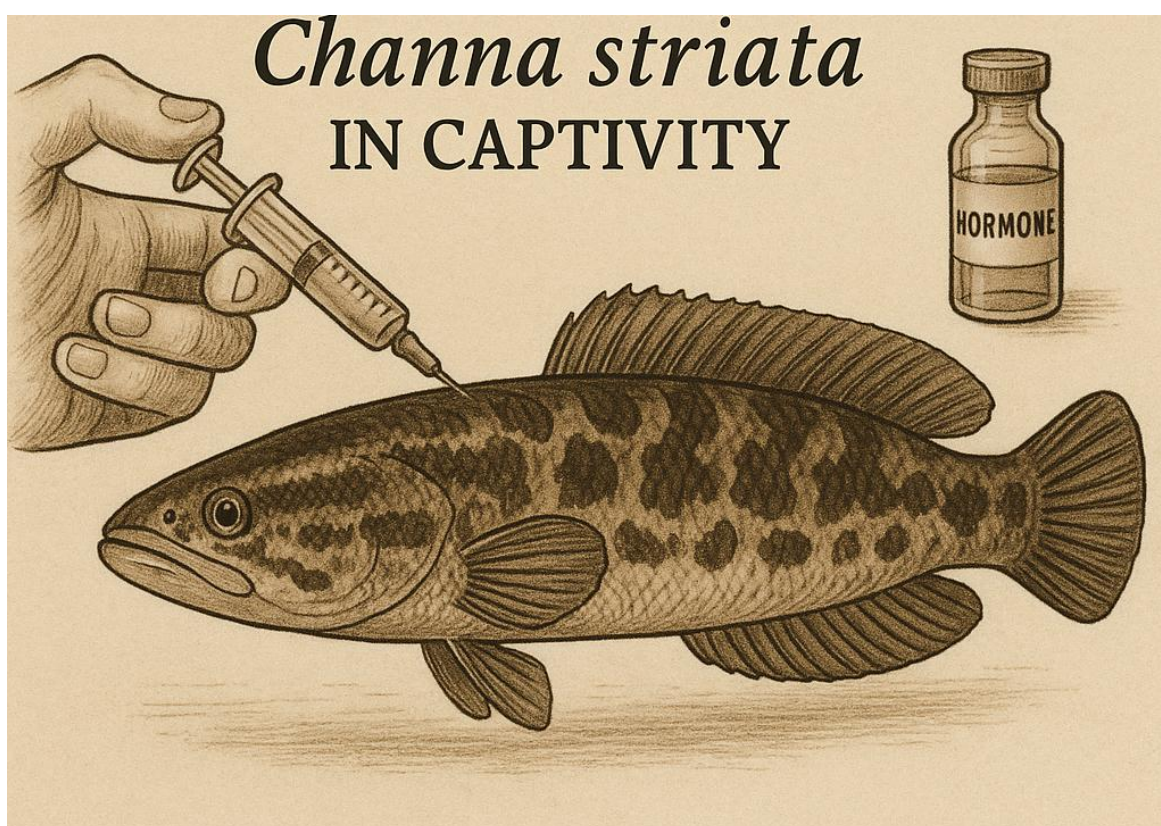
The study also demonstrates that wild *C. striata* populations in the Atrai River possess strong reproductive potential. High fecundity ($33,833 \pm 13,533$ eggs), well-defined gonadal

maturation stages confirmed by histology, and a strong correlation between GSI and fecundity ($R^2 = 0.74$) underscore their suitability as broodstock for induced breeding. The sexual dimorphism in size at first maturity further reinforces the reproductive robustness of this population. These outcomes confirm hypothesis 2 and highlight the value of wild stocks for supporting climate-resilient aquaculture.

From a management perspective, enforcing seasonal fishing bans during the identified peak spawning period (April–June), establishing minimum catch size regulations, and protecting critical spawning habitats are essential for sustaining natural populations. The reproductive benchmarks established in this study can also guide hatchery operations and broodstock selection, contributing to the development of a reliable seed supply system. Finally, engaging local communities through education and participatory management will be crucial to the conservation and sustainable use of *C. striata* in the Atrai River basin.

Chapter 4

Hormonal Dose Optimization for Induced Breeding of *Channa striata* in Captivity



Abstract

Present study describes the spawning induction of striped Snakehead, *Channa striata* using carp pituitary extract (CPE) and LH-RH agonist i.e. Buserelin (Glp-His-Trp-Ser-Tyr-Ser-tBu-Leu-Arg-Pro-NHEt). Total four treatments were designed under both hormones trail and treated as control group, T₁, T₂ and T₃ with three replications of each treatment. While breeders under all hormone treatments showed spawning performances, no spawning performance was observed in control group. Latency time after hormonal treatment was lowest (20-24 hrs) in case of CPE than Buserelin (25-30 hrs). Regarding to CPE, spawning, fertilization and hatching rate were higher with the increasing doses of CPE in different treatments. The highest mean $\pm$ Standard deviation spawning, fertilization and hatching rate were $85.60 \pm 8.58\%$, $79.38 \pm 4.89\%$ and $64.33 \pm 6.60\%$ respectively in T₃ where dose of CPE was 80 mg kg^{-1} . Similarly, in case of Buserelin hormone highest spawning rate was found in T₃ (80.61 ± 5.59) where dose of Buserelin was $0.80 \mu\text{g kg}^{-1}$ body weight. Fertilization rate was on the level 48.57 ± 5.99 , 70.62 ± 5.33 and 90.32 ± 4.79 respectively for T₁, T₂ and T₃. Whilst, hatching rate was found 20.81 ± 4.91 , 37.11 ± 4.50 and 61.33 ± 6.61 in T₁, T₂ and T₃ treatments respectively. However, T₃ exhibited best performance regarding spawning, fertilization and hatching rate which were significantly higher than other two treatments. The current study revealed that spawning induction using carp pituitary extract and Buserelin is effective and might be useful for artificial breeding of *C. striata*. Regarding to dose application i.e. 80 mg kg^{-1} of CPE and $0.80 \mu\text{g kg}^{-1}$ of Buserelin may be successfully applied to ovulation stimulation of *C. striata*.

4.1 Introduction

Along with other species of *Channa* genus, *Channa striata* (Bloch, 1793) is an economically significant freshwater indigenous fish species that is abundant naturally in large quantities throughout Bangladesh (Rahman, 2005; Ahmed & Akhter, 2008). The *C. striata* is locally investigated as Shoal fish that belongs to the Channidae family (Nelson et al., 2016). This species is also found in other Southeast Asian countries in addition to Bangladesh (Talwer and Jhingran, 1991; Rainboth, 1996; Rahman, 2005). The flesh of *C. striata* is considered as high nutritional and economic value (Suwandi et al., 2014). For this reason, it is thought to be one of the most promising freshwater species for intensive and semi-intensive cultures in Southeast Asia such as Thailand, Vietnam, Philippines, Cambodia, India, Malaysia, Pakistan and Indonesia (Mollah et al., 2009; Rahman et al., 2012; Wee, 1982; Rahman et al. 2013). Naturally, during the rainy season, snakehead fish spawn once a year. It is quite difficult for this species to spontaneously breed in an aquaculture setting. However, because to human activities including overfishing, dam construction and the degradation of spawning sites, the species' natural populations have gradually declined. As a result, the species was added to the IUCN red list of threatened species in 2015 (IUCN, 2015).

Despite this, the lack of proper induced breeding technique and poor feeding management in cultured ponds remain the main obstacles to successful domesticating of wild broodstock in captivity. Those ultimately prevent *C. striata* from being successfully cultured in Bangladesh. The production of snakehead fish in Bangladesh has primarily relied on wild capture until now. Attempts to culture the fish have not yet reached a satisfactory level due to the lack of large-scale hatchery production of fish fingerlings (Mollah et al., 2009). The mass seed production and culture at commercial scale are still not established in Bangladesh.

Carp pituitary extracts (CPE) is a natural hormone that has been reported to use as spawning induction in many species with mixed results (Kujawa et al., 2022; Souza et al., 2018). To date, some previous study where induced breeding of *C. striata* with CPE has been reported in other countries. For instance, Parmeshwaran and Murugesan attempted captive breeding of striped snakehead in India when they injected carp pituitary extracts to both male and female with varying success (Parmeshwaran et al. 1976). Although CPE has successful result in spawning induction in many species but it has also some disadvantages. Some common disadvantages of CPE have been reported are in some situation females do not respond to CPE treatments (Zadmajid et al., 2017). To overcome these problems many synthetic hormones are used adjunct to the natural hormone now-a- days. Synthetic hormone, that induce the release of gonadotropins (in pituitary) such as GnRH may be considered more appropriate for use in rheophilic species (Martins et al., 2017; Souza et al., 2018). In addition, GnRH is simple to synthesis, stable in storage, and has a constant hormonal content among preparations.

Considering the effectiveness of GnRH, a GnRH analogue, buserelin acetate which is peptide with a serine inserted in dextroposition to replace the sixth peptide ([D-Ser(tBu)⁶, Pro⁹ -NEt]-GnRH_a) are used in spawning induction of many fish (Arabaci et al., 2004). There were several reports of successful induced breeding when fish are injected with Buserelin acetate (Paulino et al., 2011; Arabaci et al., 2004). There, however, are no studies on the efficacy of CPE and buserelin in induction of reproduction *C. striata* in Bangladesh. Hence, in this study following two hypothesis were tested:

Hypothesis 1 (H₁): Carp pituitary extract (CPE) and buserelin acetate are both effective in inducing reproduction in *Channa striata* under captive conditions.

Hypothesis (H₂): Buserelin acetate induces more favourable reproductive outcomes (higher fertilization and hatching rates, lower mortality) than carp pituitary extract (CPE) in *Channa striata*.

The purpose of this study, therefore, was to evaluate the efficacy of CPE and buserelin in induction of reproduction in *C. striata* and also comparing the response when there was imposing of different doses of the conventional CPE-treatment and synthetic hormone buserelin.

4.2 Materials and Methods

4.2.1 Research station

Induced breeding study was conducted in the hatchery unit of the freshwater station of Bangladesh Fisheries Research Institute, Mymensingh (24.7214° N, 90.4212° E), Bangladesh during the breeding period. The embryonic development stage of *C. striata* was studied in the laboratory of Bangladesh Fisheries Research Institute.

4.2.2 Fish collection and broodstock management

A total number of 250 fish species were collected from different natural sources such as river, *Haor* and *beel* of the districts Netrokona and Kishoriganj. Collected brood fishes were transported to the hatchery of the BFRI, Mymensingh by using oxygenated polyethene bags and plastic drums. After being transported, fish were acclimatized for 30 minutes and then kept in the brood rearing pond. The brood fish had initial average mean length and weight ($\pm$ SD) of 30.5 $\pm$ 4.4 cm and 428.3 $\pm$ 109.18g, respectively. The fishes were fed on live fish fry and a mixture of fish meal, rice bran, wheat flour, and vitamin.

4.2.3 Broodstock selection and maturity assessment

For artificial breeding trials mature and healthy brood fish were arbitrarily caught by cast net from the rearing pond in the morning between 8:00- 9:00 among the day of breeding and

then they were weighed. From a total of 72 gravid fish, 48 males and 24 females were selected based on maturation criteria and they were immediately transferred to the cisterns for two separate breeding trials. The morphological characteristics of the brooders were used to evaluate their maturity (Figure 4.1). The maturity was assessed by looking at its belly, the colour and shape of its gonadal orifice and the distance between the beginnings of its anal fin and orifice. The mature females were recognized by their protruding abdomen as well as oval genital papillae. Compared to female fish, male fish were generally smaller. However, mature males were identified by their elongated genital papillae and also flattened abdomens. Selected mature female and male fishes were taken and weighed. After that they were kept in completely different cisterns for 5-6 hours for conditioning prior to being treated with inducing agents. A continuous flow of water was supplied for conditioning to ensure that there was adequate oxygen for fish to survive through the porous PVC pipe on the upper portion of the cistern.

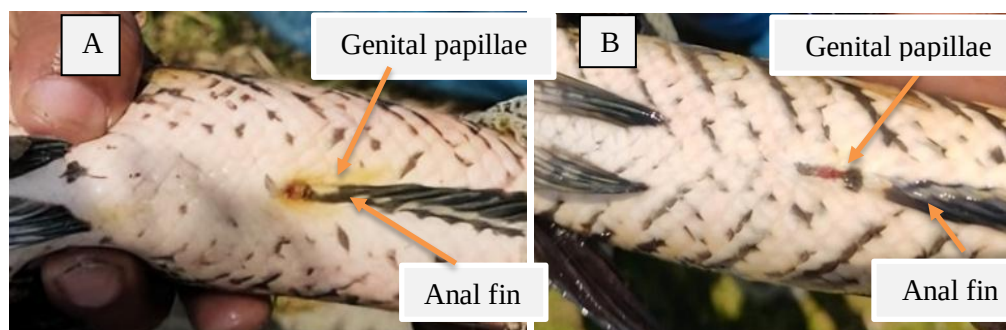


Figure 4.1 Characteristics of matured *C. striata*; A) Female (Oval genital papillae which is very close to the anal fin), B) Male (Elongated genital papilla which is slightly away from the anal fin).

4.2.4 Hormonal treatment

Commercially available carp pituitary extract CPE (United Agro Fish, Jessore, Bangladesh) was first meticulously weighed in a digital balance. The following method was used to calculate the weight on the basis of the total body weight of all the fish according to previous literature (Mollah et al., 2008).

$$W_t = W_b \times P_t$$

Where W_t is the weight in mg of the necessary amount of CPE, W_b stands for the total body weight measured in kg of all the fish that will be injected, and P_t is the rate measured in mg of CPE to be injected per kg body weight that will be used for a given treatment.

The following formula was used to determine the total amount of the extract needed:

$$\text{Volume of extract (ml)} = W_t \times 1.0 \text{ (Mollah et al., 2008)}$$

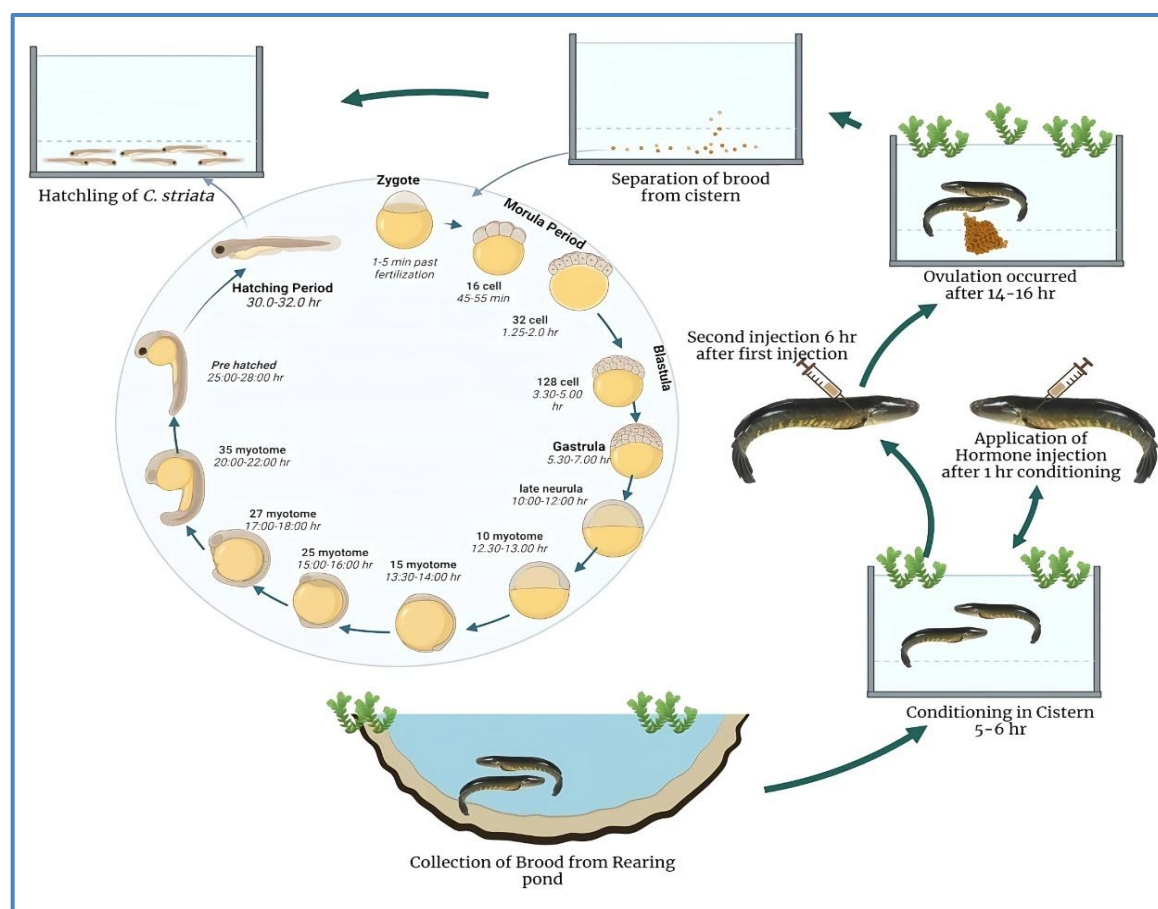
Where W_t is the CPE weight in mg and 1.0 is the amount of extract to be injected per kg of fish. A little amount of distilled water was applied to homogenize the weighed CPE before being used to meticulously transfer the homogenate to a centrifuge tube to assure proper transfer. The mixture was then centrifuged at 6000 rpm for 3-4 minutes. A vial was filled to a predetermined volume with distilled water after the supernatant solution was transferred there. Regarding Buserelin, a total of 14.4 µg Buserelin powder (Buserelin Acetate manufactured by SAMARTH life science PVT. LTD., India) and 3 tablet of Domperidone (10mg) were taken in a small Petri dish and then added 32 ml distilled water to make a solution and grounded well (Souza F.N. et al., 2018).

For both the hormones brooders were divided into four groups consisting of one female and two male into each group and considered as four treatments as control group, T_1 , T_2 , and T_3 with three replications of each treatments. In control group fish are injected with sodium chloride (0.9 %). To estimate the required doses of inducing agent weight of the brooders were taken. The various doses of hormones and the experimental design are mentioned in the Table 4. 1 and Figure 4.2.

Table 4.1 Dosage of hormones applied for *C. striata* induced breeding.

Treatments	Hormones	Number of fish	Sex ratio F: M	Doses*		GSI	Fecundity
				1 st d	2 nd d		
Control	CPE (mg kg ⁻¹)	F: 3 M:6	1:2	-	-	5.12±0.49	18626±592
T ₁		F:3 M:6	1:2	20	40		
T ₂		F:3 M:6	1:2	10	20		
T ₃		F:3 M:6	1:2	25	45		
		F:3 M:6	1:2	10	25		
		F:3 M:6	1:2	30	50		
		F:3 M:6	1:2	15	25		
Control		F: 3 M:6	1:2	-	-		
	Buserelin (µg kg ⁻¹)	F: 3 M:6	1:2	-	-		
T ₁		F:3 M:6	1:2	0.40	-		
		F:3 M:6	1:2	0.20	-		
T ₂		F:3 M:6	1:2	0.60	-		
		F:3 M:6	1:2	0.30	-		
T ₃		F:3 M:6	1:2	0.80	-		
		F:3 M:6	1:2	0.40	-		

* Time interval (hrs.) between the doses: 06

Figure 4.2 Hormonal treatment process of *C. striata* induced breeding.

4.2.5 Breeding parameter assessment

After three to four hours of injection fishes were checked to observe any kind of spawning behavior were shown. Further after six-hours of injection, all females were checked for ovulatory response in every three hours while having their abdomens gently massaged. After fertilization eggs were then transferred into hatching trays very carefully to avoid any damage and contamination. The number of eggs released into each hapa were estimated using the method adapted from Legendrea et al., (2000) and Unuma et al., (2004). To ensure the environmental condition for hatching process a continuous water flow was maintained. Water quality parameter of the breeding hapa were measured by following the standard procedure (APHA, 2005) and mentioned in table 2. There was no appreciable difference ($p < 0.05$) in the mean values of the water quality parameters among the treatments (Table 4. 2).

Table 4.2 Water quality parameters in cistern during the induced breeding of *C. striata*

Parameters	Observed values	
	Mean $\pm$ SD	Range
Water temperature ($^{\circ}$ C)	28.22 $\pm$ 1.32	27.26 - 29.22
pH	7.22 $\pm$ 0.12	7.05 - 7.54
DO (mg/l)	6.32 $\pm$ 0.38	5.88 - 6.77
Total ammonia (mg/l)	0.015 $\pm$ 0.01	0.01-0.04
Total alkalinity (mg/l)	57.82 $\pm$ 1.21	56.77-60.01
Total hardness	82.15 $\pm$ 0.89	81.45-83.44

Ovulation rate, fertilization rate, hatching rate were estimated using following formula (Galo et al., 2015):

$$\text{Ovulation rate (\%)} = 100 \times \frac{\text{Number of fish ovulated}}{\text{Total number of fish injected}}$$

$$\text{Fertilization rate (\%)} = 100 \times \frac{\text{Number of fertilized eggs}}{\text{Total number of fertilized and unfertilized eggs}}$$

$$\text{Hatching rate (\%)} = 100 \times \frac{\text{Number of eggs hatched}}{\text{Total number of fertilized eggs}}$$

4.2.7. Embryonic development

In order to determine the timing of development, eggs were placed onto 9-cm petri dishes and samples were taken every 5 minutes throughout the cleavage and gastrula stage and every 30 minutes during the segmentation to hatching period. Using previously published literature on the embryological development of freshwater fish species as a reference, the stages of embryonic development were recorded from images captured with a digital camera (Digital Sight DS-L2, Nikon, Japan) connected to a compound microscope (Eclipse E200-LED, Nikon, Japan) (Marimuthu and Haniffa, 2007). The seven stages of embryogenesis that comprise embryonic development include the beginning development, cleavage, morula, blastula, gastrula, segmentation, and hatching phases. These stages are distinguished according to their morphological traits.

4.2.8 Statistical analysis

Values were expressed as means $\pm$ standard deviation (SD). All data were tabulated before proceeding through a one-way analysis of variance at the 95% confidence level to see whether the treatment had a significant effect statistically. Differences were regarded as statistically significant at $P < 0.05$. Tables, graphs and descriptive statistics were performed using SPSS version 21 (SPSS Inc., IBM International Business Machines), Graph Pad prism 6 version (GraphPad Software, Inc., California) and Microsoft Excel 2016.

4.3 Results

4.3.1 Latency time and breeding behavior of broodstock

It was observed that both hormones at varying degrees of intensity may encourage fish to mate and deposit eggs, while in the control group, no breeding was seen. Regarding latency time, the duration between the CPE hormone administration and ovulation varied from 20-

24 hrs in different treatments while 25-30 hrs were observed in case of Buserelin injection (Table 4.3). Latency temperature varied from 27-29°C.

The breeding behaviour of *C. striata* was noticed after 4-5 hours of both hormones administration (Figure 4.3). The male fish vicinity of the female moves to the side and starts to tremble. The male participation in the mating was observed more aggressively and actively. An elaborate courtship behaviour preceded the mating. The male continued to pursue the female and often encouraged her to move which started 8-10 hours after the hormones injection. The most interesting observation across all treatments was that male was much more actively engaged in courtship and spawning. It was further also noticed that the male touched the female's snout and vent more frequently.



Figure 4.3 Spawning behaviour of *C. striata* after injection of CPE and Buserelin hormone

During mating, the pair tilted slightly to one side, helping to bring the anal regions together. The spawning behavior was carefully monitored until the gametes were released. During courtship the male tilts his body near the female's genital papilla and the bloodstocks congregate near each other probably resulting in spawning followed by external fertilization. The unfertilized eggs were separated by the characteristics of white color, non-floating and were dispersed throughout the tank. In the contrary, fertilized eggs were yellow, floated, and visible.

Table 4. 3 Response of *C. striata* fish to different doses of CPE and Buserelin

Hormone	Treatments	Body weight		Spawning period (hrs.)	Spawning rate (%)	Fertilization rate (%)	Incubation period (hrs.)	Hatching rate (%)
		Female	Male					
CPE	Control	-	-	20-24	-	-	-	-
	T ₁	527.2±82.2 ^{ab}	446.9±57.5 ^{ab}		34.42±4.50 ^c	41.50±5.89 ^c	30-32	39.66±4.91 ^c
	T ₂	511.5±56.5 ^b	432.3±43.1 ^b		57.61±7.72 ^b	55.68±5.71 ^b		53.11±4.57 ^b
	T ₃	551.3±61.3 ^a	466.4±67.4 ^a		85.60±8.58 ^a	79.38±4.89 ^a		64.33±6.60 ^a
Buserelin	Control	-	-	25-30	-	-	-	-
	T ₁	537.2±82.2	466.9±57.5		29.51±4.11 ^c	48.57±5.99 ^c	32-36	20.81±4.91 ^c
	T ₂	519.5±56.5	452.3±43.1		45.90±4.71 ^b	70.62±5.33 ^b		37.11±4.50 ^b
	T ₃	566.3±61.3	476.4±67.4		80.61±5.59 ^a	90.32±4.79 ^a		61.33±6.61 ^a

Different superscripts (a, b, c) in each column indicates significant difference among the treatments are significantly different ($P < 0.05$).

4.3.2 Spawning rate

The results of the induced breeding of *C. striata* using various CPE and Buserelin doses were shown in Table 3. In all treatments, spawning took place between 20 and 30 hours. It proved the effective ness of the CPE extract and synthetic hormones Buserelin in the instance of *C. striata*'s induced breeding. No spawning was observed in control group. In case of CPE hormone lowest spawning rate was observed in T₁ (34.42±4.50) at the dose of 60mg kg⁻¹body weight whereas the highest rate was observed in T₃ (85.60±8.58 at a dose 80mg kg⁻¹body weight (Table 4.3). The ANOVA test results showed a significant difference among the treatments with treatment T₃ being significantly ($p < 0.05$) higher than treatments T₁ and T₂ (Figure 4.4). Similarly in case of Buserelin hormone highest spawning rate was found in T₃ (80.61±5.59) when applied dose of 0.80µg kg⁻¹body weight (Table 4.3) which was significantly higher than other two treatments (Figure 4.5).

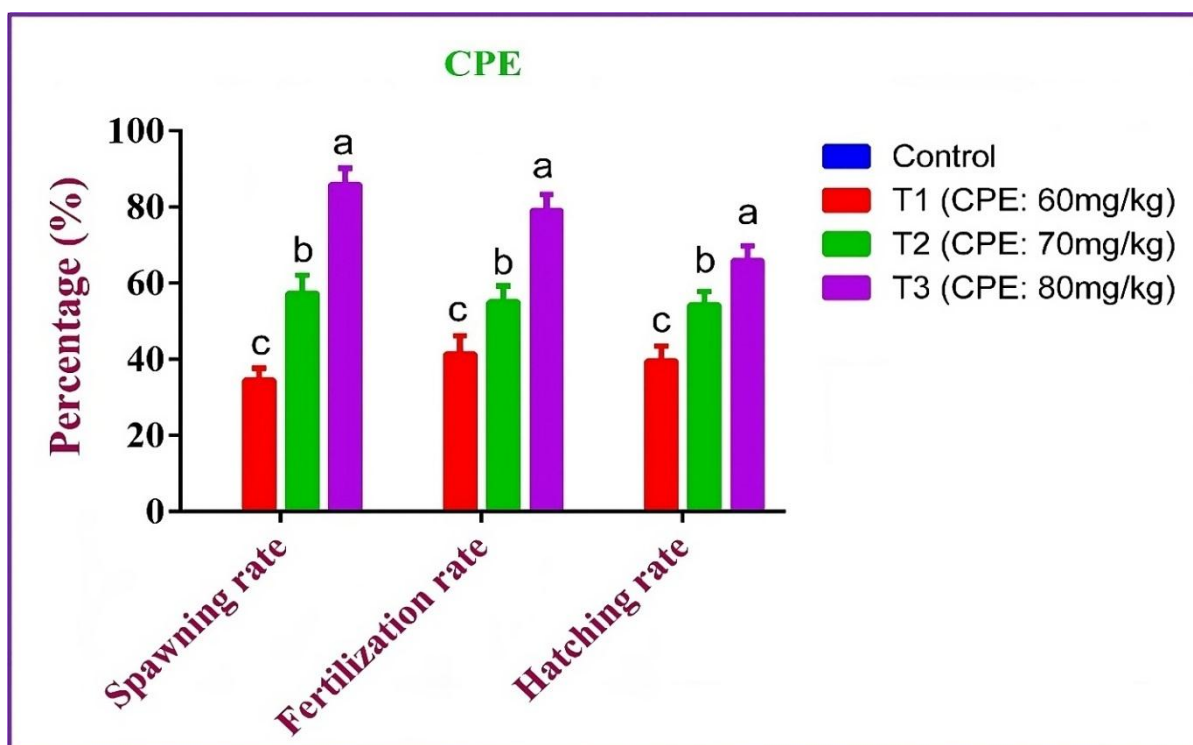


Figure 4.4 Spawning rate, fertilization rate and hatching rate (%) of *C. striata* using CPE. Values are means (±standard deviation). Different letters indicate significant differences ($P < 0.05$) by Duncan's Multiple Range Test (DMRT).

4.3.3 Fertilization rate

In case of CPE hormone, the mean fertilization rate was recorded as 41.50 ± 5.89 , 55.68 ± 5.71 and 79.38 ± 4.89 in treatments T_1 , T_2 and T_3 respectively, (Table 4.3). The highest fertilization rate 79.3% was found in the T_3 whereas the lowest, 41.5%, was found in the T_1 . The ANOVA testing results showed that the three doses significantly differed from one another with treatment T_3 being significantly ($p < 0.05$) higher than T_1 and T_2 (Figure 4.4). Whereas, in case of Buserelin, fertilization rate was observed 48.57 ± 5.99 , 70.62 ± 5.33 and 90.32 ± 4.79 in T_1 , T_2 , and T_3 respectively (Table 4.3). The three doses considerably differed from one another according to the ANOVA testing results, with treatment T_3 being significantly ($p < 0.05$) greater than T_1 , and T_2 (Figure 4.5).

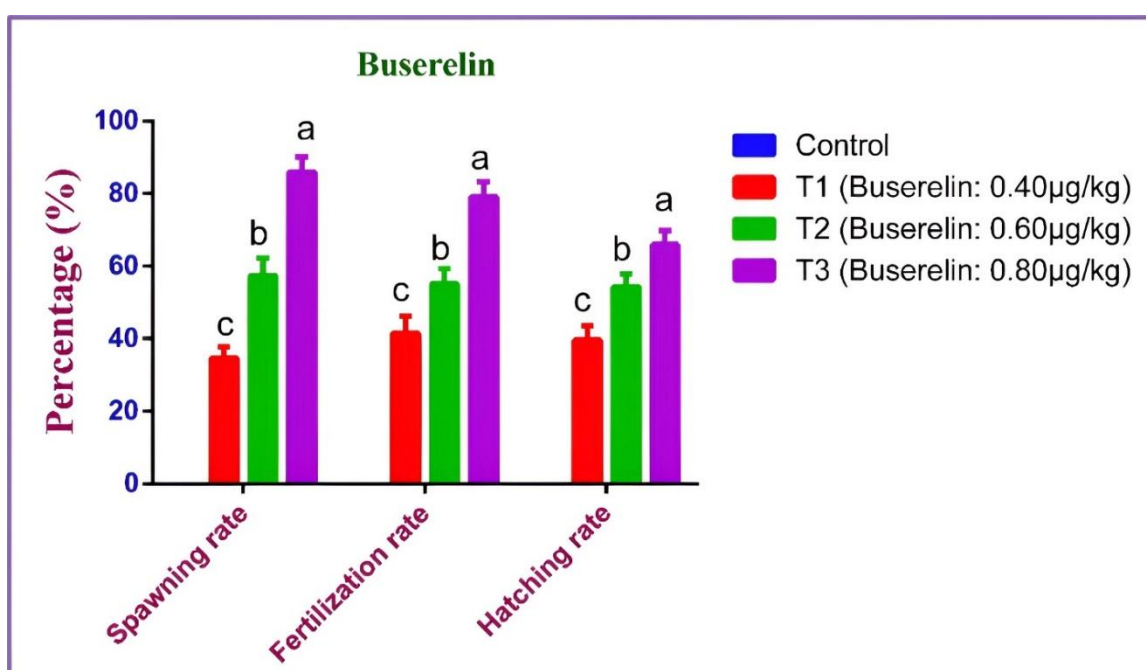


Figure 4.5 Spawning rate, fertilization rate and hatching rate (%) of *C. striata* using Buserelin. Values are means ($\pm$ standard deviation). Different letters indicate significant differences ($P < 0.05$) by Duncan's Multiple Range Test (DMRT).

4.3.4 Hatching rate

In T_1 , T_2 and T_3 treatment, the percent hatching rate was observed to be 39.66 ± 4.91 , 53.11 ± 4.57 and 64.33 ± 6.60 respectively in case of CPE hormone (Table 4.3). ANOVA test

revealed a statistically significant difference among the doses. It was noticed that the T_3 hatching rate was significantly ($p < 0.05$) higher than the T_1 and T_2 (Figure 4.4). On the other hand, in case of Buserelin, hatching rate was found 20.81 ± 4.91 , 37.11 ± 4.50 and 61.33 ± 6.61 in T_1 , T_2 , and T_3 treatment respectively (Table 4.3). Figure 4.5 showing the significant difference of hatching rate in three treatments with the highest rate ($p < 0.05$) in T_3 .

4.3.5 Embryonic development

Stage wise embryonic development was shown in Figure 4.6 (A to N). Fertilized eggs of *C. striata* were yellow in color, transparent, non-adhesive, free-floating and spherical. Lengths ranged from 1.15 mm to 1.30 mm diameters of the oil globule and the yolk sac (Figure 4.6 A). The first cleavage that divides the blastodisc into two blastomeres occurred within 20-25 min after fertilization (Figure 4.6A). The third cleavage followed within 25min and within another 20 min the 8 celled stage was reached (Figure 4.6A). The six-cleavage followed within 1.0-1.20 hours when the 64 celled stage was reached (Figure 4.6 B). As successive cleavages occurred the blastomeres decreased in size and the morula stage was reached between 1.25-2.0 hrs after fertilization (Figure 4.6 C). At about 3.30-5.0 hrs of fertilization the embryo attained blastula stage (Figure 4.6 D). After that at about 5.30-7.0 hrs of fertilization the embryo attained gastrula stage (Figure 4.6E). The late neurula was observed 10.0-12.0 hrs after fertilization where eight myotomes are formed (Figure 4.6 F). The cephalic region has expanded even more revealing a primitive brain, 10 myotomes, a tail that reaches the yolk's outer edge and a heart that is only minimally developed after 12.30-13.0 hours after fertilization (Figure 4.6 G). After 13.30-14.0 hours after fertilization, embryo's length continues to increase about 15 myotomes are visible, the heart has formed and the rudimentary Kupfer's vesicles' eye lenses have developed (Figure 4.6 H). The embryo's length continues to increase; about 22 myotomes are visible; the heart has fully formed and started to pump blood. The eyes are still in their primitive state after 14.30-15.0

hours after fertilization (Figure 4.6 I). After 15.0-16.0 hours of fertilization, the embryo's length continues to increase and now covers about 80% of the yolk's surface. The heart is well-developed and has started to pump blood but its heart rate is very slow. Around 25 myotomes are visible. Melanophores are visible over the yolk sac and the tail is narrow (Figure 4.6 J). The notochord is laid and the heart's primitive structure is visible. Melanophores is also appeared in this stage. After 17.0-18.0 hrs of fertilization an additional 85% of the yolk surface is covered by the embryo's length which also widens the cephalic and tail regions. The heart also beats quickly and rhythmically and the number of melanophores has increased (Figure 4.6 K). The embryo completely covers the yolk, the cephalic region has grown larger and the tail has begun to separate from the yolk. The number of melanophores has increased and there are about 35 myotomes observable after 20.0-22.0 hours of fertilization (Figure 4.6 L). After 25.0-28.0 hrs of fertilization the entire yolk was surrounded by the embryo. Heartbeats are 170-180 beats per minute, olfactory pits and concretions on the auditory vesicles have formed, blood is colorless and Kupfer's vesicles have vanished. A few melanophores have appeared throughout the body and the embryo frequently twitches (Figure 4.6 M). The embryo begins to hatch after 30.0-32.0 hours after fertilization (Figure 4.6 N).

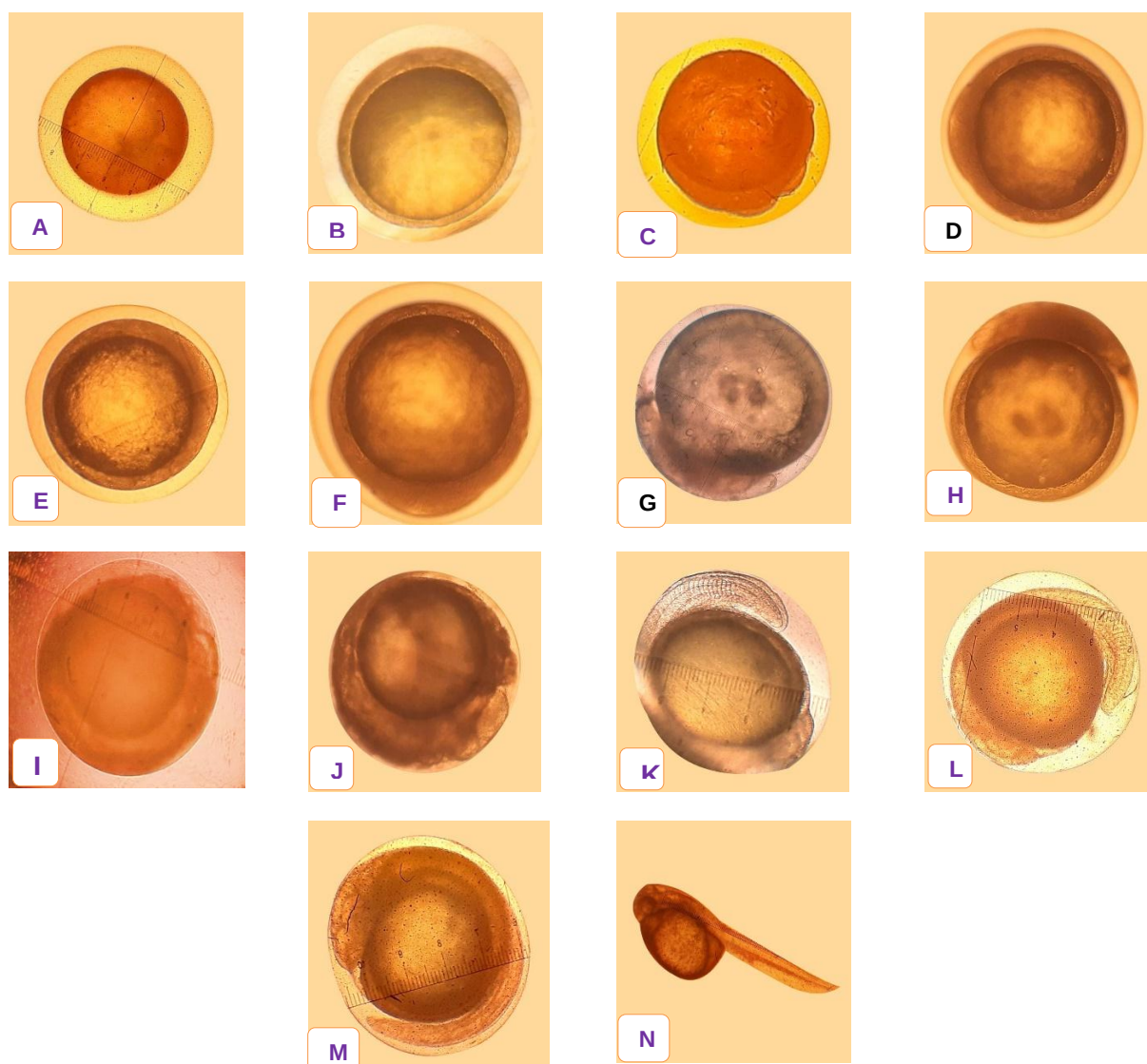


Figure 4.6 Stages of embryonic development in the CPE hormone treatment (T_1 , T_2 and T_3); A) Fertilized eggs, B) 16 cell stage, C) Morula, D) Blastula, E) Gastrula, F) Late neurula, G) 10 myotomes, H) 15 myotomes, I) 22 myotomes, J) 25 myotomes, K) 27 myotomes, L) 35 myotomes, M) Pre hatched embryo and N) Hatching.

4.4 Discussion

In our present study utilization of CPE and Buserelin were found to be effective in stimulating the spawning process of *C. striata*. The spawning, fertilization and hatching rates of the various treatments varied significantly based on the dose variation of both hormones. It was shown that higher dosages produced greater results in the treatments for

both hormones. This result provides the efficacy of these two hormones in successful induced breeding in *C. striata*.

With regards to pituitary extract (CPE), there are several reports confirming the effects of CPE extract for induction of ovulation and spawning in wild mature *Channa* species in different countries (Dayal et al.2013, Haniffa et al., 2000 and Paray et al., 2013).In current study, the reproductive behavior of *C. striata* was monitored from 4 hours immediately after CPE hormone injection until spawning and up to 30-32 hours. This observation was corroborated with the findings of Dayal et al.(2013) who studied reproductive behavior of *C. striata* and noticed that mating behavior began 1-2 days before spawning. However, since the fish spawn at night, it was difficult to observe reproductive behavior during the actual spawning period. The spherical, non-adhesive, free-floating and yellow fertilized eggs were separable with white, unattached and spread unfertilized eggs reported by previous study (Roy et al., 2016).

In our study latency time was similar to that reported by Roy et al., (2016), that spawning began 12-14 hours after CPE hormone administration, although the doses of CPE used were different. Haniffa et al.,(2000) administered CPE hormone for *C. striata* and found complete ovulation within 22-23 h which is close to our finding. Fish treated with higher doses of CPE (80 mg kg^{-1}) of female body weight in the experiment showed the maximum spawning efficiency, while fish treated with 60 mg kg^{-1} showed the lowest spawning percentage. Fishes were reared together under control, so discrepancies in spawning rates may be due to different CPE dosages. With regards to Buserelin, spawning was observed after 25-30 hrs post injection. However induced spawning of *channa* species with Buserelin are not found yet but success of this hormone has noticed in case of other fish species such as Indian major carp (Arabaci et al.,2004;Konzen et al.,2020; Nuurai et al.,

2010). In this experiment, administration of CPE and Busereline hormone at different doses significantly affected spawning rate ($p < 0.05$) but not spawning duration.

Treatment T₃ had a significantly ($p < 0.05$) higher fertilization rate than the other treatments in both hormonal trail. The fertilization rates measured in this experiment were higher compared to the other studies conducted by Roy et al., (2016), Paray et al., (2012) and Hossain et al., (2008), where they observed a fertilization rate of 40-62%, 58.83% and 40-80%, respectively. Similar results were obtained by Yulintine et al., (2017) who found fertilization rate in snakehead fish to be approximately 57.4-69.4%. The fertilization rate of present study was also similar to that of Hossain et al., (2008), averaging 58.8% but however, Marimuthu and Haniffa, (2011) found a different result for *C. punctata* with fertilization rates from 78.3-97.6%. Similarly, the fertilization rate found in this study was comparable to that found by Dayal et al., (2013), but significantly lower than that in fish injected with Ovaprim (Haniffa et al., (2000)). This may be due to the tendency to spawn in small groups where there is more mixing of gametes, these fish had higher fertilization rates (Dayal et al., 2013).

Incubation period of both hormonal treatments did not vary significantly. In T₃ treatment the maximum hatching rate were observed ($p < 0.05$) in both hormone when doses was increased suggesting that higher dose is more effective. After hatching, the hatchlings are black, but soon turn yellow and swim in schools. The hatchling passively swims near the surface of the water as soon as it hatches. It was observed that the hatching rate in this experiment was relatively similar to the hatching rate in studies by Roy et al., (2016) and Paray et al., (2012) who found the hatching rate of 60-65% and 55-80%, respectively. Hossain et al., (2008) found an average hatchability of 62.33% but with a range of 45-82%. Hatching was occurred within 30-32 hours at 27-29°C for all treatments in this experiment, which is significantly different from the results of Dayal et al., (2013) and Haniffa et al.,

(2000). They found the duration were from 22–26 hrs and 21–23 hrs when using synthetic hormones and Ovaprim but Haniffa et al., (2000) did not report water temperature in their study. The hatching time of *C. punctata* was 24 hours at 28°C (Banerji, 1974) is also inconsistent with our recent findings. In another study Yulintine et al., (2017) found opposite results where the rate of hatching of *C. striata* at 27-29°C was estimated about 23.5-28.2%. Moreover, the hatchability of *C. striata* in the current study was significantly lower than that of *C. punctata* with a hatchability of ~90.6% observed by Marimuthu and Haniffa, (2011). Parameshwaran and Murugesan, 1976) reported a significant difference in hatch rate from 28 to 100% using CPE hormone. A higher hatching rate of 92-98% was reported when using WOVA FH for induced breeding (Pati et al., 2004).

However, to notice the post effect after hormone injection, fish were monitored for two days following spawning to see whether the effects of hormones had caused any deaths or other physical issues in the fish. It was found that mortality occurred 33.30% and 67.11% in T₂ and T₃ in case of CPE hormone. On the other hand no mortality was found in T₁ of CPE hormone and in all treatments of Busereline hormone. Therefore, it may be presumed that Buserelin will be more suitable than CPE hormone for hormonal induction of *C. striata* considering mortality and any other post effects.

The results on embryonic development of *C. striata* are consistent with the results reported by Marimuthu & Haniffa (2007), and Parameshwaran and Marugesan, (1976). The first cleavage in the present observation begins about 20-25 minutes after fertilization, the 16-cell stage between 45-55 minutes, the morula stage between 1.25-2.0 hours, the morula stage between 1.25-2.0 hours, blastocyst stage between 3.30 and 5.0 hours and gastric phase between 5.30 and 7.0 hours. In this experiment, the overall egg length was found to be 3.28 mm, and the yolk length and width were 1.13 mm and 0.77 mm respectively. Our results are consistent with those of Rawat et al., (2020), where the total egg length was 3.14 mm, while

the yolk length and width were 1.20 mm and 0.80 mm, respectively. Marimuthu & Haniffa, (2007) found slightly different observations where the size of newly hatched larvae was 3.4 mm. Rawat et al., (2020) observed that the larvae initiated vigorous swimming behavior at 48.45 ± 0.55 hph which is more or less similar to our current findings. After reaching a length of 6.34 mm, the hatchlings began to move freely while actively feeding on live food, completely consuming the yolk sac at 73.35 ± 0.40 hph. Marimuthu & Haniffa, (2007) found that the length of the *C. striata* yolk sac-absorbing larvae was shorter (5.8 mm) than that of the current experiment, but otherwise the above improvements were more or less similar. Similar results with larvae absorbed into the yolk sac were noted by Rawat et al., (2020), where newly hatched hatchlings reached a length of 6.20 mm and the yolk sac was completely consumed at 77.15 ± 0.30 hph.

The breeding capacity of *C. striata* is expected to be stimulated and enhanced by the induction agent. Recent research shows that CPE and Buserelin can induce spawning of *C. striata*, enabling mass production of high quality fish seed. Induced spawning of *C. striata* is expected to reduce the dependence on natural spawning for harvesting juvenile fish and potentially provide timely access to fish spawn for farmers and hatcheries.

4.5 Conclusion

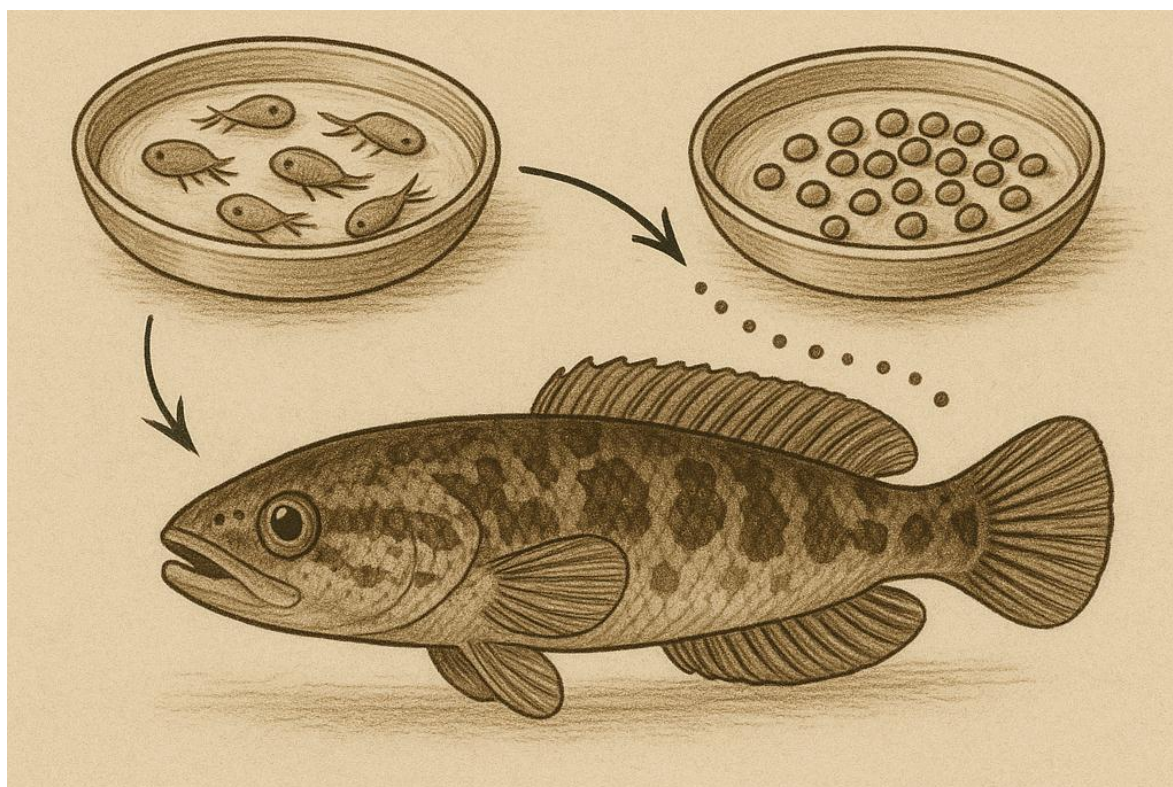
This study confirms the successful induction of reproduction in *Channa striata* using both Carp Pituitary Extract (CPE) and the synthetic GnRH analogue Buserelin acetate. Higher doses of both hormones significantly enhanced spawning, fertilization, and hatching rates, supporting hypothesis 1 that both are effective agents for induced breeding under captive conditions. Notably, Buserelin acetate at $0.8 \mu\text{g/kg}$ demonstrated superior performance with higher fertilization and hatching rates, and most importantly, no observed post-spawning mortality, in contrast to significant broodstock mortality in high-dose CPE treatments.

These findings support hypothesis 2, suggesting Buserelin as a more suitable and broodstock-safe alternative to CPE for commercial-scale seed production of *C. striata*.

The ability to induce spawning reliably with Buserelin opens the door for large-scale hatchery-based seed production, reducing dependence on wild populations and supporting conservation efforts. Future optimization of dose schedules and broodstock conditioning under hatchery conditions may further improve seed quality and larval survival, contributing to sustainable aquaculture of this economically and ecologically important species.

Chapter 5

Impact of Gradual Weaning and Feeding Regimes on Survival and Growth of *Channa striata* Larvae



Abstract

This study evaluated weaning methods for *Channa striata* larvae using a three-phase feeding trial with four treatments that included the T₁ (control), T₂ (100% Artemia), T₃ (100% fish paste), and T₄ (50% formulated feed + 50% Artemia). In Phase I (Days 1–15), larvae were fed the respective treatments, followed by a gradual transition to formulated feed in Phase II (Days 16–30). By Phase III (Days 31–45), all groups received 100% formulated feed. Initial average length and weight were 3.26 ± 0.23 cm and 0.56 ± 0.07 g. Upon completion of the experiment, T₂ had the highest final survival (89%), with T₂ and T₃ showing significantly higher weight gain and specific growth rate (SGR). A strong positive correlation between dissolved oxygen and final weight ($r = 0.85$) highlighted the importance of oxygen for growth, while elevated ammonia concentrations negatively impacted growth ($r = -0.98$). The findings suggest that *C. striata* larvae can be effectively weaned to formulated feed either through initial exclusive live feeding followed by gradual transition or by using a combination of live and formulated feeds from the beginning, both leading to enhanced survival and growth performance.

5.1 Introduction

The domestication of new fish species for aquaculture heavily depends on developing effective larval rearing and nursing techniques. A critical phase in this process is the successful transition of larvae from live to formulated feeds. This weaning stage is essential in determining cultured species' survival, growth, and long-term performance (Quin and Fast 1997; Kolkovski 2001). Larval rearing success relies significantly on the availability of appropriate feeds that are readily ingested, easily digested, and nutritionally sufficient to promote optimal development and health (Hamre et al. 2013; Kumar et al. 2008).

Several factors influence the success of weaning, including larvae's age and initial size, feed type, feeding strategy, environmental conditions, and cannibalistic behavior (Kubitza and Lovshin 1999; Papoutsoglou et al. 2001). Since feeding strategies often vary with species-specific physiological and behavioral traits, tailored weaning protocols are crucial (Cahu and Zambonino Infante 2001). Given that weaning has a direct impact on hatchery efficiency, extensive research has been conducted to optimize weaning in a variety of fish species, including both carnivorous and omnivorous types (Lall and Dumas 2015; Gisbert and Williot 2002).

Live feeds such as *Artemia*, rotifers, and zooplankton are commonly used in the early stages of larviculture due to their high palatability and digestibility (Dhert et al. 2001). However, large-scale application is limited by their high production cost, labor-intensive culture, and inconsistent quality (Sorgeloos et al. 2001; Person-Le Ruyet et al. 1993). While microdiets have been developed as alternatives, their early introduction often results in reduced growth and survival—primarily due to the immature digestive system of larvae and inadequate attractants in formulated feeds (Kolkovski S 2001; Saleh et al. 2015).

Additionally, delayed acceptance of formulated feeds can exacerbate cannibalism, especially in carnivorous and piscivorous species (Baras and Jobling 2002).

C. striata, the striped snakehead, is a high-value freshwater species widely distributed in Southeast Asia and known for its air-breathing ability, medicinal value, and market demand (Aliyu-Paiko et al. 2010; Courtenay and Williams 2004). In Thailand, India, and Vietnam, efforts to culture *C. striata* have moderately succeeded (Marimuthu et al. 2007a; Marimuthu et al. 2007b). In Bangladesh, however, commercial cultivation remains in its early stages, primarily due to poor quality fry, low spawning success, and limited knowledge of early life feeding and rearing practices (Mollah et al. 2009; Roy et al. 2020). Unlike carp species, the rearing of *C. striata* hatchlings and post-larvae poses more significant challenges due to their highly carnivorous and cannibalistic nature. These characteristics require carefully designed feeding protocols to prevent high mortality and improve performance (Qin and Fast 1996; Keawputorn et al. 2011).

From a practical and economic perspective, one of the primary considerations in snakehead farming is whether the prolonged use of expensive live feeds, which improves survival and growth, outweighs the cost-saving benefits of earlier transition to formulated feeds that may slightly compromise performance (Teshima et al. 2004; Dossou et al. 2011). Hence, the study tested following two hypotheses:

Hypothesis 1 (H₁): Gradual weaning of *Channa striata* larvae from live feed to formulated feed significantly improves growth performance and survival compared to abrupt weaning or exclusive live/formulated feeding.

Hypothesis (H₂): The timing of weaning transition (e.g., starting from day 5 post-hatch vs. day 10 post-hatch) has a significant effect on cannibalism incidence, survival rate, and growth performance in *C. striata* larvae.

Therefore, the present study aimed to evaluate the effectiveness of weaning strategies for *C. striata* larvae by gradually replacing live feed with formulated diets. The overarching goal was to develop a cost-effective and biologically sound weaning protocol to support the domestication and expansion of snakehead aquaculture in Bangladesh.

5.2 Materials and Methods

5.2.1 Study Area and Study Period

The study was conducted at the Fisheries Biology and Genetics Hatchery, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. The experiment was designed to replicate standard commercial fry production practices used in Southeast Asian countries. The feeding trial lasted six weeks and was carried out from June to July 2024.

5.2.2 Fish Larvae Collection

Channa striata larvae were obtained through induced breeding of captive-bred broodstock maintained at the freshwater station of Bangladesh Fisheries Research Institute (BFRI), Mymensingh. Following hatching, larvae were initially fed with plankton soup after complete yolk sac absorption (approximately 3 days post-hatch) for 10 days. At 13 days of age, larvae were transported to the HSTU hatchery in oxygenated polyethylene bags. Upon arrival, they were acclimatized in circular tanks and fed boiled trash fish paste prior to the experimental setup. All larvae used in the experiment were from a single batch to ensure uniformity.

5.2.3 Fish Stocking and Rearing Conditions

Larvae were stocked in 100-liter plastic tanks, each filled with 50 liters of dechlorinated water. Fifty larvae were stocked per tank (1 fish/L). Prior to stocking, initial body length (3.26 ± 0.23 cm) and weight (0.56 ± 0.07 g) were recorded. The tanks were cleaned daily with partial water exchange (approximately two-thirds of the volume) before the first feeding. Residual feed and fecal matter were siphoned out daily. Dead fish were removed and recorded regularly.

5.2.4 Experimental Design

The feeding trial consisted of three weaning phases over a total period of six weeks. Four dietary treatments were applied, with each treatment having three replicates (4 treatments $\times$ 3 replicates = 12 tanks). The treatment groups were as T₁ (Control): 100% formulated feed throughout the trial; T₂: Initial 100% *Artemia*, gradually replaced with formulated feed; T₃: Initial 100% fish paste, gradually replaced with formulated feed, and T₄: Mixed live and formulated feed initially, gradually transitioned. The feeding regime and weaning schedule for each treatment group are summarized in Table 5.1.

Table 5.1. Experimental design of feeding trial for *c. striata* larvae

Phase	Treatment	Feeding Composition	Duration	Age of Fish (Days)	Feeding Rate
I	T ₁ (Control)	100% Formulated feed	2 weeks	17–30 days	Up to satiation
	T ₂	100% <i>Artemia</i>			
	T ₃	100% Fish paste			
	T ₄	50% Formulated + 50% <i>Artemia</i>			

Phase	Treatment	Feeding Composition	Duration	Age of Fish (Days)	Feeding Rate
II	T ₁	100% Formulated feed	2 weeks	31–45 days	10% of body weight
	T ₂	50% Formulated + 50% <i>Artemia</i>			
	T ₃	50% Formulated + 50% Fish paste			
	T ₄	75% Formulated + 25% <i>Artemia</i>			
III	All groups	100% Formulated feed	2 weeks	46–60 days	8% of body weight

5.2.5 Water Quality Monitoring

Water quality parameters were monitored weekly following standard methods (APHA, 2005). Temperature, pH, and dissolved oxygen (DO) were measured using a portable multimeter (HM Digital COM-300). Total alkalinity was measured in the laboratory using the methyl orange indicator method. Ammonia, nitrite (NO_2^-), and nitrate (NO_3^-) concentrations were measured using a HACH water testing kit (Model: DR-210, USA). Water quality was maintained within optimal ranges for larval rearing.

5.2.6 Diet Preparation

5.2.6.1 Formulated Feed

A commercially available micro-particulate starter feed (Ultra Mash Powder, Tiger Brand, Yan Yan Aquaculture Ltd.) was used. The feed contained fishmeal, soybean meal, wheat flour, vitamins, minerals, enzymes, and essential oils. Proximate composition: Protein 35%, Moisture 12%, Fat 6%, Carbohydrate 28%, Fiber 5%, Ash 16%, Calcium 2.3%, Phosphorus 0.8%. The powder was mixed with water, kneaded, and sieved to 250–500 μm particle size before feeding.

5.2.6.2 *Artemia* Nauplii

Decapsulated *Artemia* cysts (Dancing Crane brand) were hatched using standard protocols. Hatching occurred in conical flasks under constant aeration and strong illumination from a 1000-watt bulb. Salinity was maintained at 25–30 ppt, temperature at 30 °C, and pH between 7.5–8.5. Hatching occurred within 24–36 hours, with an average hatching success rate of 90%. After hatching, nauplii were collected via siphoning and confirmed microscopically.

5.2.6.3 Fish Paste

Trash fish were boiled and minced to form a fine paste suitable for larval ingestion.

5.2.7 Sampling and Growth Monitoring

Weekly sampling (n = 10 per tank) was conducted to monitor growth and adjust feeding rates accordingly. Mortality and signs of cannibalism were recorded daily. Missing individuals were assumed to be victims of cannibalism. The following parameters were calculated:

$$\text{Survival Rate (\%)} = (\text{No. of fish alive} / \text{No. of fish stocked}) \times 100$$

$$\text{Weight Gain (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{Specific Growth Rate (SGR, \%/day)} = [(\ln \text{ Final weight} - \ln \text{ Initial weight}) / \text{Number of days}] \times 100$$

$$\text{Observed Mortality (\%)} = (\text{Dead fish with no sign of cannibalism} / \text{Total fish stocked}) \times 100$$

Cannibalism (%) = [(Total dead – Observed mortality) / Total stocked] × 100

Total Mortality = Observed mortality + Cannibalism

5.2.8 Statistical Analysis

All data were first tested for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965) to ensure the assumptions of parametric analysis were met. A one-way Analysis of Variance (ANOVA) was conducted to compare the growth and survival of larvae among the four treatment groups to determine if feeding regime had a significant effect on growth and survival performance of *C. striata*. Post-hoc comparisons (Tukey's HSD) were performed where significant differences were found (Tukey 1953). Variables that satisfied the assumption of normality were further analyzed using Pearson's correlation coefficient (r) test (Pearson 1896) to assess the strength and direction of linear relationships between final weight and each independent variable. This analysis was performed using Python 3 (Virtanen et al. 2020), which also provided p-values to assess statistical significance. All statistical visualizations, including correlation plots and ANOVA summary graphs, were created using Matplotlib (Hunter 2007). Statistical significance was determined at the 0.05 probability level.

5.3. Results

5.3.1 Water Quality Parameters

Throughout the experimental period, water temperature remained within acceptable ranges for *C. striata* larval rearing (Table 5.2). The water temperature did not show significant variation across treatments ($p > 0.05$), ranging from $29.13 \pm 0.59^\circ\text{C}$ in T_2 and T_3 to $29.36 \pm 0.59^\circ\text{C}$ in T_4 , indicating uniform thermal conditions in all experimental

tanks. However, significant differences were observed in pH levels among treatments ($p < 0.05$). The highest pH values were recorded in T₂ (7.68 ± 0.12) and T₃ (7.67 ± 0.13), both significantly higher than the control group T₁ (7.29 ± 0.27). Treatment T₄ exhibited an intermediate pH value (7.50 ± 0.24), which was not significantly different from T₁ and T₃. Dissolved oxygen (DO) levels also differed significantly among treatments ($p < 0.05$). The highest DO was recorded in T₂ (6.90 ± 0.48 mg/L), followed by T₃ (6.80 ± 0.76 mg/L) and T₄ (6.69 ± 0.85 mg/L), while the lowest was observed in T₁ (6.03 ± 0.99 mg/L). Total alkalinity varied significantly between treatments ($p < 0.05$), with T₁ exhibiting the highest value (118.22 ± 0.41 mg/L), followed by T₃ (116.51 ± 0.50 mg/L), T₂ (115.96 ± 0.33 mg/L), and the lowest in T₄ (115.21 ± 0.66 mg/L). Ammonia (NH₃) levels were significantly higher in T₁ (0.04 ± 0.017 mg/L) compared to other treatments ($p < 0.05$). The lowest ammonia concentration was observed in T₂ (0.01 ± 0.002 mg/L). T₃ and T₄ had intermediate values of 0.02 ± 0.004 mg/L and 0.03 ± 0.009 mg/L, respectively. In contrast, nitrate (NO₃⁻) and nitrite (NO₂⁻) levels did not differ significantly across treatments ($p > 0.05$). Nitrate concentrations ranged from 4.39 ± 0.49 mg/L in T₁ to 4.72 ± 0.60 mg/L in T₂, while nitrite concentrations remained between 0.43 ± 0.04 mg/L (T₁) and 0.47 ± 0.07 mg/L (T₃).

Overall, treatments incorporating live or mixed feeding strategies (T₂, T₃, and T₄) maintained better water quality parameters compared to the control (T₁), which relied solely on formulated feed.

Table 5.2 Water quality parameters during the feeding trial of *Channa striata* larvae under different treatments.

Parameters	T ₁ (Control)	T ₂ (100% <i>Artemia</i>)	T ₃ (100% Fish Paste)	T ₄ (50% Formulated + 50% <i>Artemia</i>)
Temperature (°C)	29.26 ± 0.61	29.13 ± 0.59	29.13 ± 0.72	29.36 ± 0.59
pH	7.29 ± 0.27 ^b	7.68 ± 0.12 ^a	7.67 ± 0.13 ^a	7.50 ± 0.24 ^{ab}
DO (mg/L)	6.03 ± 0.99 ^b	6.90 ± 0.48 ^a	6.80 ± 0.76 ^{ab}	6.69 ± 0.85 ^{ab}
Total Alkalinity (mg/L)	118.22 ± 0.41 ^a	115.96 ± 0.33 ^c	116.51 ± 0.50 ^b	115.21 ± 0.66 ^d
NH ₃ (mg/L)	0.04 ± 0.017 ^a	0.01 ± 0.002 ^c	0.02 ± 0.004 ^c	0.03 ± 0.009 ^b
NO ₃ (mg/L)	4.39 ± 0.49	4.72 ± 0.60	4.62 ± 0.31	4.59 ± 0.39
NO ₂ (mg/L)	0.43 ± 0.04	0.47 ± 0.05	0.47 ± 0.07	0.46 ± 0.06

Note: Values are presented as mean ± standard deviation. Different superscript letters (a, b, c, d) within the same row indicate significant differences among treatments (Tukey HSD, $p < 0.05$).

5.3.2 Growth and survival of *C. striata* larvae

The growth performance and survival of *C. striata* larvae varied significantly among the different treatment groups after six weeks (Table 5.3). Initial length and weight were consistent across all treatments (3.26 ± 0.23 cm and 0.56 ± 0.07 g, respectively), indicating uniformity at the beginning of the trial. Upon completion of the experiment survival rates ranged from 66% to 89%, with the highest survival observed in treatment T₂ (89%), followed by T₃ (86%) and T₄ (89%). The lowest survival was recorded in T₁ (66%) (Figure 5.1).

Final length and weight of larvae differed significantly among treatments ($P < 0.05$). The highest final length (8.10 ± 0.18 cm) and final weight (2.70 ± 0.34 g) were observed in T₂, followed closely by T₃ (8.04 ± 0.16 cm; 2.46 ± 0.26 g). These values were significantly greater than those recorded in T₁ (7.10 ± 0.24 cm; 1.52 ± 0.21 g) and T₄ (7.05 ± 0.22 cm;

1.79 ± 0.55 g), which were statistically similar. Weight gain and specific growth rate (SGR) followed a similar trend. T₂ exhibited the highest weight gain (2.14 ± 0.27 g) and SGR (3.75 ± 0.27%/day) that is significantly higher than T₁ and T₄ (P < 0.05). Following T₂, T₃ also showed higher weight gain performance (1.90 ± 0.19 g; 3.52 ± 0.19%/day) which is not significantly different from T₂ but significantly higher than other two treatment. T₁ recorded the lowest weight gain (0.96 ± 0.14 g) and SGR (2.38 ± 0.14%/day) which is significantly lower than other treatment.

T₂ (100% Artemia) and T₃ (100% fish paste) were the most effective treatments for maximizing survival and minimizing mortality where T₁ (100% formulated feed) resulted in the lowest survival and highest mortality, both total and observed, likely due to poor palatability, digestibility, or nutritional adequacy. Cannibalism was relatively low and uniform, suggesting that feeding regime had a greater effect on survival than aggression-related mortality. Overall, treatment T₂ demonstrated the most favorable combination of high survival and superior growth performance, suggesting its effectiveness in promoting the growth and survival of *C. striata* larvae during weaning.

Table 5.3 Growth parameters (Mean ± SD) of *C. striata* larvae in different treatments after Phase III.

Treatment	Survival (%)	Initial length (cm)	Initial weight (g)	Final length (cm)	Final weight (g)	Weight gain (g)	SGR (%/day)
T ₁	66	3.26 ± 0.23	0.56 ± 0.07	7.10 ± 0.24 ^b	1.52 ± 0.21 ^b	0.96 ± 0.14 ^b	2.38 ± 0.14 ^b
T ₂	89	3.26 ± 0.23	0.56 ± 0.07	8.10 ± 0.18 ^a	2.70 ± 0.34 ^a	2.14 ± 0.27 ^a	3.75 ± 0.27 ^a
T ₃	86	3.26 ± 0.23	0.56 ± 0.07	8.04 ± 0.16 ^a	2.46 ± 0.26 ^a	1.9 ± 0.19 ^a	3.52 ± 0.19 ^a
T ₄	82	3.26 ± 0.23	0.56 ± 0.07	7.05 ± 0.22 ^b	1.79 ± 0.55 ^b	1.23 ± 0.48 ^b	2.76 ± 0.48 ^b

Values are presented as mean ± standard deviation. Different superscript letters (a, b) within the same column indicate significant differences among treatments (Tukey HSD, *p* < 0.05)

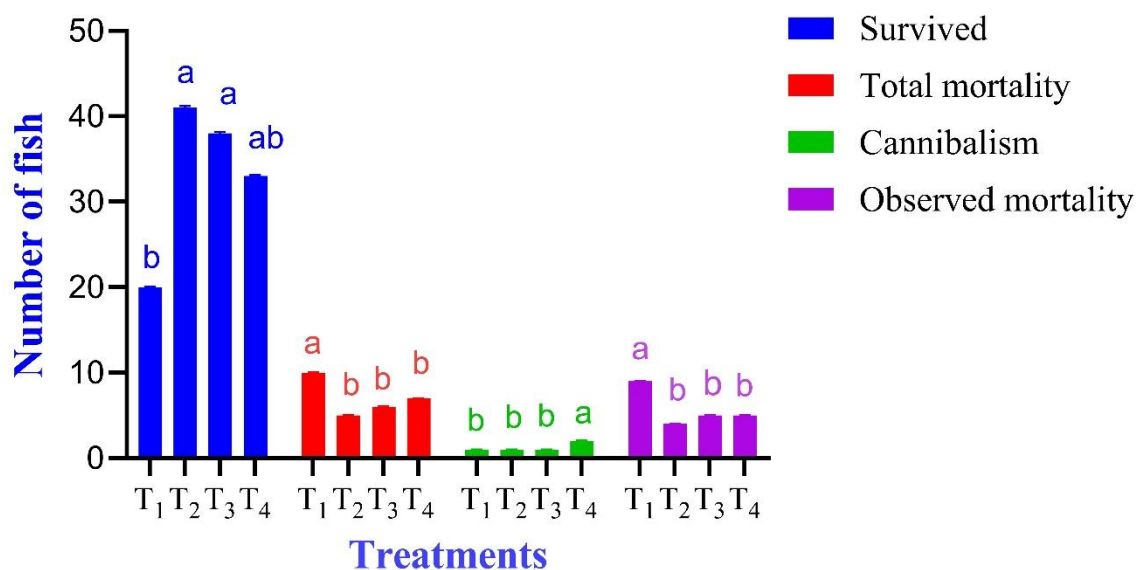


Figure 5.1 Survival, total mortality, cannibalism, and observed mortality of fish under different treatments (T₁ –T₄). Bars represent the number of fish in each category. Different letters above the bars indicate statistically significant differences between treatments ($p < 0.05$)

5.3.4 Relationship between of growth and survival rate with quality parameters

A correlation analysis was conducted to assess the relationship between final weight of *C. striata* larvae and key water quality parameters as well as survival rate across treatments (Figure 5.2). A very strong positive correlation was observed between dissolved oxygen and final weight ($r = 0.85$), indicating that higher levels of dissolved oxygen significantly supported larval growth. Similarly, survival rate also exhibited a strong positive correlation with final weight ($r = 0.92$), suggesting that treatments with higher survival outcomes were also associated with improved growth performance. In contrast, ammonia concentration showed a strong negative correlation with final weight ($r = -0.98$), implying that elevated ammonia levels adversely affected larval growth. Additionally, pH had strong positive correlation with final weight ($r = 0.95$), reflecting that pH levels closer to optimal values

were beneficial for larval development. These findings highlight the critical role of water quality and survival in influencing the weaning success and growth performance of *C. striata* larvae.

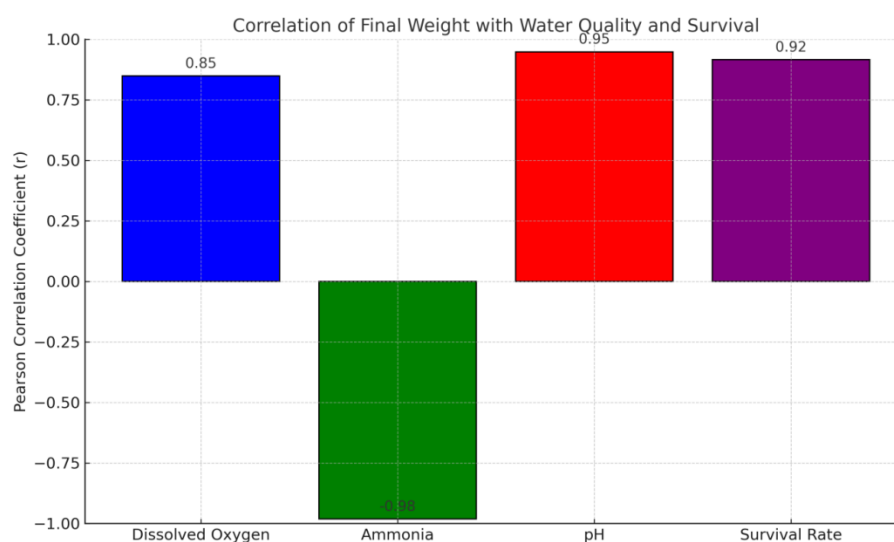


Figure 5.2 Pearson correlation coefficients between final weight and water quality parameters (dissolved oxygen, ammonia, pH) as well as survival rate. Ammonia showed a strong negative correlation, whereas pH, dissolved oxygen, and survival rate were strongly positively correlated with final weight.

5.4 Discussion

This study evaluated the water quality parameters, survival, and growth performance of *C. striata* larvae under different dietary treatments including varying combinations of formulated feed, live *Artemia* and fish paste. The feeding trial commenced when the larvae were 17 days old, based on literature indicating the optimal onset of digestive enzyme development in *C. striata* (Hien et al. 2017). The results demonstrate how dietary strategies influence water quality and larval development, with significant variations in survival, growth, and cannibalism observed across the treatments.

5.4.1 Water Quality Parameters

Water quality is a crucial factor influencing the health and growth of aquatic organisms in aquaculture (Boyd and Tucker 2012). In this study, water temperature remained consistent across treatments (29.13–29.36°C), well within the optimal range for *C. striata* larvae (28–30°C) (Ali et al. 2013). The variations in pH among treatments with significantly higher values in T₂ and T₃ compared to T₁, suggested that live and semi-moist feeds like Artemia and fish paste may act as pH buffers (Sampaio et al. 2011). This buffering effect could result from the organic matter in live feeds, which may neutralize acids produced during microbial degradation of organic waste (Kwon et al. 2020). Dissolved oxygen (DO) levels were significantly higher in treatments with live feeds (T₂ and T₃), which could be attributed to the improved water quality and reduced microbial load in these treatments (Zhou et al. 2022). In contrast, the lower DO observed in the control group (T₁) could be linked to the higher organic load resulting from uneaten formulated feed, leading to greater oxygen consumption due to microbial degradation (Vivekanandan et al. 2021). The ammonia levels were also significantly lower in T₂, likely due to the lower nitrogenous waste produced by Artemia which is known for its high digestibility and low nitrogen excretion (Bai et al. 2019). Ammonia accumulation is a major concern in aquaculture, as high concentrations can be toxic to larvae, affecting their survival and growth (Wu et al. 2020). The significant difference in ammonia levels highlights the importance of incorporating live feeds in reducing nitrogenous waste in the initial stage of weaning.

5.4.2 Survival and Mortality

The survival rates observed in this study highlight the beneficial effects of live and semi-live feeds at the beginning. Treatment T₂ (100% Artemia) demonstrated the highest survival (89%) at the end of the experiment with low cannibalism, consistent with previous studies that show live feeds such as Artemia can enhance the survival of fish larvae by improving

nutritional intake and reducing stress (Figueiredo et al. 2014). In contrast, the control group (T₁) exhibited significantly lower survival (66%) and the highest mortality and cannibalism, which aligns with findings from Liu et al. (2017), who noted that reliance on formulated feed alone could lead to poor survival and increased competition and cannibalism among larvae.

The reduced survival and higher cannibalism in T₁ could also be linked to the poor nutritional quality of formulated feeds compared to live prey, which provides essential fatty acids and other nutrients critical for larval development (Salze and Davis 2015). Takagi et al. (2016), who showed that *Artemia* and other live feeds improved the overall survival and behavior of fish larvae by reducing stress and aggressive interactions, supports this conclusion.

5.4.3 Growth Performance

The growth performance of *C. striata* larvae, measured in terms of weight gain and specific growth rate (SGR), was significantly higher in T₂ (100% *Artemia*) and T₃ (100% fish paste) compared to T₁ (formulated feed). The highest mean weight and length were recorded in T₂ followed by T₃, suggesting that live *Artemia* and fish paste, provides a high-quality protein source essential for larval growth. These findings are consistent with those of Saravanan et al. (2018), who reported superior growth performance in fish larvae fed live or semi-live feeds compared to those fed solely formulated feed.

The growth differences between treatments may also be related to the digestibility and nutritional composition of the feeds. Live *Artemia*, which contains high levels of essential fatty acids and proteins is known to improve the growth of fish larvae by providing more bioavailable nutrients than formulated feeds (Vera-Cruz et al. 2021). In contrast, T₁, which relied solely on formulated feed, provided suboptimal nutrition for the larvae, resulting in lower weight gain and SGR, consistent with the findings of Shi et al. (2020).

5.4.4 Relationship of growth and survival with quality parameters

The correlation analysis in this study provides important insights into how water quality parameters and survival rates influence the growth performance of *C. striata* larvae under different weaning regimes. The strong positive correlation between dissolved oxygen (DO) and final weight emphasizes the critical role of oxygen availability in larval growth. Adequate DO is essential for supporting metabolic functions, feed assimilation, and overall physiological development in fish larvae (Buentello et al. 2000). Previous studies have also demonstrated that low DO levels can impair growth and increase stress sensitivity in early life stages of many freshwater species (Boyd and Tucker 1998).

Conversely, a strong negative correlation was observed between ammonia concentration and final weight indicating that even slight elevations in ammonia adversely affected larval growth. Ammonia, especially in its un-ionized form (NH_3), is toxic to fish and can disrupt osmoregulation and gill function (Randall and Tsui 2002). This finding aligns with the reports of Azim and Little (2008), who noted that maintaining low ammonia levels is crucial for larval rearing success in intensive aquaculture systems.

Further, the strong positive correlation between pH and final weight suggests that maintaining pH within an optimal range (around 7.5–7.8) favors larval development. Extremes in pH are known to affect enzyme activity, ion exchange, and nutrient availability, thereby impacting growth and survival (Boyd 1990). Although all treatments in this study, maintained pH within an acceptable range for tropical freshwater species, even small differences appeared to influence growth outcomes.

Furthermore, the strong positive correlation between survival rate and final weight highlights the interconnectedness of survival and growth performance. Higher survival often indicates favorable environmental and nutritional conditions, which concurrently support better weight gain. This observation is consistent with the findings of Gurung et al.

(2010), who reported a positive link between survival and biomass yield in larval rearing of *Channa* spp. The best-performing treatment in this study T₂ (100% Artemia) demonstrated both the highest survival and growth, suggesting that early-stage feeding with live prey not only improves nutrient uptake but also mitigates cannibalism and stress.

Collectively, these findings highlight how crucial it is to maintain ideal water quality parameters, especially dissolved oxygen and ammonia, during the crucial weaning stages. Result also suggested that the integration of live feed, such as *Artemia*, in early weaning strategies to enhance both survival and growth performance in *C. striata* larvae. These findings also align with the broader body of literature on the advantages of live feeds in the early larval stages of various fish species (Zhou et al. 2022; Bai et al. 2019).

5.5 Conclusion

This study demonstrates that incorporating live and semi-live feeds, particularly *Artemia* and fish paste, during the early larval stages of *Channa striata* significantly enhances survival, growth performance, and water quality compared to exclusive reliance on formulated feeds. The highest survival rates, best growth outcomes, and lowest cannibalism were observed in larvae fed with 100% Artemia, underscoring the importance of live feed in early weaning protocols.

Water quality parameters such as dissolved oxygen, pH, and ammonia levels were strongly associated with larval performance, highlighting the critical role of maintaining optimal environmental conditions alongside appropriate feeding strategies. These findings support the hypothesis that gradual weaning incorporating live feed improves larval rearing success and suggest that the timing and nature of feed transition are key factors in reducing mortality and cannibalism.

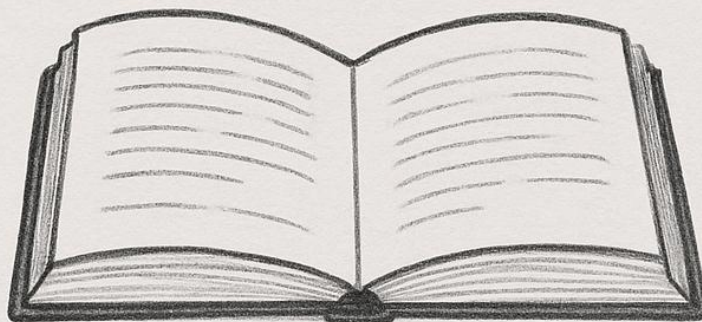
Overall, a feeding strategy that begins with live or semi-live feeds followed by gradual introduction of formulated diets appears to be the most effective approach for larval rearing in *C. striata*. This approach not only optimizes growth and survival but also helps maintain favorable water quality, essential for intensive aquaculture systems.

Future research should investigate the optimal timing for initiating feed transitions and evaluate the long-term effects of weaning strategies on juvenile growth, health, and stress resilience. Implementing these findings can improve hatchery production efficiency and support the sustainable domestication and commercial culture of *C. striata* in Bangladesh and similar tropical aquaculture settings.

Chapter 6

Summary, Conclusion and Recommendation

Summary Conclusion Recommendation



GENETIC IDENTIFICATION AND INDUCED
BREEDING OF THREATENED INDIGENOUS
SPECIES, *Channa striata* (Bloch, 1793)
FOR SEED PRODUCTION

6.1 Summary

Bangladesh is endowed with rich freshwater biodiversity, yet many indigenous fish species including *Channa striata* (striped snakehead) are facing rapid population declines due to habitat degradation, overfishing and the unregulated introduction of exotic strains. Despite its significant aquaculture potential and commercial value, the culture of *C. striata* in Bangladesh remains underdeveloped due to critical knowledge gaps in genetic identification, reproductive biology and standardized hatchery techniques. This study was undertaken to address these gaps and contribute to the sustainable aquaculture development and conservation of this threatened native species. The research was structured around four major experimental components:

6.1.1 Geometric Morphometry and COI-Based Genetic Characterization

A combination of landmark-based geometric morphometric analysis and COI (cytochrome c oxidase subunit I) gene barcoding was employed to differentiate native and exotic populations of *C. striata*. This study highlights the limitations of traditional morphometrics in distinguishing closely related *Channa* species due to overlapping characteristics and potential misidentifications. In contrast, geometric morphometric approaches utilizing PCA, scatter plots and UPGMA clustering proved effective in revealing distinct body shape variations among *Channa* species in Bangladesh. Notably, native *C. striata* and Vietnamese *C. striata* clustered in different groups, suggesting clear phenotypic divergence. The most prominent morphological variation was observed in the snout region, particularly between indigenous and Vietnamese *C. striata*, likely influenced by geographical isolation, environmental factors, and differences between wild and captive rearing systems.

The COI gene proved to be a reliable molecular marker for assessing genetic variation and phylogenetic relationships among *Channa* species. Its high proportion of conserved (61.5%), variable (36.9%) and parsimony-informative (32%) sites underscores its effectiveness for species identification. The genetic distinction between native and Vietnamese *C. striata* was clearly demonstrated, aligning with previous findings using Cyt *b* and PCR-RFLP techniques. Disparity index analyses and heatmaps revealed notable evolutionary divergence, particularly between *C. striata* and *C. gachua*, while substitution pattern analysis showed a transition bias (A↔G, T↔C) typical of mitochondrial DNA. Most species displayed homogeneous substitution patterns, suggesting genetic stability, and Phylogenetic analysis clearly separated native and exotic *C. striata* into different clades, validating their evolutionary divergence. The accuracy of reference sequences further supported the reliability of COI-based phylogenetic inferences. These findings underscore the utility of integrative taxonomic approaches in species identification, biodiversity conservation, and management of introduced strains in aquaculture.

Geometric morphometric tools should be routinely used for precise species identification in biodiversity surveys, aquaculture, and conservation initiatives. To strengthen species delimitation, future research should integrate morphometric data with molecular techniques such as DNA barcoding and phylogenetic analysis. The COI gene, as a reliable and informative marker, should be consistently applied alongside other mitochondrial and nuclear markers (e.g., Cyt *b*, RAG1) to resolve species complexes and trace exotic lineages. Such an integrated approach will enhance biodiversity monitoring, safeguard native *Channa* populations from genetic dilution and inform sustainable fisheries and aquaculture management practices.

6.1.2 Reproductive Biology and Growth Dynamics of Wild Populations

A year-long field investigation was carried out in the Atrai River system to assess the reproductive cycle, growth pattern, and gonadal development of wild *C. striata*. The species exhibited negative allometric growth, with energy preferentially allocated to reproduction rather than somatic growth during the breeding season. This study comprehensively investigated the growth and reproductive biology of *C. striata* in the drought-prone Atrai River system over a one-year period from April 2022 to March 2023. A total of 239 specimens were analyzed to elucidate seasonal growth patterns, reproductive cycles, and their implications for conservation and aquaculture management.

Both male and female *C. striata* exhibited negative allometric growth ($b < 3.0$), indicating that body weight increased at a slower rate than body length. This pattern likely reflects seasonal energy allocation toward reproductive processes. Peak body weight was observed in males during September and in females during April, suggesting sex-specific temporal investment in gonadal development. Fulton's condition factor (K) varied seasonally, with elevated values recorded during the post-monsoon months (September–November) and lower values during the peak reproductive season (April–July). This seasonal fluctuation in K reflects the physiological trade-off between somatic growth and reproductive investment.

The overall sex ratio was 1:0.67 (M:F), with a slight female bias recorded early in the year. The gonadosomatic index (GSI) of females peaked in May and June, corresponding to the pre-monsoon and early monsoon periods, whereas males showed a moderate increase in GSI, suggesting synchronized reproductive timing between sexes. Reproductive activity was strongly associated with seasonal hydrological dynamics, particularly monsoon-driven flooding, which enhances the availability of suitable spawning and nursery habitats.

Fecundity was highest in May, with a mean value of $33,833 \pm 13,533$ eggs, and exhibited a strong positive correlation with GSI ($R^2 = 0.74$), while correlations with total length ($R^2 = 0.10$) and body weight ($R^2 = 0.18$) were weak. These results underscore the importance of GSI as a robust indicator of reproductive maturity and fecundity, thus serving as a useful criterion for broodstock selection. Sexual dimorphism was evident in the size at first maturity, with males maturing at 25.8 cm and females at 34.2 cm. The larger L_{50} value in females is consistent with greater fecundity and supports the implementation of sex-specific size-based fisheries regulations to ensure sustainable exploitation. Ova diameter peaked in June (12.9 μm) and decreased following the spawning season in July and August, consistent with the progression of vitellogenic growth and subsequent oocyte resorption. The presence of asynchronous oocyte development supports a batch spawning strategy, which may enhance reproductive success under environmentally variable conditions. Five distinct gonadal maturation stages were documented in both sexes, and these were validated through histological examination. The peak reproductive period extended from May to July, as confirmed by both histological and macroscopic analyses, aligning closely with monsoon-related environmental cues.

Overall, the study provides critical insights into the reproductive phenology and growth dynamics of *C. striata* in a hydrologically variable river system, offering valuable information for the formulation of effective conservation and aquaculture management strategies. These insights provide a scientific basis for sustainable management, such as enforcing closed seasons during breeding months and implementing sex-specific minimum catch sizes to conserve reproductive stocks.

6.1.3 Induced Breeding and Hormonal Standardization

Standardized protocols for hormone administration, broodstock conditioning and hatchery management were developed as practical guidelines for large-scale hatchery-based seed production of *C. striata*. The present study assessed the efficacy of two hormonal agents carp pituitary extract (CPE) and Buserelin in inducing spawning in *C. striata* under controlled conditions. The findings demonstrate differential responses in spawning latency, fertilization and hatching success, post-spawning broodstock mortality, and embryonic development, depending on the type and dosage of hormone administered. Spawning induction with CPE hormone resulted in the onset of spawning behavior within 12–14 hours post-injection, with complete ovulation observed by 22–23 hours. Among the tested doses, a higher concentration (80 mg/kg) significantly enhanced spawning performance compared to the lower dose (60 mg/kg), indicating a dose-dependent effect on reproductive success. In contrast, Buserelin hormone induced spawning at a delayed interval of 25–30 hours post-injection. While Buserelin use has not been previously documented in *Channa* species, its effectiveness in Indian major carps supports its broader applicability in teleost breeding programs.

Fertilization rates were highest in Treatment T₃ with statistically significant differences ($p < 0.05$) compared to other treatments, and values exceeding those reported in earlier literature. Similarly, hatching success improved with increasing hormone dose, with T₃ again exhibiting superior performance. Hatching was observed 30–32 hours after spawning at an incubation temperature of 27–29°C, representing a slight delay relative to previously reported timings.

Post-spawning broodstock mortality showed a stark contrast between treatments: high mortality levels (33.30%–67.11%) were recorded in high-dose CPE treatments, whereas no

mortality was observed with Buserelin administration. This finding suggests that Buserelin may offer a safer alternative to CPE, particularly in terms of broodstock health and survivability. Embryonic development followed typical cleavage patterns and larval behavioral milestones previously reported in *C. striata*. Yolk sac absorption was completed approximately 73–77 hours post-hatching, at which point larvae initiated active exogenous feeding, marking the transition to early larval stages.

This study demonstrates the comparative efficacy of CPE and Buserelin in inducing spawning in *C. striata* under controlled conditions. While both hormones successfully triggered ovulation and embryonic development, Buserelin emerged as a safer and effective alternative, offering high fertilization and hatching rates without the post-spawning mortality observed with CPE. These findings highlight Buserelin as a potentially safer and viable alternative to traditional CPE, particularly for sustainable hatchery operations of *Channa striata* for sustainable hatchery operations and underscore the importance of optimizing hormone protocols to enhance seed production and broodstock welfare in *Channa striata* aquaculture.

6.1.4 Weaning Protocol for Hatchery-Produced Larvae

A three-phase weaning protocol was developed to facilitate the transition of larvae from live to formulated feed. This study evaluated the effects of different weaning diets formulated feed, live *Artemia* and fish paste on water quality, survival, growth performance, and cannibalism in *C. striata* larvae. The trial began at 17 days post-hatch, a stage associated with the development of digestive enzymes in the species. The results demonstrated that dietary choice significantly influenced larval performance and environmental parameters within the rearing system. Treatments with live or semi-live feeds (especially 100% *Artemia*) maintained better water quality, including higher dissolved oxygen and lower

ammonia concentrations, likely due to improved digestibility and reduced organic waste. Temperature remained stable across all treatments (29.13–29.36°C), within the optimal range for *C. striatalarvae*. The pH was significantly higher in T₂ (100% *Artemia*) and T₃ (100% fish paste), suggesting a buffering effect of live and semi-moist feeds. Dissolved Oxygen (DO) was significantly higher in T₂ and T₃, likely due to lower microbial degradation and better feed utilization. Ammonia levels were lowest in T₂, indicating reduced nitrogenous waste from the highly digestible *Artemia*. Growth performance, measured by weight gain and specific growth rate, was also superior in the live and semi-live feed groups (T₂). T₂ showed the highest growth, followed by T₃ and T₁ where live *Artemia* provided better digestibility and essential nutrients, leading to improved weight gain and specific growth rate (SGR). The highest survival (89%) was recorded in T₂ (*Artemia* only), with the lowest cannibalism. T₁ (formulated feed only) had lowest survival (66%) and highest mortality and cannibalism, due to poorer feed quality and greater competition. The water quality conditions correlated with higher survival rates (up to 89%) and reduced cannibalism. In contrast, the group fed only formulated feed showed the lowest survival and highest cannibalism, underscoring the limitations of dry feed during early larval stages. Correlation analysis revealed strong positive relationships between final weight and both dissolved oxygen and pH, while ammonia showed a strong negative correlation with growth. These results emphasize the critical influence of water quality and survival on growth during early development.

Results indicated that larvae could be effectively weaned using a gradual replacement strategy, starting with live feed and transitioning to formulated diets. This protocol supports larval survivability, reduces cannibalism, and paves the way for cost-effective commercial hatchery operations. However, further optimization of grow-out techniques remains essential for maximizing productivity.

6.4 Conclusion

This multi-faceted study has addressed key knowledge gaps in the biology, taxonomy and aquaculture of *C. striata*, a valuable yet underutilized indigenous fish species in Bangladesh. The integrative approach employed spanning morphometric and genetic identification, reproductive biology, hormonal breeding trials and larval weaning experiments offers a robust framework for both conservation and aquaculture development. Geometric morphometrics combined with COI gene barcoding provides a reliable and integrative method for distinguishing native and exotic *C. striata* populations. The evidence of phenotypic and genetic divergence between Vietnamese and Bangladeshi populations supports the implementation of traceability and biosecurity protocols to prevent genetic dilution of native stocks. The study elucidates the reproductive cycle of *C. striata* in the Atrai River system, establishing May–July as the peak breeding season and confirming batch spawning behavior. The findings recommend protection of spawning habitats and enforcement of seasonal fishing bans during critical reproductive periods. Comparative hormonal induction trials reveal that Buserelin is a promising alternative to traditional CPE, offering high fertilization and hatching rates with reduced broodstock mortality. These outcomes support the development of safer, standardized hormonal protocols for hatchery-based seed production. A phase-wise weaning strategy, particularly incorporating *Artemia* and fish paste, enhances larval survival, growth, and water quality, while reducing cannibalism. This highlights the necessity for tailored nutritional transitions in the early life stages to ensure hatchery success.

6.3 Recommendations

Based on the study findings, the following recommendations are proposed for future research, hatchery practice and policy implementation:

For aquaculture practice

- 1. Adoption of Integrated Identification Protocols:** Hatcheries and biodiversity surveys should incorporate geometric morphometric tools and COI-based DNA barcoding to ensure accurate species identification and prevent genetic contamination from exotic strains.
- 2. Implementation of Breeding Season Regulations:** Establishment and enforcement of fishing restrictions during the peak reproductive months (May–July) and introduce sex-specific size limits (e.g., 34 cm for females) to protect broodstocks.
- 3. Standardization of Hormonal Protocols:** Promoting the use of Buserelin in hatchery breeding programs due to its safety and efficacy. Further trials with combinations or lower doses may optimize outcomes.
- 4. Optimization of Weaning Strategies:** Utilization of a three-phase weaning protocol beginning with *Artemia*, transitioning to fish paste and finally to formulated feeds to maximize larval survival and reduce mortality and cannibalism in nursery phases.

For Conservation and Management

- 1. Protection of Natural Habitats:** Prioritizing the restoration and protection of monsoon-flooded spawning habitats in rivers like Atrai. Introduce habitat mapping and seasonal monitoring programs.

- 2. Prevention of Genetic Introgression:** Enforcement of quarantine and registration of exotic *C. striata* strains introduced for aquaculture to safeguard the genetic integrity of native populations.

For Future Research

- 1. Expanding Molecular Marker Use:** Integration of nuclear markers such as RAG1 and microsatellites with mitochondrial COI and Cyt b to resolve species complexes and monitor genetic drift.
- 2. Study on Environmental Cues:** Investigation of the physiological responses of *C. striata* to environmental cues (e.g., photoperiod, temperature, and hydrology) to further refine breeding and conservation protocols.
- 3. Enhancement Larval Nutrition Research:** Development of species-specific microdiets and evaluate gut microbiota development during weaning to optimize larval nutrition and disease resistance.

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APPENDICES

Appendix 1 Experimental fishes for identification and evolutionary relationship study



Channa striata (Native)



Channa striata (Exotic)



Channa marulius

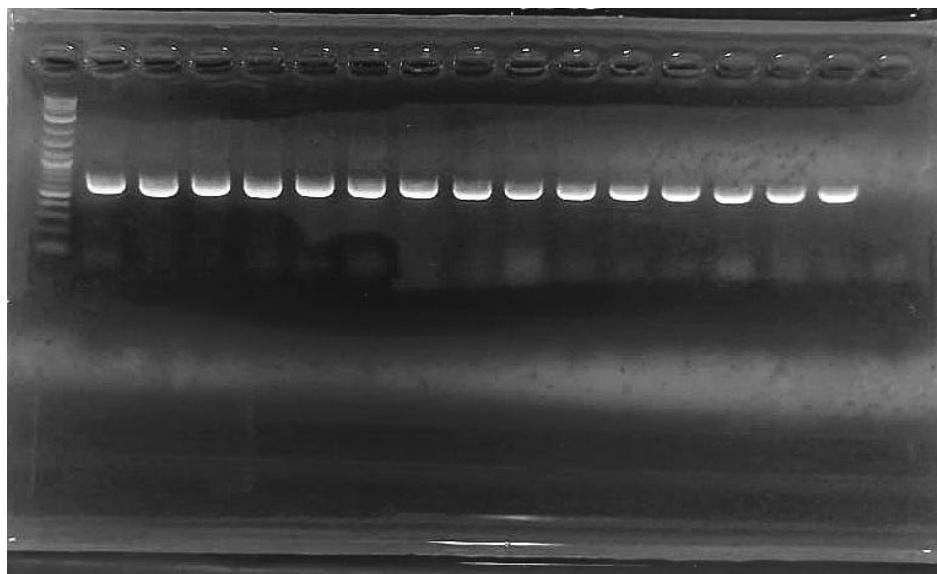


Channa punctata



Channa gachua

Appendix 2: Electrophoretic band of COI sequences



Appendix 3. Partial COI sequences with accession number

a. *Channa striata* (Native) sequence

OR864290 (*C. striata*) seq_1

ACATTGGCACCCCTTTATCTCGTATTCGGTGCCTGAGCCGGAATAGTGGGCACAGCCCTAAGCCTTCTAATTTCG
AGCAGAACTAAGCCAACCTGGCGCTCTCCTCGGAGACGATCAAATTTATAATGTAATTGTAACAGCACACGC
CTTTGTAATAATCTTTTTCATGGTTATGCCAATAATAATTGGAGGCTTCGGAAATTGACTTATTCCTCTTATGA
TTGGCGCCCCGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGATTGCTCCCTCCATCATTCTCCTT
TACTAGCCTCTTCTGCTGTAGAAGCCGGGGCCGGAACCGGGTGAACAGTTTACCCACCCCTAGCCAGCAAT
TAGCCACGCAGGGGCCTCCGTCGATCTAACCATTTTCTCCCTACACCTGGCTGGTGTATCCTCAATTTTAG
GGGCCATTAACCTTTATTACTACTATTATTAACATAAAACCCCTGCTATTTCTCAATATCAAACCCCTATTG
TATGGGCTATCTTAATTACAGCCGTACTACTTTTACTTTCCCTCCCAGTATTAGCTGCAGGCATTACAATGCTA
CTCACAGACCGAAATCTCAACACCACCTTCTTCGACCCTGCTGGC

OR864291 (*C. striata*) Seq_2

ACATTGGCACCCCTTTATCTCGTATTCGGTGCCTGAGCCGGAATAGTGGGCACAGCCCTAAGCCTTCTAATTTCG
AGCAGAACTAAGCCAACCTGGCGCTCTCCTCGGAGACGATCAAATTTATAATGTAATTGTAACAGCACACGC
CTTTGTAATAATCTTTTTCATGGTTATGCCAATAATAATTGGAGGCTTCGGAAATTGACTTATTCCTCTTATGA
TTGGCGCCCCGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGATTGCTCCCTCCATCATTCTCCTT
TACTAGCCTCTTCTGCTGTAGAAGCCGGGGCCGGAACCGGGTGAACAGTTTACCCACCCCTAGCCAGCAAT
TAGCCACGCAGGGGCCTCCGTCGATCTAACCATTTTCTCCCTACACCTGGCTGGTGTATCCTCAATTTTAG
GGGCCATTAACCTTTATTACTACTATTATTAACATAAAACCCCTGCTATTTCTCAATATCAAACCCCTATTG
TATGGGCTATCTTAATTACAGCCGTACTACTTTTACTTTCCCTCCCAGTATTAGCTGCAGGCATTACAATGCTA
CTCACAGACCGAAATCTCAACACCACCTTCTTCGACCCTGCTGGC

OR864293 (*C. striata*) Seq_3

ACATTGGCACCCCTTTATCTCGTATTCGGTGCCTGAGCCGGAATAGTGGGCACAGCCCTAAGCCTTCTAATTTCG
AGCAGAACTAAGCCAACCTGGCGCTCTCCTCGGAGACGATCAAATTTATAATGTAATTGTAACAGCACACGC
CTTTGTAATAATCTTTTTCATGGTTATGCCAATAATAATTGGAGGCTTCGGAAATTGACTTATTCCTCTTATGA
TTGGCGCCCCGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGATTGCTCCCTCCATCATTCTCCTT
TACTAGCCTCTTCTGCTGTAGAAGCCGGGGCCGGAACCGGGTGAACAGTTTACCCACCCCTAGCCAGCAAT
TAGCCACGCAGGGGCCTCCGTCGATCTAACCATTTTCTCCCTACACCTGGCTGGTGTATCCTCAATTTTAG
GGGCCATTAACCTTTATTACTACTATTATTAACATAAAACCCCTGCTATTTCTCAATATCAAACCCCTATTG
TATGGGCTATCTTAATTACAGCCGTACTACTTTTACTTTCCCTCCCAGTATTAGCTGCAGGCATTACAATGCTA
CTCACAGACCGAAATCTCAACACCACCTTCTTCGACCCTGCTGGC

OR864294_ (*C. striata*) Seq_4

ACATTGGCACCCCTTTATCTCGTATTCGGTGCCTGAGCCGGAATAGTGGGCACAGCCCTAAGCCTTCTAATTTCG
AGCAGAACTAAGCCAACCTGGCGCTCTCCTCGGAGACGATCAAATTTATAATGTAATTGTAACAGCACACGC
CTTTGTAATAATCTTTTTCATGGTTATGCCAATAATAATTGGAGGCTTCGGAAATTGACTTATTCCTCTTATGA
TTGGCGCCCCGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGATTGCTCCCTCCATCATTCTCCTT
TACTAGCCTCTTCTGCTGTAGAAGCCGGGGCCGGAACCGGGTGAACAGTTTACCCACCCCTAGCCAGCAAT

TTAGCCACGCAGGGGCTCCGTCGATCTAACCATTTCTCCCTACACCTGGCTGGTGTATCCTCAATTTTAG
GGGCCATTAACCTTTACTACTATTATTAACATAAAACCCCTGCTATTTCTCAATATCAAACCCCTATTTG
TATGGGCTATCTTAATTACAGCCGACTACTTTTACTTTCCCTCCCAGTATTAGCTGCAGGCATTACAATGCTA
CTCACAGACCGAAATCTCAACACCACCTTCTTCGACCCTGCTGGC

b. *Channa striata* (Exotic) sequence

OR863890 (*C. striata*) Seq_1

CACAAAGACATTGGCACCCCTTTATCTCGTATTTGGTGCCTGAGCCGGAATAGTGGGCACAGCCCTCAGCCTT
CTAATTCGAGCAGAACTAAGCCAACCTGGCGCTCTCCTCGGAGACGATCAAATTTATAATGTAATTGTAACA
GCACACGCCTTTGTAATAATCTTTTCATGGTTATGCCAATAATAATTGGAGGCTTCGGAAATTGACTTGTTT
CTCTTATGATCGGTGCCCCGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGATTGCTCCCCCATC
ATTCTGCTTTTACTAGCCTCTTCTGCTGTAGAAGCCGGGGCCGGAACCGGATGAACAGTTTACCCACCCCTA
GCCAGCAACTTAGCCACGCAGGGGCTCCGTCGACCTAACGATTTTCTCCCTACACCTAGCTGGTGTGTCCT
CAATCTAGGGGCCATTAACCTTTACTACTATTATTAACATAAAACCCCTGCTATTTCTCAATATCAAACC
CCACTGTTTGATGGGCTATTTAATTACAGCCGACTACTTCTACTTTCCCTCCCAGTATTAGCTGCAGGCAT
TACAATGCTACTCACAGACCGAAATCTCAACACCACCTTCTTCGACCCTGCTGGTGGAGGGGACCC

OR863892 (*C. striata*) Seq_2

CACAAAGACATTGGCACCCCTTTATCTCGTATTTGGTGCCTGAGCCGGAATAGTGGGCACAGCCCTCAGCCTT
CTAATTCGAGCAGAACTAAGCCAACCTGGCGCTCTCCTCGGAGACGATCAAATTTATAATGTAATTGTAACA
GCACACGCCTTTGTAATAATCTTTTCATGGTTATGCCAATAATAATTGGAGGCTTCGGAAATTGACTTGTTT
CTCTTATGATCGGTGCCCCGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGATTGCTCCCCCATC
ATTCTGCTTTTACTAGCCTCTTCTGCTGTAGAAGCCGGGGCCGGAACCGGATGAACAGTTTACCCACCCCTA
GCCAGCAACTTAGCCACGCAGGGGCTCCGTCGACCTAACGATTTTCTCCCTACACCTAGCTGGTGTGTCCT
CAATCTAGGGGCCATTAACCTTTACTACTATTATTAACATAAAACCCCTGCTATTTCTCAATATCAAACC
CCACTGTTTGATGGGCTATTTAATTACAGCCGACTACTTCTACTTTCCCTCCCAGTATTAGCTGCAGGCAT
TACAATGCTACTCACAGACCGAAATCTCAACACCACCTTCTTCGACCCTGCTGGTGGAGGGGACC

OR863894 (*C. striata*) Seq_3

CACAAAGACATTGGCACCCCTTTATCTCGTATTTGGTGCCTGAGCCGGAATAGTGGGCACAGCCCTCAGCCTT
CTAATTCGAGCAGAACTAAGCCAACCTGGCGCTCTCCTCGGAGACGATCAAATTTATAATGTAATTGTAACA
GCACACGCCTTTGTAATAATCTTTTCATGGTTATGCCAATAATAATTGGAGGCTTCGGAAATTGACTTGTTT
CTCTTATGATCGGTGCCCCGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGATTGCTCCCCCATC
ATTCTGCTTTTACTAGCCTCTTCTGCTGTAGAAGCCGGGGCCGGAACCGGATGAACAGTTTACCCACCCCTA
GCCAGCAACTTAGCCACGCAGGGGCTCCGTCGACCTAACGATTTTCTCCCTACACCTAGCTGGTGTGTCCT
CAATCTAGGGGCCATTAACCTTTACTACTATTATTAACATAAAACCCCTGCTATTTCTCAATATCAAACC
CCACTGTTTGATGGGCTATTTAATTACAGCCGACTACTTCTACTTTCCCTCCCAGTATTAGCTGCAGGCAT
TACAATGCTACTCACAGACCGAAATCTCAACACCACCTTCTTCGACCCTGCTGGTGGAGGGGGCCC

OR863891 (*C. striata*)Seq_4

CACAAAGACATTGGCACCCCTTTATCTCGTATTTGGTGCCTGAGCCGGAATAGTGGGCACAGCCCTCAGCCTT
CTAATTCGAGCAGAACTAAGCCAACCTGGCGCTCTCCTCGGAGACGATCAAATTTATAATGTAATTGTAACA
GCACACGCCTTTGTAATAATCTTTTCATGGTTATGCCAATAATAATTGGAGGCTTCGGAAATTGACTTGTTT

CTCTTATGATCGGTGCCCCGACATGGCCTTCCCCGAATAAATAACATGAGCTTCTGATTGCTCCCCCATC
ATTCCTGCTTTTACTAGCCTCTTCTGCTGTAGAAGCCGGGGCCGGAACCGGATGAACAGTTTACCCACCCCTA
GCCAGCAACTTAGCCACGCAGGGGCCTCCGTCGACCTAACGATTTTCTCCCTACACCTAGCTGGTGTGTCTT
CAATTCTAGGGGCCATTAACTTTATTACTACTATTATTAACATAAAACCCCTGCTATTTCTCAATATCAAACC
CCACTGTTTGTATGGGCTATTTAATTACAGCCGTACTACTTCTACTTTCCCTCCCAGTATTAGCTGCAGGCAT
TACAATGCTACTCACAGACCGAAATCTCAACACCACCTTCTTCGACCCTGC

c. *Channa marulius* sequence

OR864288 (*C. marulius*) Seq_1

ATCGGCACCCTTTATTTAGTATTTGGTGCTTGAGCCGGAATAGTAGGCACCGCCCTAAGTCTCTTAATTCGGG
CAGAACTAAGCCAGCCCCGGCGCCCTATTAGGCGATGATCAGATCTATAATGTAATTGTAACAGCGCATGCCT
TTGTAATAATTTTCTTCATGGTCATGCCAATAATAATTGGAGGCTTTGGAACTGACTAGTCCCACTAATAAT
TGGCGCCCCTGACATAGCATTCCCCGCATGAATAACATAAGCTTCTGATTACTTCTCCCTCTTTTCTTCTCT
GCTAGCCTCCTCCGCAGTAGAAGCCGGAGCCGGCACTGGGTGGACCGTTTACCCGCCCTGGCCAGCAACC
TAGCTCACGCAGGGGCTTCTGTAGATTTAACTATCTTCTCCCTGCATCTTGCAGGTGTTTCTCAATCCTAGGT
GCTATTAACCTCATTACAACCTATTATTAACATAAAACCCCTGCCATTTCTCAGTATCAGACACCCCTGTTTGT
CTGAGCCATTCTGATTACTGCCGTACTTCTACTCCTCTCCCTCCCCGTACTGGCCGCCGGCATTACAATGCTAC
TCACAGACCGAAACCTAAACACCACCTTCTTCGACCCCGCCGGCGGAGGAGATCCAATTCTT

OR864286 (*C. marulius*) Seq_2

ATCGGCACCCTTTATTTAGTATTTGGTGCTTGAGCCGGAATAGTAGGCACCGCCCTAAGTCTCTTAATTCGGG
CAGAACTAAGCCAGCCCCGGCGCCCTATTAGGCGATGATCAGATCTATAATGTAATTGTAACAGCGCATGCCT
TTGTAATAATTTTCTTCATGGTCATGCCAATAATAATTGGAGGCTTTGGAACTGACTAGTCCCACTAATAAT
TGGCGCCCCTGACATAGCATTCCCCGCATGAATAACATAAGCTTCTGATTACTTCTCCCTCTTTTCTTCTCT
GCTAGCCTCCTCCGCAGTAGAAGCCGGAGCCGGCACTGGGTGGACCGTTTACCCGCCCTGGCCAGCAACC
TAGCTCACGCAGGGGCTTCTGTAGATTTAACTATCTTCTCCCTGCATCTTGCAGGTGTTTCTCAATCCTAGGT
GCTATTAACCTCATTACAACCTATTATTAACATAAAACCCCTGCCATTTCTCAGTATCAGACACCCCTGTTTGT
CTGAGCCATTCTGATTACTGCCGTACTTCTACTCCTCTCCCTCCCCGTACTGGCCGCCGGCATTACAATGCTAC
TCACAGACCGAAACCTAAACACCACCTTCTTCGACCCCGCCGGCGGAGGAGATCCAATTCTT

OR864285 (*C.marulius*) Seq_3

ATCGGCACCCTTTATTTAGTATTTGGTGCTTGAGCCGGAATAGTAGGCACCGCCCTAAGTCTCTTAATTCGGG
CAGAACTAAGCCAGCCCCGGCGCCCTATTAGGCGATGATCAGATCTATAATGTAATTGTAACAGCGCATGCCT
TTGTAATAATTTTCTTCATGGTCATGCCAATAATAATTGGAGGCTTTGGAACTGACTAGTCCCACTAATAAT
TGGCGCCCCTGACATAGCATTCCCCGCATGAATAACATAAGCTTCTGATTACTTCTCCCTCTTTTCTTCTCT
GCTAGCCTCCTCCGCAGTAGAAGCCGGAGCCGGCACTGGGTGGACCGTTTACCCGCCCTGGCCAGCAACC
TAGCTCACGCAGGGGCTTCTGTAGATTTAACTATCTTCTCCCTGCATCTTGCAGGTGTTTCTCAATCCTAGGT
GCTATTAACCTCATTACAACCTATTATTAACATAAAACCCCTGCCATTTCTCAGTATCAGACACCCCTGTTTGT
CTGAGCCATTCTGATTACTGCCGTACTTCTACTCCTCTCCCTCCCCGTACTGGCCGCCGGCATTACAATGCTAC
TCACAGACCGAAACCTAAACACCACCTTCTTCGACCCCGCCGGCGGAGGAGATCCAATTCTTTATCAACACCT

OR864287 (*C. marulius*) Seq_4

ATCGGCACCCTTTATTTAGTATTTGGTGCTTGAGCCGGAATAGTAGGCACCGCCCTAAGTCTCTTAATTCGGG
CAGAACTAAGCCAGCCCCGGCGCCCTATTAGGCGATGATCAGATCTATAATGTAATTGTAACAGCGCATGCCT
TTGTAATAATTTTCTTCATGGTCATGCCAATAATAATTGGAGGCTTTGGAACTGACTAGTCCCACTAATAAT
TGGCGCCCCTGACATAGCATTCCCCGCATGAATAACATAAGCTTCTGATTACTTCTCCCTCTTTTCTTCTCCT
GCTAGCCTCCTCCGCAGTAGAAGCCGGAGCCGGCACTGGGTGGACCGTTTACCCGCCCCTGGCCAGCAACC
TAGCTCACGCAGGGGCTTCTGTAGATTTAACTATCTTCTCCCTGCATCTTGCAGGTGTTTCCTCAATCCTAGGT
GCTATTAACCTCATTACAACCTATTATTAACATAAAACCCCCTGCCATTTCTCAGTATCAGACACCCCTGTTTGT
CTGAGCCATTCTGATTACTGCCGTACTTCTACTCCTCTCCCTCCCCGTACTGGCCGCCGGCATTACAATGCTAC
TCACAGACCGAAACCTAAACACCACCTTCTTCGACCCCGCCGGCGGAGGAGATCCAATTC

OR864289.1 (*C.marulius*) Seq_5

ATCGGCACCCTTTATTTAGTATTTGGTGCTTGAGCCGGAATAGTAGGCACCGCCCTAAGTCTCTTAATTCGGG
CAGAACTAAGCCAGCCCCGGCGCCCTATTAGGCGATGATCAGATCTATAATGTAATTGTAACAGCGCATGCCT
TTGTAATAATTTTCTTCATGGTCATGCCAATAATAATTGGAGGCTTTGGAACTGACTAGTCCCACTAATAAT
TGGCGCCCCTGACATAGCATTCCCCGCATGAATAACATAAGCTTCTGATTACTTCTCCCTCTTTTCTTCTCCT
GCTAGCCTCCTCCGCAGTAGAAGCCGGAGCCGGCACTGGGTGGACCGTTTACCCGCCCCTGGCCAGCAACC
TAGCTCACGCAGGGGCTTCTGTAGATTTAACTATCTTCTCCCTGCATCTTGCAGGTGTTTCCTCAATCCTAGGT
GCTATTAACCTCATTACAACCTATTATTAACATAAAACCCCCTGCCATTTCTCAGTATCAGACACCCCTGTTTGT
CTGAGCCATTCTGATTACTGCCGTACTTCTACTCCTCTCCCTCCCCGTACTGGCCGCCGGCATTACAATGCTAC
TCACAGACCGAAACCTAAACACCACCTTCTTCGACCCCGCCGGCGGAGGAGATCCAATTC

d. *Channa Gachua* Sequence

OR864281 (*C. gachua*) Seq_1

AACCACAAAGACATTGGCACCCCTTTATCTAGTATTTGGTGCTTGGGCCGGAATAGTCGGCACCGCACTGAGC
CTACTGATCCGGGCTGAACTTAGCCAGCCCCGGTGCTCTTCTAGGCAACGACCAAATTTATAATGTAATTGTTA
CGGCCCACGCCTTCGTCATGATCTTCTTCATGGTAATGCCAATAATAATCGGGGGCTTTGGAACTGACTGG
TCCCGCTTATGATCGGCGCCCCTGACATAGCCTTCCCTCGAATAAACAATATGAGTTTTTGACTTCTCCCCCT
TCTTCTCCTTCTTCTGGCCTCTTCTGCAGTAGAAGCCGGAGCTGGGACAGGCTGGACAGTTTACCCACCTT
TAGCTGGCAATCTGGCTCACGCGGGGGCATCCGTAGACCTAGCCATCTTCTTTTACACCTTGCAGGTGTCTC
TTCAATTTTAGGGGCAATTAACCTCATCACAACGATCATTAACATGAAACCCCCAGCCATCTCTCAGTACCAG
ACACCTCTGTTTGTATGGGCCATCCTAATCACTGCCATCCTTCTACTTCTTCTCTTCCCGTTTTAGCCGCCGGT
ATCACAATACTATTAACAGACCGAACTTAACACAACCTTCTTTGACCCCGTGGGG

OR864278 (*C.gachua*) Seq_2

AACCACAAAGACATTGGCACCCCTTTATCTAGTATTTGGTGCTTGGGCCGGAATAGTCGGCACCGCACTGAGC
CTACTGATCCGGGCTGAACTTAGCCAGCCCCGGTGCTCTTCTAGGCAACGACCAAATTTATAATGTAATTGTTA
CGGCCCACGCCTTCGTCATGATCTTCTTCATGGTAATGCCAATAATAATCGGGGGCTTTGGAACTGACTGG
TCCCGCTTATGATCGGCGCCCCTGACATAGCCTTCCCTCGAATAAACAATATGAGTTTTTGACTTCTCCCCCT
TCTTCTCCTTCTTCTGGCCTCTTCTGCAGTAGAAGCCGGAGCTGGGACAGGCTGGACAGTTTACCCACCTT
TAGCTGGCAATCTGGCTCACGCGGGGGCATCCGTAGACCTAGCCATCTTCTTTTACACCTTGCAGGTGTCTC
TTCAATTTTAGGGGCAATTAACCTCATCACAACGATCATTAACATGAAACCCCCAGCCATCTCTCAGTACCAG

ACACCTCTGTTTGTATGGGCCATCCTAATCACTGCCATCCTTCTACTTCTTTCTCTTCCCGTTTTAGCCGCCGGT
ATCACAATACTATTAACAGACCGAACTTAACACAACCTTCTTTGACCCCGTG GGGG

OR864280 (*C.gachua*) Seq_3

AACCACAAAGACATTGGCACCCCTTTATCTAGTATTTGGTGCTTGGGCCGGAATAGTCGGCACCGCACTGAGC
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CGGCCCACGCCTTCGTCATGATCTTCTTCATGGTAATGCCAATAATAATCGGGGGCTTTGGAACTGACTGG
TCCCGCTTATGATCGGCGCCCCTGACATAGCCTTCCCTCGAATAAACAATATGAGTTTTTGACTTCTCCCCCT
TCTTTCCTCCTTCTTCTGGCCTCTTCTGCAGTAGAAGCCGGAGCTGGGACAGGCTGGACAGTTTACCCACCTT
TAGCTGGCAATCTGGCTCACGCGGGGGCATCCGTAGACCTAGCCATCTTCTTTACACCTTGCAGGTGTCTC
TTCAATTTTAGGGGCAATTAACCTTCATCACAACGATCATTAAACATGAAACCCCCAGCCATCTCTCAGTACCAG
ACACCTCTGTTTGTATGGGCCATCCTAATCACTGCCATCCTTCTACTTCTTTCTCTTCCCGTTTTAGCCGCCGGT
ATCACAATACTATTAACAGACCGAACT

OR864283 (*C. gachua*) Seq_4

AACCACAAAGACATTGGCACCCCTTTATCTAGTATTTGGTGCTTGGGCCGGAATAGTCGGCACCGCACTGAGC
CTACTGATCCGGGCTGAACTTAGCCAGCCCGGTGCTCTTCTAGGCAACGACCAAATTTATAATGTAATTGTTA
CGGCCCACGCCTTCGTCATGATCTTCTTCATGGTAATGCCAATAATAATCGGGGGCTTTGGAACTGACTGG
TCCCGCTTATGATCGGCGCCCCTGACATAGCCTTCCCTCGAATAAACAATATGAGTTTTTGACTTCTCCCCCT
TCTTTCCTCCTTCTTCTGGCCTCTTCTGCAGTAGAAGCCGGAGCTGGGACAGGCTGGACAGTTTACCCACCTT
TAGCTGGCAATCTGGCTCACGCGGGGGCATCCGTAGACCTAGCCATCTTCTTTACACCTTGCAGGTGTCTC
TTCAATTTTAGGGGCAATTAACCTTCATCACAACGATCATTAAACATGAAACCCCCAGCCATCTCTCAGTACCAG
ACACCTCTGTTTGTATGGGCCATCCTAATCACTGCCATCCTTCTACTTCTTTCTCTTCCCGTTTTAGCCGCCGGT
ATCACAATACTATTAACAGACCGAACTTAACACAACCTTCTTTGACCCCGTG GGGG

OR864284 (*C.gachua*) Seq_5

AACCACAAAGACATTGGCACCCCTTTATCTAGTATTTGGTGCTTGGGCCGGAATAGTCGGCACCGCACTGAGC
CTACTGATCCGGGCTGAACTTAGCCAGCCCGGTGCTCTTCTAGGCAACGACCAAATTTATAATGTAATTGTTA
CGGCCCACGCCTTCGTCATGATCTTCTTCATGGTAATGCCAATAATAATCGGGGGCTTTGGAACTGACTGG
TCCCGCTTATGATCGGCGCCCCTGACATAGCCTTCCCTCGAATAAACAATATGAGTTTTTGACTTCTCCCCCT
TCTTTCCTCCTTCTTCTGGCCTCTTCTGCAGTAGAAGCCGGAGCTGGGACAGGCTGGACAGTTTACCCACCTT
TAGCTGGCAATCTGGCTCACGCGGGGGCATCCGTAGACCTAGCCATCTTCTTTACACCTTGCAGGTGTCTC
TTCAATTTTAGGGGCAATTAACCTTCATCACAACGATCATTAAACATGAAACCCCCAGCCATCTCTCAGTACCAG
ACACCTCTGTTTGTATGGGCCATCCTAATCACTGCCATCCTTCTACTTCTTTCTCTTCCCGTTTTAGCCGCCGGT
ATCACAATACTATTAACAGACCGAACTTAACACAACCTTCTTTGACCCCGTG GGGG

OR864279 (*C. gachua*)Seq_6

AACCACAAAGACATTGGCACCCCTTTATCTAGTATTTGGTGCTTGGGCCGGAATAGTCGGCACCGCACTGAGC
CTACTGATCCGGGCTGAACTTAGCCAGCCCGGTGCTCTTCTAGGCAACGACCAAATTTATAATGTAATTGTTA
CGGCCCACGCCTTCGTCATGATCTTCTTCATGGTAATGCCAATAATAATCGGGGGCTTTGGAACTGACTGG
TCCCGCTTATGATCGGCGCCCCTGACATAGCCTTCCCTCGAATAAACAATATGAGTTTTTGACTTCTCCCCCT
TCTTTCCTCCTTCTTCTGGCCTCTTCTGCAGTAGAAGCCGGAGCTGGGACAGGCTGGACAGTTTACCCACCTT
TAGCTGGCAATCTGGCTCACGCGGGGGCATCCGTAGACCTAGCCATCTTCTTTACACCTTGCAGGTGTCTC
TTCAATTTTAGGGGCAATTAACCTTCATCACAACGATCATTAAACATGAAACCCCCAGCCATCTCTCAGTACCAG

ACACCTCTGTTTGTATGGGCCATCCTAATCACTGCCATCCTTCTACTTCTTTCTCTTCCCGTTTTAGCCGCCGGT
ATCACAATACTATTAAACAGACCGAACTTAAACACAACCTTCTTTGACCCCGTGGGGG

e. *Channa Punctata* sequence

OR864277 (*C.punctata*) Seq_1

CTCTATCTTGTATTTGGTGCTTGAGCCGGAATAGTAGGCACAGCTCTAAGCCTTCTAATTCGAGCAGAACTTA
GCCAGCCCGGCGCTCTTCTAGGTGACGACCAGATTATAATGTAATCGTCACAGCACACGCCTTTGTAATAA
TTTTCTTCATGGTTATGCCAATAATAATTGGGGGATTGGTAATTGACTAGTCCCTTTAATAATTGGCGCCCC
CGACATAGCCTTCCCCGAATGAACAACATAAGCTTCTGACTCCTTCCCCCTTCTTTTCTTTACTACTGGCCT
CTTCGCGAGTTGAAGCTGGGGCCGGAACCGGGTGGACAGTTTACCCACCTCTAGCCAGCAATCTGGCCAC
GCAGGGGCCTCTGTAGACTTAACCATCTTCTCATTACACCTTGCAGGTGTCTCCTCTATTTTAGGCGCCATCA
ACTTCATTACGACGATTATTAACATGAAACCCCTGCCATCTCTCAGTACCAAACCTCCCTTATTTGTGTGAGCT
ATTTAATTACAGCCGTACTTCTCCTTCTGTCCCTTCCAGTCTTAGCCGCCGGAATCACAATATTGCTCACCGA
CCGAAACCTAAACACCACCTTCTTTGACCCCG

OR864276 (*C.punctata*) Seq_2

CTTGTATTTGGTGCTTGAGCCGGAATAGTAGGCACAGCTCTAAGCCTTCTAATTCGAGCAGAACTTAGCCAG
CCCGGCGCTCTTCTAGGTGACGACCAGATTATAATGTAATCGTCACAGCACACGCCTTTGTAATAATTTCT
TCATGGTTATGCCAATAATAATTGGGGGATTGGTAATTGACTAGTCCCTTTAATAATTGGCGCCCCGACAT
AGCCTTCCCCGAATGAACAACATAAGCTTCTGACTCCTTCCCCCTTCTTTTCTTTACTACTGGCCTCTTCGC
AGTTGAAGCTGGGGCCGGAACCGGGTGGACAGTTTACCCACCTCTAGCCAGCAATCTGGCCACGCAGGG
GCCTCTGTAGACTTAACCATCTTCTCATTACACCTTGCAGGTGTCTCCTCTATTTTAGGCGCCATCAACTTCAT
TACGACGATTATTAACATGAAACCCCTGCCATCTCTCAGTACCAAACCTCCCTTATTTGTGTGAGCTATTTAA
TTACAGCCGTACTTCTCCTTCTGTCCCTTCCAGTCTTAGCCGCCGGAATCACAATATTGCTCACCGACCGAAAC
CTAAACACCACCTTCTTTGACCCC

Appendix 4: Data collection sheet for Growth and Reproductive Biology Study of *Channa striata* in the Drought-Stressed Atrai River

Month	monthNo	FishNo	TL	SL	BW	HL	Sex	GW	GL	NE	LW	OvaDiam	MatStg
January	1	180	36.42	31.36	335.45	10.19	F	1.68	5.16		1.66		Imature
January	1	179	36	31	327	10.1	F	1.31	5		1.62		Imature
January	1	196	34.4	29.31	318.35	9.13	F	1.25	4.31		2.01		Imature
January	1	195	34	29	311	9	F	0.87	4.1		2		Imature
January	1	184	33.45	28.24	287.82	9.1	F	1.72	4.2		1.05		Imature
January	1	183	33	28	286	9	F	1.34	4		1.04		Imature
January	1	198	32.83	29.9	322.21	9.73	F	1.05	3.25		1.78		Imature
January	1	197	32.5	29.5	314	9.6	F	0.95	3.2		1.75		Imature
January	1	188	32.4	27.32	282.86	8.98	M	0.66	2.24		2.3		Imature
January	1	187	32	27	281	8.9	M	0.24	2.1		2.29		Imature
January	1	194	31.45	27.26	241.94	9.01	F	0.67	3.63		1.06		Imature
January	1	182	31.03	26.37	222.43	8.9	M	1.73	4.22		1.36		Imature

January	1	193	31	27	238	8.9	F	0.41	3.5		1.03		Imature
January	1	186	30.92	26.74	241.51	7.23	F	0.22	2.29		1.17		Imature
January	1	185	30.5	26.5	232	7.1	F	0.18	2.2		1.15		Imature
January	1	181	30.5	26	218	8.8	M	1.3	4.2		1.34		Imature
January	1	192	30.47	25.8	263.22	8.72	F	0.64	2.33		1.64		Imature
January	1	191	30	25.5	257	8.6	F	0.4	2.3		1.6		Imature
January	1	200	29.93	26.29	314.78	9.28	F	1.38	4.31		2.24		Imature
January	1	190	29.78	25.79	226.38	8.38	F	1.04	3.51		1.32		Imature
January	1	189	29.5	25.5	218	8.3	F	0.7	3.5		1.3		Imature
January	1	199	29.5	26	309	9.2	F	1.24	4.1		2.2		Imature
February	2	218	38.41	33.42	471.35	10.72	F	2.07	5.63		2.31		Imature
February	2	217	38	33	468	10.6	F	1.9	5.5		2.3		Imature
February	2	214	37.85	32.81	404.62	9.93	F	0.2	1.2		2.09		Imature
February	2	220	37.51	32.24	424.14	10.28	M	0.64	1.39		2.12		Imature
February	2	213	37.5	32.5	399	9.8	F	0.17	1		2.05		Imature
February	2	219	37	32	420	10.2	M	0.6	1.3		2.1		Imature
February	2	210	36.36	31.29	398.41	10.01	F	0.39	1.75		2.76		Imature

February	2	204	36.33	31.43	407.87	9.52	M	0.23	0.89		3.06		Imature
February	2	209	36	31	390	9.9	F	0.19	1.5		2.73		Imature
February	2	203	36	31	399	9.4	M	0.12	0.7		3.03		Imature
February	2	216	35.31	30.3	399.94	9.99	M	0.24	3.01		1.92		Imature
February	2	215	35	30	390	9.9	M	0.2	3		1.89		Imature
February	2	202	34.52	29.91	326.78	9.31	F	0.56	1.6		2.3		Imature
February	2	201	34	29.5	317	9.2	F	0.16	1.5		2.26		Imature
February	2	206	33.49	28.4	329.28	9.53	F	0.65	1.02		1.82		Imature
February	2	205	33	28	323	9.4	F	0.22	1		1.8		Imature
February	2	212	32.85	28.29	287.63	9.19	F	0.29	1.41		2.08		Imature
February	2	211	32.5	28	285	9.1	F	0.12	1.2		2.07		Imature
February	2	208	30.34	26.29	244.36	9.13	M	0.52	0.95		1.39		Imature
February	2	207	30	26	239	9	M	0.1	0.9		1.37		Imature
March	3	230	39.88	33.26	486.08	11.8	M	1.18	3.67		3.08		Maturing
March	3	229	39.5	33	481	11.7	M	0.75	3.5		3.05		Maturing
March	3	222	36.32	31.34	339	10.5	M	0.46	3.45		1.73		Maturing
March	3	221	36	31	335	10.4	M	0.29	3.4		1.72		Maturing

March	3	240	33.53	28.34	272.71	9.23	M	0.18	0.85		1.67		Maturing
March	3	239	33	28	263	9.1	M	0.13	0.8		1.63		Maturing
March	3	226	35.39	30.34	299.41	10.18	F	0.98	3.7		1.98		Maturing
March	3	225	35	30	294	10.1	F	0.89	3.6		1.95		Maturing
March	3	236	31.37	27.25	246.88	8.41	M	0.23	4.76		1.42		Maturing
March	3	224	31.3	27.42	257.74	9.08	M	0.29	4.1		1.43		Maturing
March	3	234	34.85	29.87	355.66	10.03	F	2.45	4.84		2		Maturing
March	3	232	34.52	29.39	306.74	9.61	F	3.68	6.56		1.7		Maturing
March	3	233	34.5	29.5	348	9.9	F	2.11	4.8		1.97		Maturing
March	3	231	34	29	301	9.5	F	3.27	6.3		1.67		Maturing
March	3	238	33.88	28.94	287.03	9.92	F	2.3	5.68		1.55		Maturing
March	3	223	31	27	255	9	M	0.16	4		1.4		Maturing
March	3	237	33.5	28.5	283	9.8	F	2.02	5.6		1.52		Maturing
March	3	235	31	27	245	8.3	M	0.14	4.7		1.38		Maturing
March	3	228	28.41	24.24	178.16	7.49	M	0.5	3.81		0.97		Maturing
March	3	227	28	24	174	7.4	M	0.12	3.6		0.93		Maturing
May	5	25	46.5	39.33	746.09	11.83	F	34.26	12.68	866	3.58	11.66	Spawning

April	4	12	46.41	39.41	588.91	12	M	0.71	1.97		3.49		Mature
May	5	26	46	39	738	11.7	F	34	12.5	858	3.54	11.55	Spawning
April	4	11	46	39	581	11.9	M	0.7	1.8		3.46		Mature
April	4	5	45.95	38.87	756.65	12.59	F	18	11.1	543	4.4	11.34	Spawning
April	4	1	45.84	39.84	794.08	10.7	F	29.06	10.57	614	3.16	12.23	Spawning
April	4	2	45.5	39.5	785	10.6	F	28.5	10.5	610	3.12	12.12	Spawning
April	4	6	45.5	38.5	749	12.5	F	18.21	10.9	560	4.37	11.23	Spawning
April	4	16	45.41	38.32	661.4	12.11	M	3.12	2.06		4.83		Mature
April	4	15	45	38	658	12	M	3	2		4.8		Mature
April	4	18	41.36	36.38	359.14	9.82	M	3.2	3.3		3.12		Mature
April	4	17	41	36	352	9.7	M	3	3.2		3.1		Mature
April	4	10	35.45	30.3	362.66	9.91	M	1.64	2.75		2.86		Maturing
April	4	14	35.34	30.35	326.06	9.58	M	1.4	2.17		3.32		Maturing
April	4	20	35.34	30.29	328.98	8.29	M	2.36	2.73		3.33		Maturing
April	4	9	35	30	354	9.8	M	1.5	2.5		2.82		Maturing
April	4	13	35	30	321	9.5	M	1.2	2.1		3.3		Maturing
April	4	19	35	30	325	8.2	M	2.2	2.7		3.3		Maturing

April	4	3	41.92	36.42	526.41	9.63	F	19.7	10.06	655	3.14	14.69	Maturing
April	4	4	41.5	36	520	9.5	F	19.6	10	650	3.1	14.57	Maturing
May	5	28	46.32	40.28	726.78	11.59	M	1.21	4.36		4.38		Maturing
May	5	27	46	40	722	11.5	M	1.09	4.2		4.36		Maturing
May	5	36	34.91	30.36	365.21	10.13	M	6.12	6.24		3.53		Mature
May	5	35	34.5	30	357	10	M	6.1	6.21		3.5		Mature
May	5	34	32.47	27.26	269.67	9.1	M	5.33	5.37		2.55		Mature
May	5	33	32	27	262	9	M	5.12	5.24		2.52		Mature
May	5	30	30.37	26.76	213.54	8.23	M	1.38	4.31		1.88		Maturing
May	5	29	30	26.4	209	8.1	M	1.18	4.1		1.85		Maturing
May	5	32	28.88	24.75	189.14	8.1	M	1.69	4.46		1.86		Maturing
May	5	31	28.5	24.5	185	8	M	1.6	4.3		1.83		Maturing
June	6	52	35.41	28.6	352.95	10.33	M	1.41	2.19		1.62		Spent
June	6	51	35	28.2	350	10.2	M	1.4	2.1		1.6		Spent
May	5	23	41.31	34.94	474.6	11.03	F	39	9.05	1208	4.63	12.71	Maturing
June	6	48	34.84	29.3	366.03	10.52	M	1.21	2.51		3.12		Spent
June	6	47	34.5	29	361	10.4	M	1.2	2.3		3.1		Spent

May	5	24	41	34.5	465	10.9	F	29	9	540	4.6	12.59	Maturing
May	5	22	40	33.9	468.6	10.1	F	43.7	8.8	1200	3.2	13.75	Maturing
May	5	21	35.34	29.25	369.85	9.7	F	35.96	9.04	805	3.24	13.87	Maturing
June	6	54	34.44	28.84	404.14	11.29	M	1.82	3.31		2.63		Maturing
June	6	50	34.4	29.33	316.23	9.89	M	1.31	2.19		1.97		Spent
June	6	53	34	28.5	400	11.2	M	1.8	3.1		2.6		Maturing
June	6	49	34	29	313	9.8	M	1.3	2.1		1.95		Spent
July	7	60	46.3	40.29	772.58	10.89	M	1.67	5.24		4.25		Spawning
July	7	59	46	40	770	10.8	M	1.32	5		4.21		Spawning
July	7	64	44.31	37.31	655.87	12.22	M	0.41	2.12		3.56		Spend
April	4	7	39.37	33.37	571.12	12.41	F	24.37	9.56	565	3.21	11.72	Spawning
July	7	63	44	37	652	12.1	M	0.2	2.1		3.54		Spend
June	6	41	42.34	35.46	522.01	10.21	F	19.61	9.88	465	1.63	12.41	Maturing
July	7	56	43.31	36.93	603.33	9.9	M	1.74	4.85		3.24		Mature
June	6	42	42	35.2	520	10.1	F	19.6	9.8	460	1.6	12.3	Maturing
June	6	43	41.45	35.26	526.13	10.31	F	21.36	10.18	523	1.72	13.31	Mature
June	6	44	41	35	518	10.2	F	21.3	10	520	1.7	13.2	Mature

June	6	37	40.4	34.3	475.99	10.13	F	22.43	10.26	454	2.23	12.72	Mature
July	7	55	43	36.5	600	9.8	M	1.6	4.8		3.2		Mature
June	6	38	40	34	472	10	F	22.23	10	450	2.2	12.6	Mature
June	6	45	38.37	33.91	474.4	9.71	F	18.31	9.72	484	2.23	12.81	Mature
June	6	46	38	33.6	469	9.6	F	18.2	9.7	480	2.2	12.7	Mature
July	7	62	41.47	36.38	530.36	9.42	M	1.14	4.13		3.21		Maturing
June	6	39	37.29	32.86	396.69	9.59	F	18.62	9.67	505	1.94	13.62	Mature
July	7	66	41.44	35.34	577.54	12.08	M	0.67	1.96		3.85		Imature
July	7	65	41	35	572	12	M	0.35	1.8		3.81		Imature
July	7	61	41	36	521	9.3	M	1	4		3.17		Maturing
June	6	40	37	32.5	388	9.5	F	18.5	9.5	500	1.9	13.5	Mature
July	7	58	37.36	33.28	423.73	9.61	M	1.46	4.32		3.17		Spawning
July	7	57	37	33	414	9.5	M	1.3	4.2		3.15		Spawning
August	8	80	47.37	39.76	686.61	11.82	M	6.05	6.05		1.82		Mature
April	4	8	39	33	569	12.3	F	24.26	9.3	800	3.18	11.59	Spawning
August	8	79	47	39.5	680	11.7	M	5.87	6		1.8		Mature
July	7	71	46.31	41.3	564.75	11.29	F	8.5	6.04	435	3.82	11.51	Mature

July	7	72	46	41	560	11.2	F	8.27	6	432	3.8	11.4	Mature
August	8	82	43.34	36.11	552.8	10.51	M	5.32	5.58		2.12		Mature
August	8	78	43.31	36.39	547.81	10.62	M	0.78	2.06		2.64		Spend
August	8	77	43	36	540	10.5	M	0.6	1.8		2.6		Spend
August	8	81	43	35.8	550	10.4	M	5.27	5.5		2.1		Mature
August	8	76	40.44	34.38	500.47	10.38	M	0.74	1.63		2.52		Imature
August	8	75	40	34	496	10.3	M	0.5	1.5		2.5		Imature
August	8	84	39.33	34.8	484.18	9.53	M	3.26	3.48		1.64		Mature
August	8	83	39	34.5	478	9.4	M	3.2	3.4		1.6		Mature
September	9	104	53.41	44.9	1087.66	13.03	M	2.24	3.26		3.84		Maturing
September	9	103	53	44.5	1081	12.9	M	2.02	3		3.81		Maturing
July	7	67	41.38	35.3	545.13	11.2	F	6.1	5.24	356	4.43	12.01	Maturing
July	7	74	41.31	35.26	453.39	9.51	F	2.57	3.29		2.63		Imature
July	7	73	41	35	451	9.4	F	2.5	3.2		2.6		Imature
September	9	98	48.99	41.36	969.16	12.89	M	0.77	2.81		3.43		Maturing
July	7	68	41	35	541	11.1	F	5.98	5	350	4.4	11.9	Maturing
September	9	97	48.5	41	964	12.8	M	0.74	2.6		3.4		Maturing

July	7	69	36.47	31.33	403.27	10.01	F	11.41	6.39	525	4.34	12.41	Mature
July	7	70	36	31	398	9.9	F	11.2	6.2	520	4.3	12.3	Mature
September	9	102	48.42	41.41	809.53	12.43	M	1.6	2.68		4.26		Maturing
September	9	101	48	41	805	12.3	M	1.44	2.5		4.22		Maturing
August	8	87	45.38	40.39	764.8	12.73	F	27.22	10.89	385	4.24	11.45	Maturing
August	8	90	45.34	38.33	634.11	11.61	F	0.65	2.56		4.66		Maturing
August	8	88	45	40	758	12.6	F	27.03	10.7	380	4.2	11.33	Maturing
August	8	89	45	38	630	11.5	F	0.64	2.5		4.62		Maturing
September	9	100	44.29	37.42	757.62	11.89	M	0.87	2.32		4.24		Maturing
September	9	99	44	37	755	11.8	M	0.66	2.3		4.21		Maturing
September	9	106	42.39	35.85	541.15	10.08	M	1.36	3.75		4.21		Maturing
September	9	105	42	35.5	535	10	M	1.15	3.5		4.17		Maturing
September	9	96	37.45	31.28	368	9.98	M	1.1	3.19		3.52		Maturing
September	9	95	37	31	358	9.9	M	0.83	3		3.5		Maturing
October	10	138	38.35	32.36	522.48	9.39	M	1.57	3.55		3.14		Spent
October	10	137	38	32	517	9.3	M	1.56	3.5		3.12		Spent
October	10	120	37.34	32.33	420.62	11.22	M	0.54	3.17		4.53		Spent

October	10	119	37	32	413	11.1	M	0.53	3.1		4.5		Spent
October	10	134	36.37	31.34	494.06	10.82	M	1.32	3.59		2.6		Spent
October	10	133	36	31	486	10.7	M	1.3	3.56		2.58		Spent
September	9	107	47.5	40.5	858	12.9	F	1	2		4.83		Spent
September	9	108	47.82	40.85	862.36	13	F	1.02	2.12		4.85		Spent
September	9	109	32	27.5	319	10.2	F	1.5	2.2		1.98		Spent
September	9	110	32.37	27.88	325.23	10.3	F	1.51	2.45		2.01		Spent
September	9	111	29.5	25	249	9.2	F	1.2	2.2		1.45		Spent
September	9	112	29.91	25.35	256.29	9.33	F	1.21	2.46		1.47		Spent
September	9	113	46.6	40.1	820	12.1	F	1.3	2.2		4.2		Spent
September	9	114	46.95	40.45	823.33	12.2	F	1.32	2.46		4.24		Spent
September	9	115	33	27	360	10	F	1.6	2		1.7		Spent
September	9	116	33.42	27.27	368.11	10.13	F	1.61	2.24		1.73		Spent
September	9	117	42	41	870		F	2.2	2.8		3.25		Spent
September	9	118	42.43	41.41	872.67	0.12	F	2.22	2.98		3.28		Spent
August	8	91	35.37	29.36	361.35	10.11	F	8.97	6.52	283	3.77	11.9	Maturing
October	10	126	35.36	29.33	301.57	9.72	M	0.56	2.97		1.6		Spent

October	10	125	35	29	296	9.6	M	0.54	2.9		1.58		Spent
October	10	132	31.38	26.29	255.18	9.09	M	0.55	3.16		1.71		Spent
October	10	123	30	26.5	246	10.4	F	0.62	2.8		1.88		Spent
October	10	124	30.36	26.88	251.22	10.5	F	0.63	2.9		1.9		Spent
August	8	92	35	29	355	10	F	8.95	6.5	280	3.75	11.78	Maturing
October	10	122	31.37	26.38	260.66	9.62	M	0.63	3.16		1.87		Spent
October	10	127	37.5	31.5	395	10.1	F	0.48	26		3.76		Spent
October	10	128	37.87	31.77	400.04	10.2	F	0.5	26.16		3.78		Spent
October	10	129	34	29	308	9.3	F	0.52	3.1		1.96		Spent
October	10	130	34.32	29.26	310.59	9.43	F	0.54	3.13		1.98		Spent
August	8	85	33.45	29.39	315.64	9.11	F	6.28	6.63	275	2.93	10.97	Maturing
August	8	94	33.36	28.36	358.17	8.9	F	1.28	4.36		3.03		Maturing
August	8	86	33	29	311	9	F	6.14	6.54	270	2.89	10.86	Maturing
August	8	93	33	28	353	8.8	F	1.24	4.2		3		Maturing
October	10	135	38	33	551	13	F	1.2	3.4		3.49		Spent
October	10	136	38.33	33.38	555.83	13.12	F	1.21	3.45		3.52		Spent
October	10	121	31	26	256	9.5	M	0.61	3		1.85		Spent

October	10	131	31	26	248	9	M	0.53	3.12		1.68		Spent
November	11	146	42.42	36.28	660.59	11.81	M	0.5	2.59		2.21		Spent
November	11	154	42.34	36.89	495.55	11.59	M	0.54	2.85		2.12		Spent
November	11	145	42	36	655	11.7	M	0.49	2.56		2.18		Spent
November	11	153	42	36.6	490	11.5	M	0.52	2.8		2.1		Spent
November	11	143	38.5	33	534	10.6	F	0.56	3		1.9		Spent
November	11	144	38.87	33.29	540.03	10.71	F	0.57	3.03		1.92		Spent
November	11	158	41.31	38.38	612.79	11.3	M	0.71	3.24		2.22		Spent
November	11	157	41	38	610	11.2	M	0.7	3.2		2.2		Spent
November	11	147	45.5	39	824	12.7	F	0.58	3		2.2		Spent
November	11	148	45.86	39.34	828.75	12.8	F	0.59	3.05		2.23		Spent
November	11	149	37.5	32.5	514	10.5	F	2.63	4.8		3.52		Spent
November	11	150	37.89	32.82	518.81	10.59	F	2.65	4.84		3.54		Spent
November	11	142	40.44	35.37	586.02	11.12	M	0.93	3.16		2.92		Spent
November	11	141	40	35	579	11	M	0.92	3.12		2.89		Spent
November	11	140	38.41	34.29	530.18	10.61	M	0.55	2.63		2.97		Spent
November	11	152	38.39	34.57	515.71	10.09	M	0.62	2.35		2.23		Spent

November	11	155	37	33	530	10	F	2.1	4.1		3.6		Spent
November	11	156	37.38	33.35	533.01	10.11	F	2.12	4.14		3.63		Spent
November	11	139	38	34	524	10.5	M	0.53	2.6		2.95		Spent
November	11	151	38	34.2	510	10	M	0.6	2.3		2.2		Spent
December	12	166	38.84	32.77	399.14	9.99	F	0.24	2.24		1.94		Imature
December	12	165	38.5	32.5	396	9.9	F	0.23	2.2		1.92		Imature
December	12	160	38.35	32.28	430.31	10.69	M	1.34	5.14		2.59		Imature
December	12	159	38	32	427	10.6	M	1.33	5.1		2.57		Imature
December	12	174	36.86	30.79	361.12	10.4	M	0.42	3.54		1.62		Imature
December	12	164	36.84	30.81	351.79	9.59	F	1.33	4.23		1.77		Imature
December	12	173	36.5	30.5	358	10.3	M	0.41	3.5		1.6		Imature
December	12	163	36.5	30.5	349	9.5	F	1.32	4.2		1.75		Imature
December	12	162	36.31	30.82	332.93	10.29	M	1.41	4.14		1.87		Imature
December	12	161	36	30.5	329	10.2	M	1.4	4.1		1.85		Imature
December	12	172	35.34	30.3	353.99	9.4	F	0.61	2.63		1.22		Imature
December	12	171	35	30	351	9.3	F	0.6	2.6		1.2		Imature
December	12	178	34.34	29.29	298.96	9.3	F	0.96	3.24		1.82		Imature

December	12	177	34	29	296	9.2	F	0.95	3.2		1.8		Imature
December	12	176	33.84	29.29	294.95	9.5	F	0.88	4.14		1.77		Imature
December	12	175	33.5	29	292	9.4	F	0.87	4.1		1.75		Imature
December	12	170	31.83	27.78	274.38	9	F	0.71	3.54		1.6		Imature
December	12	169	31.5	27.5	271	8.9	F	0.7	3.5		1.58		Imature
December	12	168	31.36	27.3	255.44	9.2	M	0.25	2.14		1.68		Imature
December	12	167	31	27	252	9.1	M	0.24	2.1		1.66		Imature

Appedix 5. Characteristics of gonad maturation stages of male *C. striata*

	Immature (Stage I)	Maturing (Stage II)	Mature (Stage III)	Spawning (Stage IV)	Spent (Stage V).
Macroscopic/ morphological appearance	The testes in this stage were transparent, minuscule, and rich with spermatogonia. Primary spermatocytes surround by testicular wall were visible microscopically (Figure 3.9A).	Larger in size than immature stage. Sperm cells are clearer and more visible. Additionally, spermatids and spermatocytes were more prevalent. During the initial phase, the testes are large and white. The testicular wall displayed capillary blood capillaries. Further become larger in size and white (Figure 3.9 B).	Large in size and weight with slightly pinkish color. There were a considerable amount of spermatozoa with visible secondary and tertiary spermatocytes . Whereas, primary spermatocytes were getting fewer (Figure 3.9 C).	In this spawning stage testes were characterized by dark and opaque color. Soft and limp and most are whippy and very squiggly. Blood vessel is more evident and semen containing sperm cell will spread with a gentle pressure. The majority of spermatozoa go toward the lobule's edge (Figure 3.9 D).	Become short and shrinkage again whitish color. No secondary spermatozoa are found (Figure 3.9 E)

Histological observation (Figure 3.9G and 3.9H)	In this stage, largest germ cell found in the testes. Spermatogenesis is in its initial stage at this point.	Through meiotic division, spermatogonia became spermatocytes, Which appeared spherical in shape and had a nucleus in the center	Spermatocytes transformed into spermatids. Relatively smaller and spherical in shape.	Through the process of spermiogenesis, functioning male gametes were produced from spermatids	Empty lumen found
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Appendix 6. Characteristics of gonad maturation stages of female *C. striata*

	Immature (Stage I)	Maturing (Stage II)	Mature (Stage III)	Spawning (Stage IV)	Spent (Stage V)
Macroscopic/ morphological appearance	Ovary is small in size, transparent with ribbon-like structure. No oocytes were visible (Figure 3.10A).	Ovary is comparatively larger than immature stage and unbent. Oocytes are reddish-white in color and resemble white spots (Figure 3.10 B).	The size of the ovary grows, and the oocytes are visible and have a yellowish-orange color (Figure 3.10 C).	The ovary completely enlarges and produces yellowish-colored eggs that fill the abdominal cavity (Figure 3.10 D)	Sizes become smaller again with few amounts of oocyte were seen and look like membranes (Figure 3.10 E).
Histological observation (Figure 3.10 G and 3.10 H)	Multiple nucleoid steps of primary growth were observed. Ova has granule-like structures	Primary oocytes are prominent in this stage. Oil droplet is visible	Vitellogenesis started and gradually evident by late vitellogenesis. Half of the ooplasm is filled with yolk granules. Peripheral migration of the nucleus has not yet started.	Vitellogenesis is at its highest point; bigger, more hydrated cells ; The nucleus has moved to the cell's outer edge and is dissolving at this time.	Short and appeared with some oocytes undergoing atresia.