A multidisciplinary approach to study the feeding behavior of Grey-eel catfish (*Plotosus canius*) in exploring the blue economy of the Bay of Bengal

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ABSTRACT

Abstract:

The feeding ecology of fish is closely related to its population dynamics, and knowledge can help to understand resource allocation, habitat preferences, prey selection, predation, evolution, competition, and the transfer of energy within and between ecosystems. Therefore, this research aimed to examine the data on the feeding habits of a commercially important catfish, *Plotossus canius* from two different regions (Chattogram and Cox's Bazar) of Bangladesh. The live fish were collected from the local fishermen and gut content was assessed and different gastrointestinal indices like visceral somatic index (VSI), hepatosomatic index (HSI), intestine somatic index (ISI), condition factor (K), relative condition factor (Kn), and relative length of gut (RLG) were measured. Gut content analysis showed that crustaceans (45%), bivalves and mussels (36%), arthropods, and isopods (11%) were the dominant food items in *P. canius*. Measured gastrointestinal indices revealed that fishes from the Chattogram region had significantly higher VSI, HSI, ISI, K, and Kn values compared to the fishes from the Cox's Bazar region. Histologically, it was observed that the villi of the intestine are longer and more branching in the anterior part of the gut than they are short and unbranched in the posterior part. At the indoor level, *P. canius* preferred crab meat compared to the marcel (mixture of clam and mussel muscle) and trash fish (tilapia). The findings of the present study may be helpful for understanding the food and feeding habits as well as in improving the domestication and culture of this species in the future of this commercially important fish species.

Keywords: Feeding behavior, gastrointestinal indices, gut content analysis, intestinal histology, *Plotosus canius*

CHAPTER-1: INTRODUCTION

1.1. Introduction

Fish is an irreplaceable animal protein source for billions of people in the world. It also plays an important role in the human diet. According to Tidwell and Allen (2001), about one billion people use fish as their primary source of animal protein. There is an increasing trend in global fish consumption compared with other animal-oriented protein sources. Fish also contains affordable, high-quality protein that the body needs, including high-quality minerals (Solomon et al., 1999). In Bangladesh, fish is commonly used in the human diet as it meets the nutritional requirements of people at a low cost. Bangladesh is geographically located in Asia, it is considered one of the most successful fisheries nations in the world (Shamsuzzaman et al., 2017) and possesses the third largest amount of freshwater biodiversity in the world. Fisheries is the second most significant agricultural commodity having a GDP contribution of about 3.67% in Bangladesh (DoF, 2018), and the fact that billions of people depend on fishing activities for both their employment and livelihoods. The national prosperity and food security of the Bangladeshi people originate primarily from fish culture (Ghose, 2014). People prefer fish in their diet as fish protein is highly digestible than animal protein and contains all of the essential amino acids. About 60% of animal protein comes from fish which represent a crucial source of micro-nutrients that make the fish one of the priority items in Bangladesh.

Bangladesh is a riverine country that harbors many freshwater fishes. With the increasing demand of the rising population, capture fisheries have not been able to keep pace with it (Hossain et al., 2014). To meet this ever-increasing demand, aquaculture has started to expand rapidly. Along with rivers, Bangladesh has also a vast area (1,18,813 km^2) in the Bay of Bengal. Recently, Bangladesh regained its access to an additional 1.10 million square kilometers of marine water in the Bay of Bengal by the International Tribunal for the Law of the Sea (ITLOS) (DoF, 2020). Regardless of the bounty of ocean assets, just around 15.41% of all out-fish production comes from the marine waters of Bangladesh (DoF, 2017). Due to massive overfishing, environmental destruction, fishing in spawning seasons, use of fixed nets, and probably to meet the fish consumption requirements of the human population, the number of fish is decreasing the stock and species diversity of wild fishes all over the world (Worm et al., 2006; Coll, 2008; Milton et al., 2002; Nunoo et al., 2006; Fazli et al., 2007; Narges et al., 2011). Therefore, understanding the basic life history patterns, habitat, and exploitation status is required for the conservation and management of fisheries stocks. This requires a thorough understanding of basic reproductive physiology, spawning seasons and grounds, maturation size, fecundity, sex ratios, age, and growth-based population structure, especially for commercially important species.

The diets of fish have an impact on their development, health, and capacity to adapt to illnesses and physiological stresses in their environment (Oliva-Teles, 2012). When it comes to aquaculture, the diet and feeding habits of fish are crucial. This includes selecting fish that can consume all of the food in bodies of water without encountering aggressive competition and instead inhabiting a symbiotic relationship with other fish (Biswas and Takeuchi, 2003). Fish feeding behavior is influenced by both how they perceive their food and the makeup of their diet. Olfactory and gustatory signals are perceived by chemoreceptors in response to nutrition, and they affect feeding behavior. According to Alhassan and Ansu-Darko (2011), the feeding habits of fish are a crucial tool for identifying fish in terms of their nutrition, feeding methods, and food habits. According to the food they are given, certain fish raised in captivity may modify the ratio of their intestinal length to their body length over time (Namaware and Peter, 2002). Depending on the kind of food they eat, fish are classified as carnivorous, herbivorous, omnivorous, or detritivores. Fish intestines are evolutionary adapted to meet their feeding patterns, which in most carnivorous species are frequently shorter and straighter which aids in the digestion of meats. On the other hand, because of their large and heavily folded gut, herbivorous fish absorb more plant matter and take longer to digest. Therefore, understanding the intestinal morphology would be helpful to select the best potential species for aquaculture.

Gastrointestinal indices are not a single attribute rather it's a combination of several values like visceral somatic index (VSI), intestine somatic index (ISI), relative length of gut (RLG), condition factor (K), relative condition factor (Kn) hepato-somatic index (HSI) and so on (Bhaumik, 2015). All the values together create a better understanding of the habitat's condition, feeding habits as well and surrounding environment. Here higher RLG values indicate the diets of less easily digestible (De et al., 2013). VSI is an important biological index for assessing food value (Rasmussen et al., 2000). It helps to decode the existing relationship between feed size and the length of the gut followed by understanding the mechanism of feeding and diet selection (Igejongbo et al., 2022).

VSI also plays an important role in the absorption, metabolism, synthesis, and secretion of digestive enzymes in a fish (Malami et al., 2004) Condition factor has been analyzed for measuring the growth and feeding intensity from the 20th century (Froese, 2006). This value provides information on the variations of fish physiological status for comparing populations living in certain feeding, climate, and other conditions (Le-Cren, 1951; Lizama and Ambrosia, 2002). Therefore, understanding the gastrointestinal indices would provide better accuracy for determining the feeding behavior of fish.

Studying feed composition and feeding biology may be done with ease and accuracy using stomach contents analysis (Mohsin et al., 1993). Fish gut content analyses are considered as one of the most efficient techniques for determining the diet of fish. Ecology and feeding habits vary greatly between various fish species evenif within a single fish's developmental phases (MJ, 1974). Therefore, it is possible to acquire information on the trophic linkages in aquatic communities as well as changes in fish feeding methods, fish health, and habitat-related food availability from fish stomach contents. Due to the challenges involved in viewing fish feeding habits "in vivo," analyses of fish stomach contents are particularly crucial to understanding fish ecology and health (Fabay et al., 2021). An essential component of fisheries management in the aquaculture industry is the quantitative evaluation of feeding habits which can be made possible by this gut content analysis (Hyslop, 1980). The relationship between food and energy is one of the most crucial elements of a proper diet for an aquatic animal (Jobling, 2012). At extremely low or high levels, it may have an impact on fish body composition, feed efficiency, and growth performance (Wang et al., 2016). Since the animal's energy needs were already supplied by an energy-dense diet, the consumption rate was reduced. Therefore, it is crucial for fish nutrition to analyze the contents of each fish's stomach.

Several factors, such as habitat and environment, influence the feeding behavior of fish. Environmental factors that affect feeding include temperature and photoperiod, which are typically closely related to the reproductive season. For example, it has been noted that certain fish in the temperate zone stop eating as winter approaches; the shorter photoperiod and falling water temperatures are likely functioning as environmental signals (Hart, 1993). Previous studies demonstrate that there is a correlation between food intake and environmental factors (temperature and photoperiod) in halibut, Atlantic cod, tilapia, and largemouth bass (Jonassen et al., 2000; Peck et al., 2003; Biswas and Takeuchi, 2003; Petit et al., 2003). Salinity another important environmental factor also has a comparable effect on fish intake and macronutrient preferences (Rubio et al., 2004). Data indicate that fish may regulate their food intake at a predetermined point to facilitate growth (Peter, 1979). A range of fish species, including sole (Reig et al., 2003) and Atlantic salmon (Refstie et al., 2004), are stimulated to feed by a number of substances, including salts, carbohydrates, and amino acids (Carr et al., 1996). Fish have the ability to discriminate and make decisions depending on their nutritional needs. For instance, rainbow trout can distinguish between a meal containing fish oil and one containing vegetable oil, while goldfish prefer high-fat diets to low-fat ones (Narnaware and Peter, 2002). Therefore, a clear understanding of the relationship between multiple factors and feeding behavior is necessary to develop a suitable feed for commercial aquaculture.

Gray-eel catfish *Plotosus canius* under the family Plotosidae, is a spiny, testy catfish of the coastal region worldwide. This fish is local to Southeast Asia and Australia (Mohsin and Ambak, 1996; Ferraris, 2007). It is a marine fish species, however once in a while can be seen in brackish and freshwater (Riede, 2004) and even found generally in estuaries and tidal ponds. They are found in the lower portions of waterways in freshwater or saline water and in beachfront oceans (Rainboth, 1996; Kuiter and Tonozuka, 2001). *Plotosus canius* is important both economically and nutritionally (Nurnadia et al., 2011) and is typically sold fresh (Gomon, 1984; Mohsin and Ambak 1996; Ambak et al., 2010). According to Osman et al. (2001), this species is one of the top ten marine fishes consumed in Malaysia. According to Nurnadia et al. (2011), it is also one of the top five fishes reported to originate from the Malacca Straits in Malaysia. It is valued as being both expensive and extraordinarily nourishing (Gupta and Gupta, 2006; Ambak et al., 2010). Due to its heavy commercial demand, the production performance has been declining day by day due to overexploitation. Therefore, there is an urgent need to conserve this species in the natural environment and also need to develop commercial aquaculture of this species to meet the increasing demand. So, this study is undertaken to know the food and feeding behavior of this species from the Bay of Bengal, Bangladesh.

1.2. Objectives

- Analysis of feeding behavior in response to different types of feed for domestication; and
- Identifying differences in the gastrointestinal tract between regionally distinct specimens.

CHAPTER-2: REVIEW OF LITERATURE

2. Literature Review

Bangladesh is geologically situated in the arms of the Bay of Bengal (Uddin et al., 2019). Its Padma, Meghna, and Jamuna streams make fields fertile and give different water-based resources like oil, gas, rare earth elements, minerals, electricity, and so forth (Uddin and Jeong, 2021). As we have colossal water, it is similarly abundant with different species of fish like snakehead, carp, catfish, cichlid, eel, snappers, groupers, etc. These are the most generally perceived sorts of fish that may be found in both fresh and salt water. All of these group has their obvious characteristics. Their physical and meristic qualities are assorted from one another. Among these fish groups, catfish is considered one of the main ones. Catfishes are considered tasty, delightful to eat and economically important. It is regularly used as a typical feast in any Bengali house because of its high dietary benefit and affordability (Grosso et al., 2016). Here in this review, the biology and ecology of an estuarine catfish, *Plotossus canius* are discussed below.

2.1. Taxonomic classification and short description

The Kine magur, *Plotosus Canius* (Hamilton, 1822), a close connection to *Plotosus lineatus* (Asriyana and Halili, 2021) is classified into the Plotosidae family and recorded as a nearly threatened fish species in Bangladesh (IUCN, 2015). Overall, nine species from this genus were described (Usman et al., 2013).

Kingdom: Animalia

Subkingdom: Bilateria

Phylum: Chordata

Class: Teleostei

Order: Siluriformes

Family: Plotosidae

Genus: *Plotosus*

Species: *Plotosus canius*

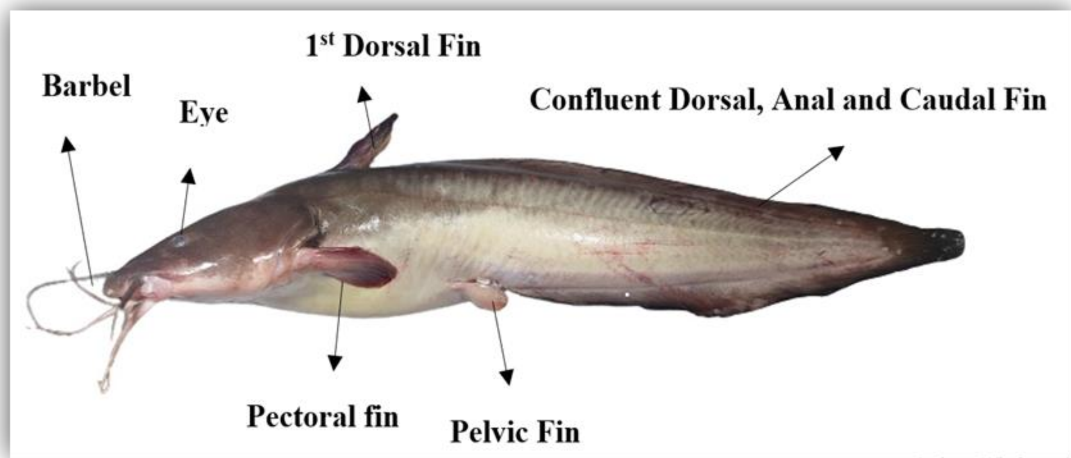

Figure 1: *Plotosus Canius*

The English name of *Plotosus canius* is "Catfish Eel" (Usman et al., 2013) and is locally known as "kain Magur" or "Gang Magur" in Bangladesh (Khan et al., 2002). The body is covered in a brownish hue (Prithiviraj et al., 2012), with a long, anteriorly sloping shape and a posteriorly compacted configuration (IUCN Bangladesh, 2000; Usman et al., 2013). The maximum size was reported 150cm from the Tamil Nadu coast of India (Prabhu et al., 2019). Dorsal and pectoral spines are cleaved (Hossain and Alam, 2015; Usman et al., 2013) can instigate a toxic venom that is harmful to humans and other animals (Usman et al., 2013; Prabhu et al., 2019). This fish exhibits a carnivore's feeding behavior in regards to feeding (Sinha, 1984; Leh et al., 2012).

2.2. Distribution, stock structure and habitat

2.2.1. Distribution

P. canius is native to the Sunda Islands, the coast of Thailand, and Southeast Asia. *P. canius* also extends to Bangladesh, Sri Lanka, Myanmar, India-Australia Islands, the Philippines, and Papua New Guinea (Ferraris, 2007; Usman et al., 2013; Kundu et al., 2019). Before entering the lower Mekong River, it had already originated in Thailand, Sulawesi, the Molucaas, and India (Sinha, 1984).

Table 1: Distribution and occurrence of *Plotosus canius* in different countries

Country	Occurrence	Reference
Andaman Island	Native	Rajan, et al. (2013)
Australia	Native	Hoese, et al. (2006)
Bangladesh	Native	Rabby, et al., (2022)
Brunei	Native	Gomon (1984)
Cambodia	Native	Gomon (1984)
Fiji	Native	Seeto and Baldwin (2010)
India	Native	Talwar and Jhingran (1991)
Indonesia	Native	Gomon (1983)
Laos	Questionable	Kottelat (2001)
Malaysia	Native	Mohsin et al. (1993)
Myanmar	Native	Gomon (1983)
Papua New Guinea	Native	Kailola and Hoese (1991)
Philippines	Native	Gomon (1983)
Thailand	Native	Suvatti (1981)

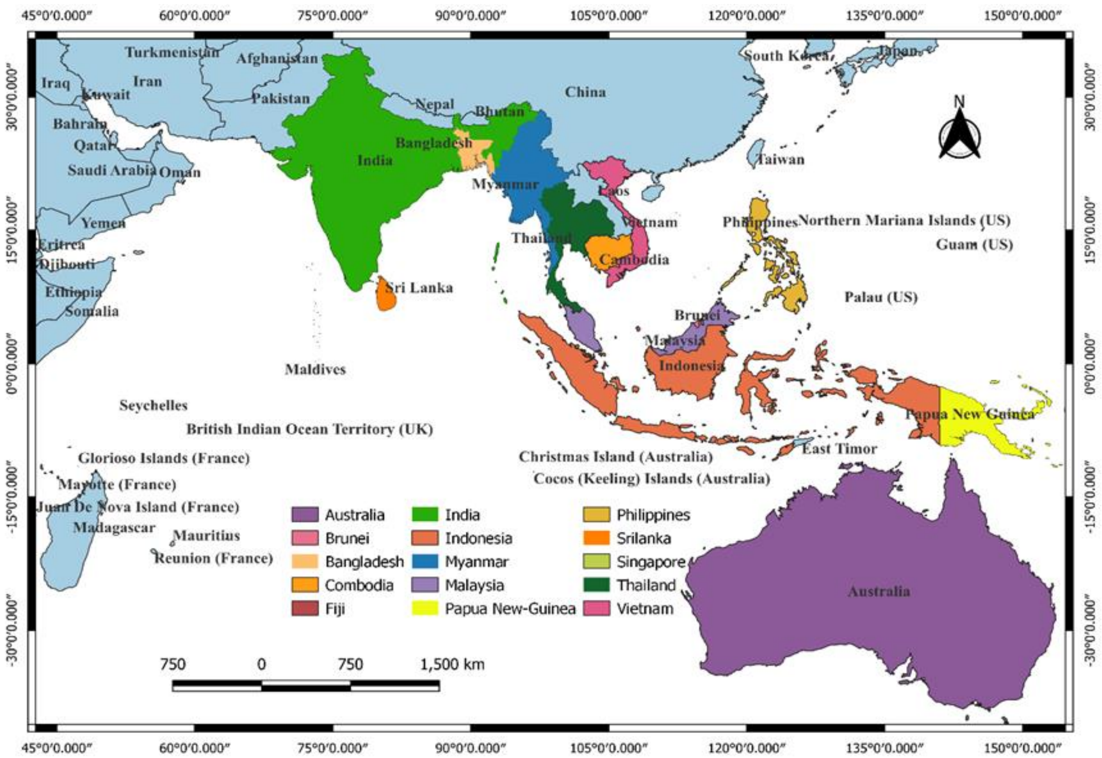

Figure 2: Worldwide geographical distribution of *Plotosus canius*

2.2.2. Stock structure

Samani et al. (2016) discovered a number of extreme response patterns across Malaysian populations in Negeri Sembilan, Selangor, Johor, Sarawak, and Sabah, indicating that the populations did not experience long-term demographic growth. When the population was analyzed, they also revealed a somewhat sluggish growth rate, especially in the female population. Additionally, the long-term stability of the *P. canius* population in Malaysia had made it impossible to investigate historical evaluations, and a lower level of heterozygosity suggested that breeding populations did not randomly mate and that juvenile fish were successively inbred (Samani et al. 2016). Additionally, Sinha (1984) discovered the morphometric homozygosity between the *Plotosus canius* populations in the Hooghly-Matlah estuary and Chilka lake. The limited sharing of haplotypes between a specific Peninsular Malaysian population and the Borneo population showed that there was no simultaneous gene flow among groups (Samani et al., 2016). In addition, *P. canius* in the Sundarbans region showed considerable genetic variation (Kundu et al., 2019).

2.2.3. Habitat

According to Abdullah et al. (2000), the species is euryhaline and capable of maintaining an internal salinity that is isotonic to its environment. *P. canius* is amphidromous in nature and lives in muddy coastal areas (Samani et al., 2016; Bray, 2020). Additionally, *Plotosus lineatus* a closely related species was identified as a euryhaline species, and the juveniles were described as moving in groups (Golani, 2002). According to Abdullah et al. (2000) and Asriyana et al. (2020), both *Plotosus canius* and *Plotosus lineatus* prefer the mangrove ecosystem as their optimal environment.

2.3. Fishery:

In Indonesia, Malaysia (Amornsakun et al., 2018, Nurnadia et al., 2011), and south-east Asia (Usman et al., 2013), canine catfish are significant socioeconomic resources that are sold fresh (Usman et al., 2016), as well as a significant source of poly-unsaturated fatty acids (Osman, 2001) for the community. *Plotosus lineatus*, is also a significant source of protein in Indonesia (Asriyana et al., 2020). Nurnadia et al. (2011) found that the Gray-eel catfish had the highest moisture content among the demersal fishes they had chosen, including golden snapper, six-bar grouper, Japanese threadfin bream, Indian threadfin, Malabar red snapper, moonfish, giant sea-perch, etc.

According to reports (Khan et al., 2002 and Ahmed and Haque, 2007), overfishing, habitat loss, illegal fishing, indiscriminate fishing of brood stocks and juveniles, pollution, and the introduction of alien species are all contributing to the reduction of *P. canius* populations. IUCN Bangladesh, 2000 and Mijkherjee, 2002 have characterized this species as one of the fishes in India and Bangladesh that are on the verge of extinction. However, its status has not been evaluated by the IUCN Red List (IUCN, 2015).

In Malaysia, the primary fishing methods for *Plotosus canius* included trawl nets (14%), gill nets, drift nets, barrier nets (50.9%), beach seines, cast nets, eel traps, and hook and line (20.9%) (Leh et al., 2012). From 1980 to 2009, the west coast of Peninsular Malaysia reported an annual output that ranged from 915 tons to 2,653 tons (0.21% of the total commercial catch in 2009) (Leh et al., 2012). Usman et al. (2013) stated that the total canine catfish landings for the west and east coasts of Malaysia were 582 tons and 403 tons, respectively. High levels of exploitation were cited by Asriyana et al. (2020) as a key factor in the reduction of this species' natural population in Indonesia. The seasonal availability of this fish has reportedly become a significant barrier to tracking its yearly capture status, according to local Malaysian fisherman (Usman et al., 2013). In Bangladesh, this species was less well-liked and its population was in decline (Islam et al., 2019; Patra et al., 2005). The main causes of the population decline of *Plotosus canius* and other fish species in India were found to be overexploitation, destruction of spawning grounds, disease breakout, bionomical changes in area, and a lack of management (Patra et al., 2005; Mijkherjee et al., 2002).

Table 2: Decade wise production trend Analysis of *Plotosus canius* fishes of North-East Sundarbans (Patra et al., 2005)-

Year	Production
1960	254.30 ton
1970	224.50 ton
1980	190.00 ton
1990	83.06 ton
2000	31.08 ton

2.4. Morphology of the digestive tract

The gastrointestinal tract and digestive system of *Plotosus canius* is not well understood, with only one paper published by Sinha (1986) on its morphology. Sinha's research also revealed that the digestive system has a rudimentary irregular shape. The mouth and Bucco-pharynx were found to be morphologically distinct from the rest of the body. The Bucco-pharynx is short and easily expanded, and contains teeth that help gently compress food. The stomach of *P. canius* is similar to that of catfish, but it cannot be easily distinguished from the intestine, except for its slightly broad shape. The pyloric region shows a slight external constriction where it connects to the cardiac stomach. The gut is composed of three areas, the anterior, mid, and hind intestines, each with a distinct breadth. There is no distinct rectum as the rectum ends at the anus, which leads to the outside (Sinha, 1984).

2.5 Gastrointestinal indices

Understanding the diets and eating habits of aquatic animals, is crucial for comprehending their biology, including growth, reproduction, migration, and other processes. It differs significantly from habitat to habitat in both time and space and at various stages of life. To determine the sort of food consumed and the eating patterns, the stomach contents of the individuals taken from their habitats should be examined. Studies on diet and feeding behavior are crucial for understanding fish biology and management (Chakraborty et al., 2021). According to Khongngain et al. (2017) and Sarkar et al. (2017), feeding intensity generally changes with seasons, the availability of favored food items, maturation stages, and spawning season. To create effective management strategies for fisheries, frequent bio-monitoring of commercially significant species foraging is required. When assessing the economic potential of stock, studies on the reproductive biology of any species are crucial (Vahneichong et al., 2017).

2.5.1. Hepatosomatic Index (HSI)

The hepatosomatic index (HSI) is used in fisheries biology and fish physiology to assess the health and condition of fish, specifically in relation to their liver. The hepatosomatic index provides information about the relative size and condition of the liver compared to the overall body weight of the fish. It is often used as an indicator of the energy reserves, metabolic activity, and overall health status of fish (Pyle et al., 2005). Since the liver is one of the most important organs for the development of the gonads and helps fish to produce vitellogenin, which is regarded as the predecessor to the yolk (Lucifora et al., 2002), understanding the functioning of the HSI is also one of the key factors for determining the better health of fish. HSI is frequently used to assess fish condition and nutritional status (Rueda-Jasso et al., 2004). Typically, a higher HSI value suggests a larger and more active liver relative to the overall body weight, indicating good health and sufficient energy reserves. Conversely, a lower HSI value may indicate a smaller or less active liver, which could be associated with poor nutrition, disease, or other physiological stressors (Kumar et al., 2017).

2.5.2. Visceral Somatic Index (VSI)

VSI is a measure of the relative size and weight of the visceral organs (such as the liver, stomach, and intestines) compared to its body size. A higher VSI may suggest increased energy reserves or an enlarged organ due to stress or disease ((Cui and Wootton, 1988; Dawood, 2016). Conversely, a lower VSI may indicate poor nutrition, reduced energy reserves, or the depletion of visceral organs. Researchers and fish farmers use VSI as a tool to monitor fish health, growth, and overall condition. By regularly assessing VSI values, they can identify potential issues and take appropriate measures to improve fish welfare and optimize production (Henderson et al., 1984).

2.5.3. Relative Length of Gut (RLG)

RLG is the most popular indicator that has been extensively used to determine the feeding habits of fish (Gupta and Banerjee, 2012). Different fish species exhibit variations in their gut length index based on their feeding habits. For example, carnivorous fish with a diet primarily consisting of animal matter tend to have shorter intestines, whereas herbivorous fish that consume plant material often have longer intestines to aid in the digestion of plant fibers. The RLG values for carnivorous (0.5–2.4), omnivorous (1.3–4.3), and herbivorous (3.7–6.0) fishes were previously listed by Al Hussaini (1947).

2.5.4. Intestine Somatic Index (ISI)

The ISI is a metric used in fisheries and aquatic biology to assess the condition and health of fish. It is a quantitative measure that compares the weight of the intestines to the total body weight of the fish. The ISI is commonly used to evaluate the impact of environmental stressors, such as pollution or changes in diet, on fish health (Wootton, 1998). Typically, fish with a higher ISI value are considered to have a larger and more developed intestine relative to their body size. This can indicate a higher capacity for nutrient absorption and digestion, which is often associated with better overall health and condition. Researchers and scientists often conduct studies to compare ISI values between different fish populations, experimental groups, or environmental conditions to assess the health and well-being of fish and understand the potential impacts of various factors on their intestinal physiology (Ahmed and Haque, 2007).

2.6. Food and feeding habits

The contents of a fish's stomach can reveal information about its feeding habits and habitats (Hynes, 1950; Hyslop, 1980; Mohan and Sankaran, 1988; Lima-Junior and Goitein, 2001). Accurate feeding habits will certainly help to evaluate the trophic level of a fish species as well as its management in aquaculture (Alhassan and Ansu-Darko, 2011; Amani et al., 2011). The feeding habits of other catfish species from other regions have been the subject of a prodigious amount of literature (Marriott et al. 1997; Solak et al., 2001; Sreeraj et al., 2006; Prasad and Ali, 2008; Offem et al., 2008; Alhassan and Ansu-Darko, 2011; Kadye and Booth, 2012). The fact that Sinha (1984) and Ahmed and Haque (2007) have cited this type of feed is a bit surprising. The majority of diets consists of fish, mollusks, amphibian bugs, plants, and amphipods. Small amounts of detritus were also discovered; these were referred to as "unplanned considerations" (Sinha, 1984). The primary foods consumed by the fingerlings were determined to be prawns, tiny fish, and bugs. Crabs, shrimp, and mollusks were the primary sources of food for *P. canius* in Bangladeshi coastal waters, (Ahmed and Haque, 2007). Other food sources were fish, creepy crawlies, green vegetation, and detritus.

In addition, Baber et al. (2003) reported that *C. batrachus*, another catfish was observed to feed a smaller number of tadpoles of four different species of frogs and toads. They mostly feed on small to moderate-sized fish, insect larvae, worms, shrimps, and organic debris to some extent (Sakhare and Chalak, 2014). Evidence of Dipteran larvae, insects, Formicides, Coleopteran, adult Hymenopetrans, Cladocera, Hemiptera, Ostracods, Plant parts, fish fry, Orthoptera, and Areneids were also recorded as the diets of *C. batrachus* (Rajagopal, 2005).

Wallago attu species are highly carnivores in nature. Babare et al. (2013) observed that the intestinal wall of this particular species is very thick, narrow, medium, and thick along with the muscular, roughly spherical, highly extensible stomach bag that helps to digest verities of prey. They mostly feed on fish, insects, crustaceans, and mollusks. Of these food items, fish (60%) possesses the highest percentage (Khaing and Khaing, 2020). The higher peak in the gastro-somatic index of both males and females was during February and September. Because of their carnivorous nature, they usually intake protein (Ranjan et al., 2009).

Heteropneustes fossilis is a benthopelagic species and prefers slow and shallow water bodies such as ponds, ditches, swamps, marshlands, and muddy rivers. As a carnivorous fish, it primarily feeds on crustaceans, worms, mollusks, and detritus plant matter according to Kohli and Goswami (2012).

2.7 Biology

2.7.1. Age and growth

Various methods have been used to evaluate the growth rate of canine eel catfish: length-weight relationship analysis, length frequency analysis, probability plot method, and study of the annual nature of vertebral rings (Usman et al., 2013, 2016;). A positive linear relationship was found between total length and total weight (Usman et al., 2022). Whereas, a negative linear relation occurred between total length and total weight for *Plotosus lineatus* (Asriyana et al., 2020). There were no differences in size between the two sexes of *Plotosus canius* in the coastal waters of Port Dickson as well as both the male and female exhibited negative allometric growth meaning different organs showed a lower growth rate than the body as whole (Usman et al., 2016). On the contrary, positive allometric growth was found in the case of *Plotosus lineatus* (Asriyana et al. 2020). Even though, the increase in total length was found proportionate to body length for both *Plotosus canius* male and females in Peninsular Malaysia, the fastest growth rates (Per month) were recorded 0.96mm/month for standard length (Usman et al., 2016).

2.7.2. Trophic ecology

The canine catfish is a predator that feeds on other animals and has a basic digestive system. It feeds on the bottom of aquatic environments, as confirmed by several studies (Islam et al., 2021; Usman et al., 2018; Sinha, 1984; Leh, 2012; Nurnadia et al., 2011).

The *P. limbatus*, found in Sri Lanka, has been observed to be omnivorous and use suction to feed (Wasala et al., 2007). The shape of the teeth differs between species (Usman et al., 2013; Sinha, 1986), and mouth size increases with body size (Usman et al., 2016). These catfish can hunt prey that is up to half their size (Usman et al., 2016). They eat various types of food, including fish, crustaceans, mollusks, sand, mud, and debris (Usman et al., 2013, 2018; Islam et al., 2019; Sinha, 1984; Leh et al. and Prithiviraj et al., 2012; Wasala et al., 2007). In Port Dickson, Malaysia, crabs were the second most important food, while mollusks were the third (Islam et al., 2019: Usman et al., 2018). Detritus affects their diet (Sinha, 1984). The *Plotosus canius* has different food preferences depending on the habitat and prey size (Leh et al., 2012). Juvenile and adult catfish have different dietary habits (Sinha, 1984; Leh et al., 2012). The diet of young canine catfish consists mainly of prawns, plankton, and insects (Usman et al., 2013; Sinha, 1984). The quality of the food they consume remains consistent, despite variations in quantity (Usman et al., 2018; Sinha, 1984). Therefore, catfish of similar sizes compete for food (Leh et al., 2012). The feeding frequency of the canine catfish is higher during September and lower from February to July, which may be related to the spawning season (Sinha, 1984; Usman et al., 2018; Islam et al., 2019). The *Plotosus limbatus* has a poorly developed stomach and a long digestive tract (Wasala et al., 2007).

2.7.3. Proximate composition

Fish is a great source of food for all ages of people because of having a good amount of all the necessary nutrients, which are directly or indirectly essential for growth. Nutritionally, catfish are well-known as a valuable source of dietary protein worldwide (Liu et al., 2016). All body parts are packed with nutrients (Manikandarajan et al., 2014).

Table 3: Proximate composition of *P. canius* muscle taken from the mangroves of Muthupet (Suganthi et al., 2015) and West Coast of Peninsular Malaysia (Nurnadia et al., 2011):

Nutrients	Amount	Amount in percentage
Carbohydrate	3.26(mg/g)	0.0%

Protein	14.69(mg/g)	16.61%
Lipids	1.48(mg/g)	3.04%
Moisture	-	81.66%
Ash	-	0.96%
Energy	-	5.14 KJ/kg

Table 4: Proximate Composition of *P. lineatus* for gm/100gm.

	Head	**Body**
Carbohydrate	0.92	1.98
Protein	21.82	32.56
Lipids	43.07	66.93
Moisture	3.01	4.03
Ash	3.98	3.46

2.8. Culture of *P. Canius*

A trial was conducted to study the effect of biological recirculation systems on the growth of *Plotosus canius* for 5 months in Thailand. *P. canius* in the density of 100-150 fish/ tank was recommended for rearing in RAS (Musikasung, 2015).

2.9 Venom found in the dorsal fin spine

The spines that connect to the anterior fins have a lot of venom (Hamilton, 1822). This venom most likely contains a deadly protein toxin. The crude venom was purified by ammonium sulfate precipitation followed by Diethyl-amino ethyl groups DEAE ion exchange chromatography. On isolated toad and guinea pig hearts, toxin PC caused cardiac arrest (Auddy et al., 1995).

2.10. Biodiversity status of freshwater catfishes in Bangladesh

In Bangladesh (Rahman, 2005) as well as all over the world (Talukder, 2021) catfish species are considered as one of the most diverse groups constituting the order Siluriformes. After the most common and large groups (Cypriniformes and

Perciformes), Siluriformes is the most prominent group of fish on the basis of species number (Talukder, 2021). According to Rahman (2005), at least 55 species under 35 genera have been recorded in Bangladesh like Boal (*Wallago attu*), Ayre (*Sperata aor*), Baghair (*Bagarius bagarius*), Rita (*Rita rita*) and Pangas (*Pangasius pangasius*) etc are the largest sized members. Whereas *Amblyceps mangois*, *Hara hara*, *Hara jerdoni,* etc. are among the smallest, rarely exceeding 5 cm in length (Sarker et al., 2008). In Khulna district, 12 species of catfish were identified among 53 freshwater fin fishes (Ali et al., 2004).

To date, very little is known about the gastrointestinal indices, food, and feeding behavior of *Plotossus canius* from the Bay of Bengal. Therefore, this study aimed to investigate the dietary analysis and food and feeding behavior analysis of this commercially important fish species in indoor conditions.

CHAPTER-3: MATERIALS AND METHODS

3.1. Sampling site and collection of samples

The experimental Grey-eel catfish both male (32) and female (12) was collected from the fishermen of the local fish market and fish landing sites of Chattogram (Foillatoli Bazar). *P. canius* was also collected from the local fish market of the Cox's Bazar. A total of 42 fish was collected from the Chittagong fish market and eighteen fish were collected from the Cox's Bazar fish market. The collected fish were transported alive either in the wet laboratory of the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram or to the hatchery of the Institute of the Coastal Biodiversity, Marine Fisheries and Wildlife Research Centre, CVASU, Cox's Bazar for further investigation.

3.2. Measurement of experimental fish

Every individual fish was caught using a fine mesh scoop net and its length and weight were recorded. The length of individual fish was measured using a meter scale and recorded in centimeters. The weight of individual fish was measured using a weight machine (Model no: SKU-RMD00164, RFL, Bangladesh) and recorded in grams.

3.3 Collection of internal organs

At first, the collected fishes were dissected to collect the internal organs including the liver, gonad, intestine, and viscera. Then, the weight of the individual organs was recorded separately in gram (g) to determine the condition factor (K), relative condition factor (Kn), hepatosomatic index (HSI), intestine somatic index (ISI), and visceral somatic index (VSI) respectively from both sources (Chittagong and Cox's Bazar). To determine the relative length of the gut (RLG), the length of the gut was also measured in centimeters (cm) by using a measuring scale.

3.4. Parameters to be assessed

3.4.1. Visceral somatic index (VSI)

Visceral somatic index (VSI) is the percent ratio of the weight of viscera to the total weight of fish. Fish abdomens were cut with scissors, and whole visceral contents were collected using forceps and kept in a petri dish. The contents were measured using a digital weighing balance. VSI was estimated by using the following formula (Hoffnagle, et al., 2017):

$$\text{VSI} = \frac{weight\ of\ viscera}{total\ weight\ of\ fish} \times 100$$

3.4.2. Intestine somatic index (ISI)

Intestine somatic index is the ratio of intestinal weight of fish and is calculated by the formula given below (Zhang et al., 2013):

$$\text{ISI} = \frac{weight\ of\ intestine}{total\ weight\ of\ fish} \times 100$$

3.4.3. Hepatosomatic index (HSI)

Hepatosomatic index (HSI) is the ratio of liver weight to body weight. Fish abdomens were cut with scissors, and livers were collected using forceps and kept in a petri-dish. Livers were measured using a digital weighing balance. Hepatosomatic index (HSI) was calculated following this equation (Htun-Han, 1978):

$$\text{HSI}=\frac{weight\ of\ liver}{total\ weight\ of\ fish} \times 100$$

3.4.4. Condition factor and Relative condition factor

Condition factor and relative condition were calculated from the monthly samples, which were used to detect the seasonal variations in the conditions of fish.

$$\text{Condition factor (K)} = \frac{W}{SL^3} \times 100$$

Where,

K = condition factor

W = body weight (gm)

SL = standard length (cm)

$$\text{Relative condition factor } (K_n) = \frac{W}{aL^b} \times 100$$

Where,

K_n = relative condition factor

W = body weight (gm)

L = total length (cm)

a = intercept

b = slope

a and b are the exponential form of the intercept and slope, respectively of the logarithmic length-weight relationship equation.

3.4.5. Relative length of gut (RLG)

Relative Length of Gut (RLG) is the ratio of the total length of the gut to the total length of the fish. The total gut length was measured by using a tap scale and the total fish length was estimated by a standard measuring scale.

RLG was estimated by using the following formula (Al-Hussani, 1949):

$$\text{RLG} = \frac{total\ length\ of\ gut}{total\ length\ of\ fish}$$

3.5. Feeding behavior observation

3.5.1. Acclimatization of fish

The feeding trail experiment was conducted in the Institute of Coastal Biodiversity, Marine Fisheries and Wildlife Research Centre, Cox`s Bazar, which is an outreach campus of Chattogram Veterinary and Animal Sciences University (CVASU). The fish was acclimated to laboratory conditions first in running freshwater for thirty minutes and then gradually adjusted the desired salinity (15 ppt) in the conditioning tank. Fish were fed with chopped crab muscle ad libitum at two-day intervals.

3.5.2. Experimental design for the feeding trial

After conditioning, the fish were put into three observation tanks in each group. Three distinct meal types- chopped crab, marcel (a combination of mussel and clam muscle), and trash fish were employed to monitor the eating behavior. Fish were fed to their body weight basis every morning after two-day intervals and continuously observed how they took the food. Time and amount of feed were also noted for each group of fish. All these data were recorded in a video camera for better understanding and further evaluation.

Fish were housed in a tank made of cement and each feed was given for a period of 15 days. The feed was dispersed throughout the tank at various locations, from one corner to the center of the tank. Each time, the fish received roughly 200 grams of each meal. The extra meal was taken out and weighed from the tank in the late afternoon (6 hours after feed delivery).

3.5.3. Feed preparation

In this study, we have provided 3 different types of feed to *P. canius*. Those are:

1. Crab muscle
2. Marcel
3. Tilapia chop muscle

3.5.4. Preparation of crab muscle

Muscle from crabs (*Scylla serrata*), which we obtained from the neighborhood market or occasionally directly from the fishermen, served as the main source of nutrition for *P. canius*. The crab was brought to the Coastal Biodiversity, Marine Fisheries and Wildlife Research Centre after collecting and maintained there under refrigeration. The crab was then defrosted and its entire body section was sliced by using a knife. The muscle component and feed were returned to the refrigerator after being weighed.

3.5.5. Preparation of marcel

Marcel is a type of feed that mostly contains the muscle from clams and mussels. This feed is mostly utilized for prawn cultivation since it is highly productive and nourishing for the young shrimp. This meal mostly originates in the Khuruskhul-Chowfalldondi area of Cox's Bazar region, where tribal people collect the mussels and clams. Fishermen take the muscular section of the wild mussel and clam, wash it, and then blend it to create marcel. They then sold it as a piece of food on the neighborhood market. It costs about (350-400) tk/kg. These feeds are gathered by the hatchery owner for a unique prawn culture. These feeds were sold to hatchery owners for shrimp or prawn culture in the Cox's Bazar area. The chopped feed was then placed into each little polythene bag, which held 200g of feed. The divided feed is then placed in the refrigerator for further use.

Figure 3: Feed preparation for feeding trial of *Plotosus canius* (A) marcel and (B) crabs' meat.

3.5.6. Preparation of Tilapia

As part of our feeding experiment, tilapia was also utilized. We cut the tilapia into little pieces after purchasing it from the neighborhood fish market. We weighed it using the weighing scale, and then for preservation we placed it in the refrigerator.

3.5.7. Observation of the feeding behavior

A minimum of 15 minutes prior to feed administration and 30 minutes afterward providing food were distributed for observing and recording the feeding behavior for each feed type. Every time the feeding habit was carefully observed and videotaped using a camera (Go Pro Hero 8, San Mateo, California). Then, behavioral analysis (feed intake, feed sharing, voraciousness, social hierarchy, amount of feed consumption, response to feed etc.) was performed based on the recorded videos.

Figure 4: Feeding behavior observation of *Plotosus canius.*

3.6. Morphological and histological analysis of the intestine

3.6.1. Collection and preservation of intestine

At first, the fish was placed on a tray and then it was cut from the anus towards the lower jaw by using a scissor. The intestine was carefully removed and weighed from the individual fish by using an electrical balance (Maks HR-250A, India) and the data were recorded for further study. Individual intestine sample was kept in a vial and preserved in Bouin’s fixative solution and kept at room temperature for histological analysis. After 24 hours, samples were replaced by 70% Ethanol. All of these steps were performed in a vial where the samples were kept earlier.

3.6.2. Washing of fixed tissue

After fixation, excess fixatives were washed out from the tissue to prevent interference with subsequent processes during the histological procedure. Washing was done in running water about 2–3 hours before dehydration.

Figure 5: Collection of samples (A) dissecting the abdominal portion of the sample fish (B) preserving intestinal sample in the Bouin's solution.

3.6.3. Dehydration

After washing, the intestines were taken out and cut into small pieces (about 1cm) and put into pre-labeled cassettes separately. Then the tissue blocks were passed through successive ascending concentrations of graded alcohol for dehydration. (Table: 5)

Table 5: Dehydration schedule:

SI. No.	Solution	Time
1	50% alcohol	2 hours
2	70% alcohol	2 hours
3	80% alcohol	2 hours
4	90% alcohol	2 hours
5	95% alcohol	2 hours
6	100% alcohol (1)	2 hours
7	100% alcohol (2)	2 hours

3.6.4. Cleaning

After dehydration, the cassettes having intestines were passed through successive changes of xylene until the alcohol from the tissue was replaced by xylene (Table-6).

Table 6: Cleaning schedule:

SI. No.	Solution	Time
1	Ethanol + Xylene (1:1)	2 hours
2	Xylene (1)	2 hours
3	Xylene (2)	2 hours

3.6.5. Infiltration

After cleaning, the tissue blocks were placed in melted paraffin in the oven at 60ºC. Heat causes evaporation of xylene and the space in the tissue became infiltrated with paraffin.

Table 7: Infiltration schedule:

SI. No.	Solution	Time
1	Xylene + Paraffin (1:1)	2 hours
2	Paraffin	2 hours
3	Paraffin	2 hours
4	Paraffin	2 hours

3.6.6. Embedding

After infiltration, each tissue sample was taken from the melted paraffin put in a block, and filled with molten paraffin. Then the embedded blocks were allowed to cool at room temperature overnight.

3.6.7. Trimming

In the process of trimming, the unwanted wax layers of the embedded blocks were cut away to produce the right blocks. Trimming helps in the easy sectioning of the samples. In this step, both side trimming and surface trimming were conducted.

3.6.8. Sectioning

The small embedded paraffin blocks with tissue were sectioned by the hand rotatory microtome (KD-2258 Rotary Microtome, China) at 5µm size. After sectioning, the ribbons-like sections were floated in a water bath at (42°C) temperature for 3-4 min.

3.6.9. Attachment of section on glass slide and drying

The well-spread ribbons of the section from the water bath were transferred to glass slides treated with an adhesive substance (gelatin, egg albumin). The glass slides with section (s) were then dried in a slide warmer (XH-2003, USA) at a temperature of 37°C overnight.

3.6.10. Staining

After drying, the slide with the tissue section was ready for staining. Following steps (Table-8) were followed in staining the tissue for hematoxylin and eosin stain.

Table 8: Staining schedule:

Sl. No.	Treatment	Time	Stages
1	Xylene-1	10 min	
2	Xylene-2	10 min	Deparaffinization
3	Xylene-3	10 min	
4	100% Ethanol-1	5 min	
5	100% Ethanol-2	5 min	
6	95% Ethanol	3 min	Rehydration
7	70% Ethanol	3 min	
8	50% Ethanol	2 min	
9	Distilled water	10-15 dips	
10	Hematoxylin	1- 1.5 min	
11	Tap wash water	15 min	
12	1% acid alcohol	2 dips	
13	Tap water wash	5 min	Staining
14	50% Ethanol	10 dips	
15	95% Ethanol	30 sec	
16	Eosin Y	3 min	

17	95% Ethanol-2	2 min	Rehydration
18	100% Ethanol-3	2 min	
19	100% Ethanol-4	2 min	
20	100% Ethanol + Xylene (50%+50%)	2 min	Cleaning and removal of alcohol
21	Xylene	20 min	
22	Xylene	20 min	

3.6.11. Mounting:

After staining, tissue section with glass slide was protected by thin cover slip. Required drop of DPX (Qualikems Fine Chemical Pvt. Ltd., India) was put on each slide followed by attachment of cover slip and mounted slides are allowed to harden.

3.6.l2. Microscopic examination:

The mounted slides were observed under a microscope (Optika; B-190 Series, Italy), which was connected to the computer with a digital camera. With the help of this mechanism, numerous photographs were taken at different magnifications.

3.7. Statistical Analysis:

The statistical test is a method of statistical inference used to determine a possible conclusion from two different and likely conflicting hypotheses. The observation has been done directly on the spot or using a video camera for further observation. The collected data, time interval, feed amount, and distance to feed from each group of fish were analyzed by one-way ANOVA and other statistical analyses were performed with the aid of the computer software SPSS programmer (V. 26). Differences between the gastrointestinal indices of *P. canius* from two different sources were analyzed student t-test. Significant differences were indicated by p-values <0.05. Values are presented as mean ± standard deviation (SD). The homogeneity and distribution of data were checked.

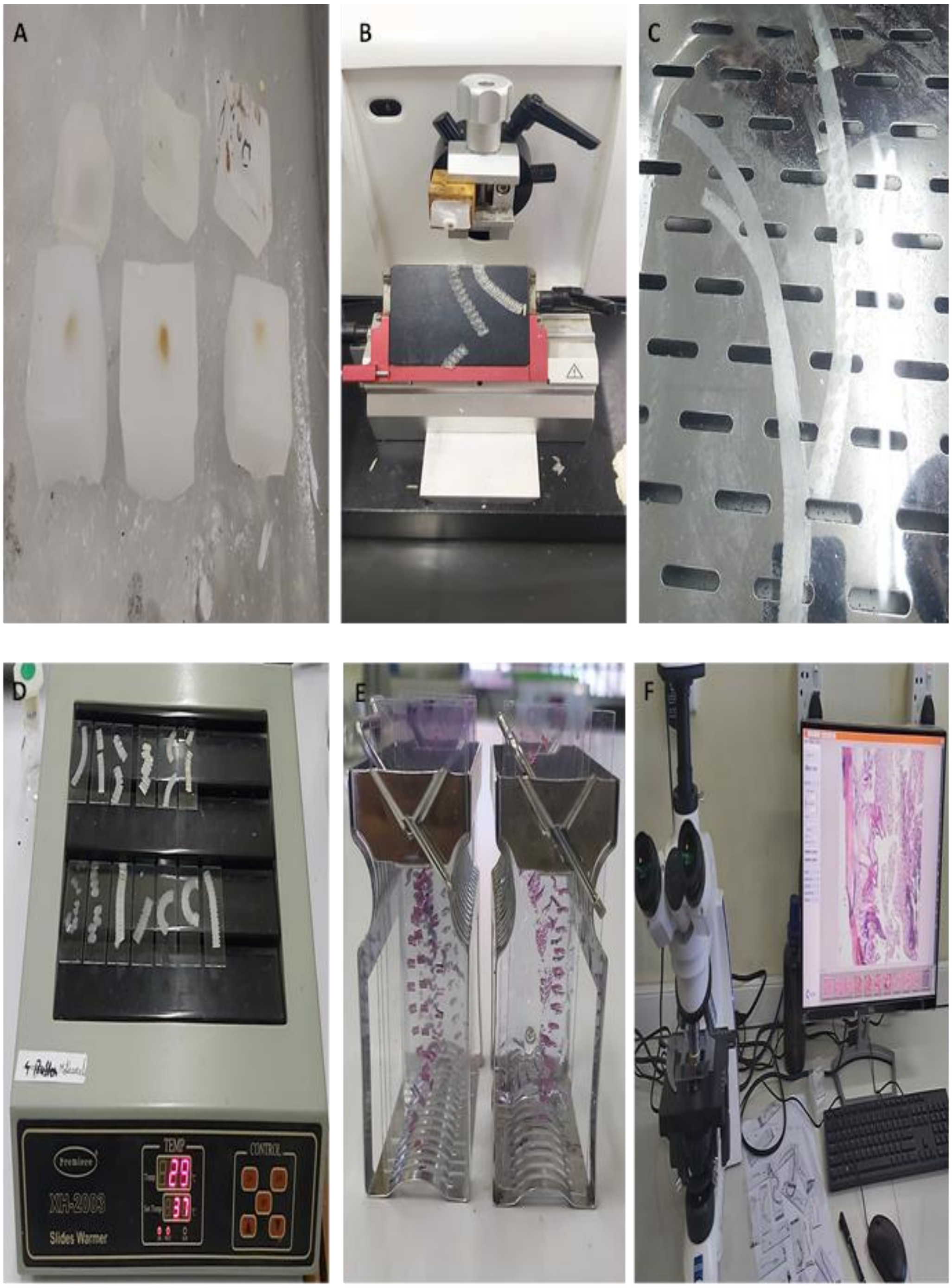

Figure 6: Different steps for performing histology (A) embedding (B) sectioning (C) floating in hot water bath (D) drying (E) stained slides (F) microscopic observation.

CHAPTER- 4: RESULTS

4.1. Length-weight distribution of *Plotosus canius* collected from two different regions of Bangladesh

In the present study, *P. canius* was collected from both the Chattogram and Cox's Bazar regions of Bangladesh. The length and weight of the collected fish were analyzed and found some significant variations in the length and weight of the collected fish. Based on the data, it appears that the mean body weight and length of fish from the Chattogram region was significantly higher than that of the Cox's Bazar region. The highest and lowest body weights recorded were 3291.6 gm and 148 gm, respectively in Chattogram, while the highest and lowest body weights in Cox's Bazar were 940.4 gm and 71.6 gm. The highest length (788 mm) was recorded in the Chattogram. The mean length of fish in Chattogram was 536.35 ± 130.37 mm, while in Cox's Bazar, it was 372.38 ± 69.31 mm (Figure 7).

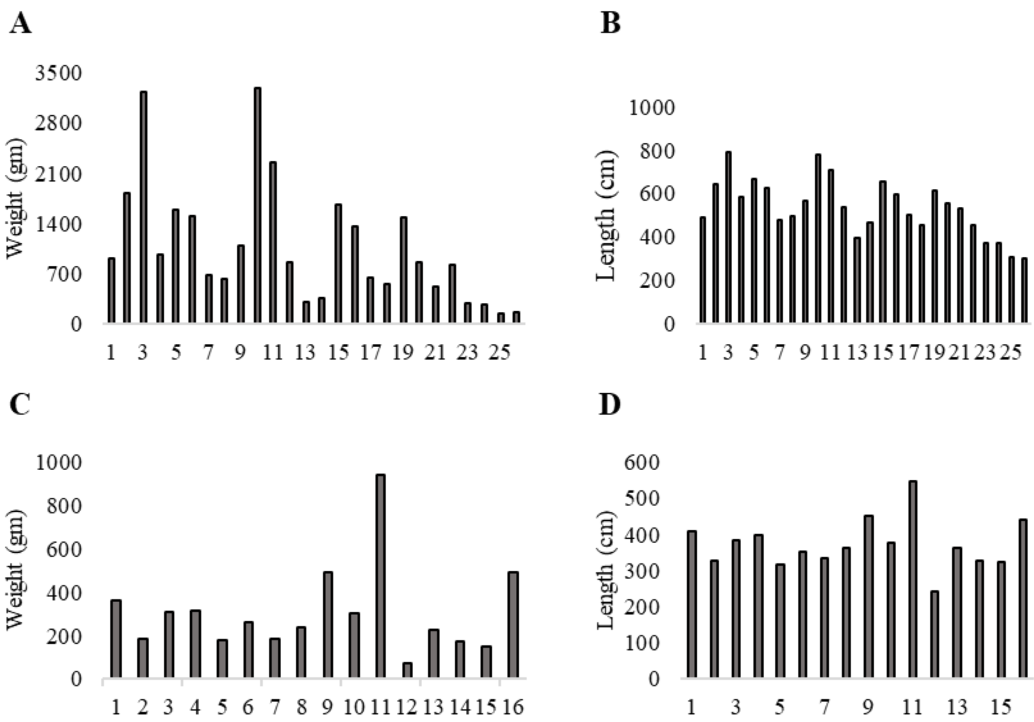

Figure 7: Length-weight distribution of *Plosotus canius* collected from two different regions of Bangladesh. Weight (A) and length (B) of the *P. canius* collected from Chattogram region and weight (C) and length (D) of the of the *P. canius* collected from Cox's bazar region.

4.2. Gut Contents analysis *Plotosus canius* collected from two different regions of Bangladesh

It is well-known that catfish are carnivorous and primarily consume crustaceans, bivalves, mussels, arthropods, and gastropods. In *P. canius* the most commonly consumed items were the appendages of crustaceans (45%) and bivalve mollusks (20%). The diet of *P. canius* collected from Chattogram consisted of small-sized crabs with some evidence of isopods (Figure 8B). Additionally, there were traces of debris and small amounts of herbs in the gastrointestinal tract of *P. canius* collected from the Chattogram regions of Bangladesh (Figure 8).

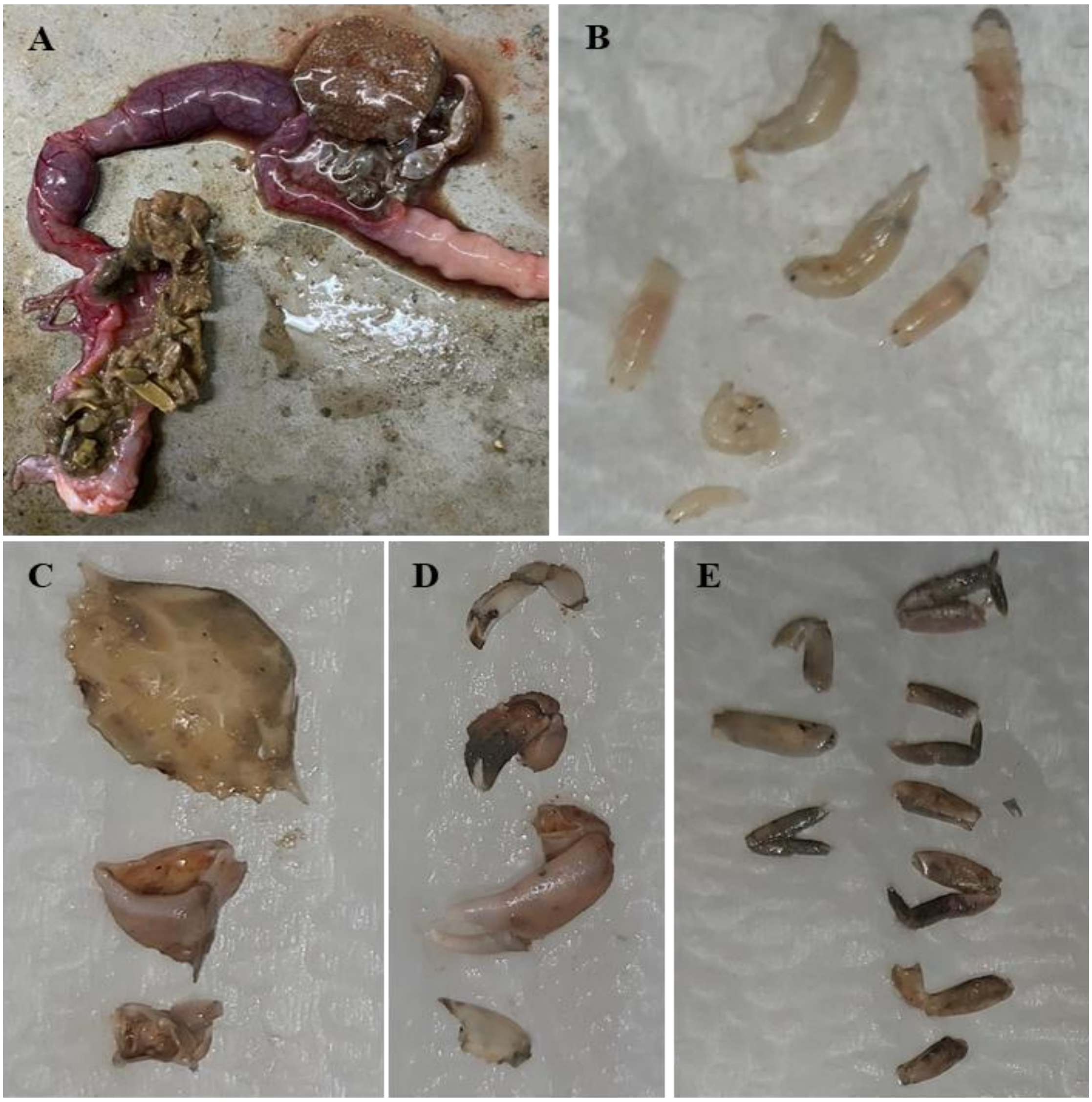

Figure 8: Gut content analysis of *P. canius* collected from the Chattogram region of Bangladesh. Intestine with crabs (A), isopods (B), carapace of crustacean (crab) (C), chelate legs of crabs (D) and walking legs of crabs (E) (n = 42).

On the other hand, the *P. canius* collected from the Cox's Bazar region were fed on crustaceans (45%) and mollusks (36%) (Figure 9). A small portion of herbs and debris was also reported from the gut of *P. canius* collected from the Cox's Bazar region. However, no isopods were recorded in the gut of *P. canius* collected from the Cox's Bazar regions (Figure 9).

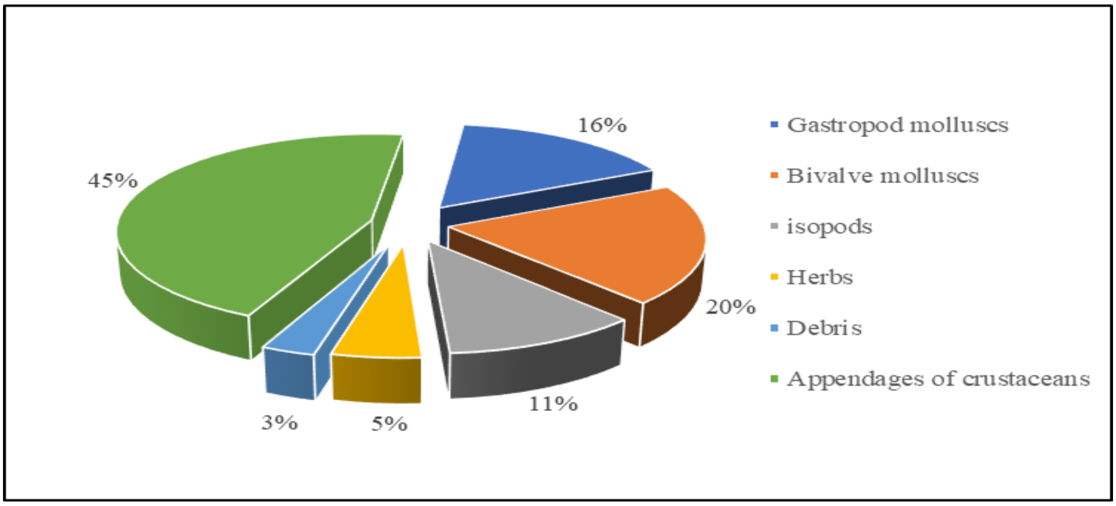

Figure 9: Quantitative percentage of different food items in the gut of *P. canius* collected from the Bay of Bengal.

Figure 10: Gut content analysis of *P. canius* collected from the Cox's Bazar region of Bangladesh. Bivalve's shell (A) and snail, plant residues, and debris (B) (n = 18).

4.3. Gastrointestinal indices of *Plotossus canius* collected from two different regions of Bangladesh

4.3.1. Visceral somatic index (VSI)

In the present study, the visceral somatic index was calculated in *P. canius* collected from the Chattogram and Cox's Bazar regions of Bangladesh. The highest (18.79) VSI value was recorded from fish collected from the Chattogram region with a mean value of 8.23 ± 1.29. On the other hand, the mean VSI was recorded as 6.77 ± 1.41 in *P. canius* collected from the Cox's Bazar region (Figure 11A). A statistically significant difference ($p < 0.05$) was observed between two regions of Bangladesh in the case of VSI values (Figure 11A).

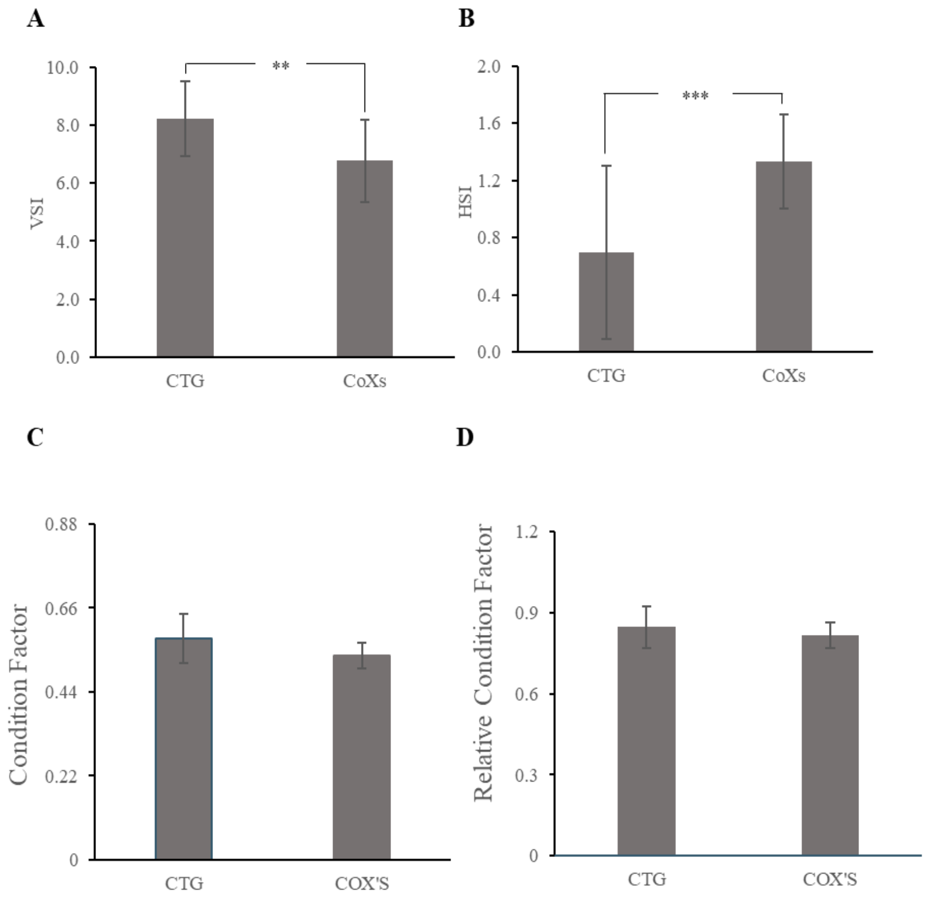

Figure 11: Gastrointestinal indices- visceral somatic index (VSI) (A), hepato-somatic index (HSI) (B), condition factor (C) and relative condition factor (D) of *P. canius* collected from the Chattogram (CTG) and Cox's Bazar (CoXs) region of Bangladesh. Asterisks denotes a significant difference between fishes from Chattogram and Cox's Bazar region (**, $p < 0.01$ and ***, $p < 0.001$) (n = 42 from Chattogram region and n = 18 from Cox's Bazar region).

4.3.2. Hepatosomatic index (HSI)

In the present study, the HSI value was recorded in *P. canius* from two different parts of Bangladesh and showed a statistically significant difference in the HSI values ($p < 0.05$) (Figure 11B). The HSI value ranged from 0.02 to 4.96 in fishes collected from Chattogram, whereas it was 0.50 − 2.08 for the Cox's Bazar region. The higher HSI value was recorded for the Chattogram region (2.08 ± 0.60) compared to the Cox's Bazar region (1.34 ± 0.33).

4.3.3. Condition factor

The condition factor for the *P. canius* from two different regions of Bangladesh has shown no statistically significant difference (p-value > 0.05). Fish of the Chattogram region have a condition factor of 0.58 ± 0.064 which is comparatively higher than the value of Coxs Bazar region 0.54 ± 0.035 (Figure 11C).

4.3.4. Relative condition factor (Kn)

On the basis of the recorded data, *P. canius* collected the mean Kn values did not vary significantly among the two regions of Bangladesh (Figure 11D). The mean Kn value was 0.85 ± 0.077 in fishes collected from the Chattogram region whereas the Kn value was 0.82 ± 0.047 for the fishes collected from the Cox's Bazar.

4.3.5. Relative length of gut (RLG)

In this study, the RLG values were also calculated and found that RLG ranged from 0.15 to 2.05 with a mean of 1.30 ± 0.20 for the *P. canius* collected from the Chattogram region. On the other hand, the RLG value ranged from 0.87 to 2.29 with a mean of 1.55 ± 0.32 in *P. canius* collected from the Cox's Bazar region (Figure 12A). A statistically significant difference (p-value < 0.01) can be observed between the RLG values of these two regions of Bangladesh (Figure 12A). It was also observed that the RLG values were significantly higher in smaller-sized fish compared to the larger-sized fish collected from the Cox's Bazar regions. However, no such kind of variation was observed in *P. canius* collected from the Chattrogram region (Table- 9).

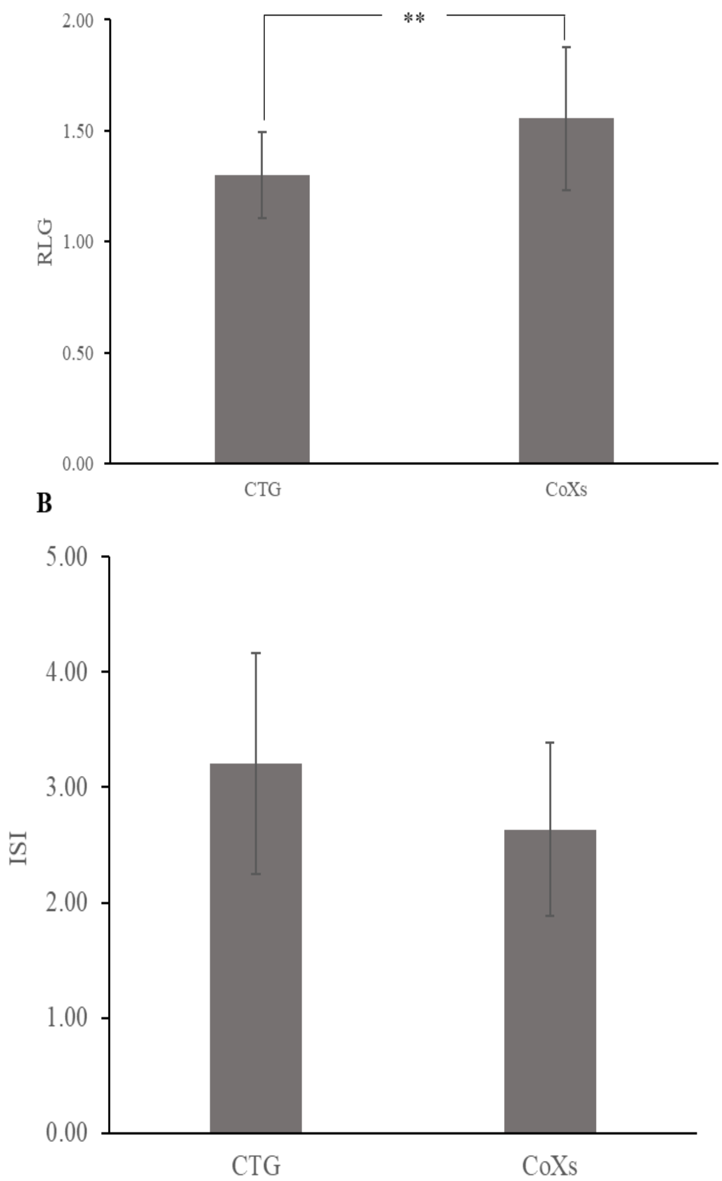

Figure 12: Relative length of gut (RLG) (A) and intestine somatic index (ISI) of *P. canius* collected from the Chattogram (CTG) and Cox's Bazar (CoXs) region of Bangladesh. Asterisks denotes a significant difference between fishes from Chattogram and Cox's Bazar region (**, $p < 0.01$) (n = 42 from Chattogram region and n = 18 from Cox's Bazar region).

Table 9: Mean variations of RLG values in different size groups for two different regions of Bangladesh:

Chattogram Region	
Size Group (mm)	**RLG**
300-400	1.35±0.21
400-500	1.37±0.31
500-600	1.26±0.16
600-700	1.49±0.36
700-800	1.38±0.22
Coxs Bazar Region	
300-400	1.69±0.43
400-500	1.33±0.33

4.3.6. Intestine somatic index (ISI)

Plotossus canius collected from both regions showed comparatively similar ISI values in which no significant differences ($p < 0.05$) were observed. The Chattogram region had an ISI value 3.21 ± 0.96 on average. Where the fishes of the Cox's Bazar had a mean ISI value of 2.64 ± 0.75 (Figure 12B).

4.4. Morphological and histological study of the intestine of *P. canius* collected from two different regions of Bangladesh

As *P. canius* is considered carnivorous, it possesses a short-length intestine. It starts from the pyloric end of the stomach (Figure 13C). Mesentery helps the convoluted intestine to be held in the proper position. The entrance of the bile duct can be addressed near its origin from the stomach. In the anterior part, numerous mucosal folds were present which were longitudinal in direction with wide reticular connection in between. The same in the posterior part is much closer with no reticular connections (Figure 13).

The intestinal structure of *P. canius* was also examined histologically. The mucosa is composed of uncomplicated, cylinder-shaped columnar cells and is folded into several groups that periodically branch.

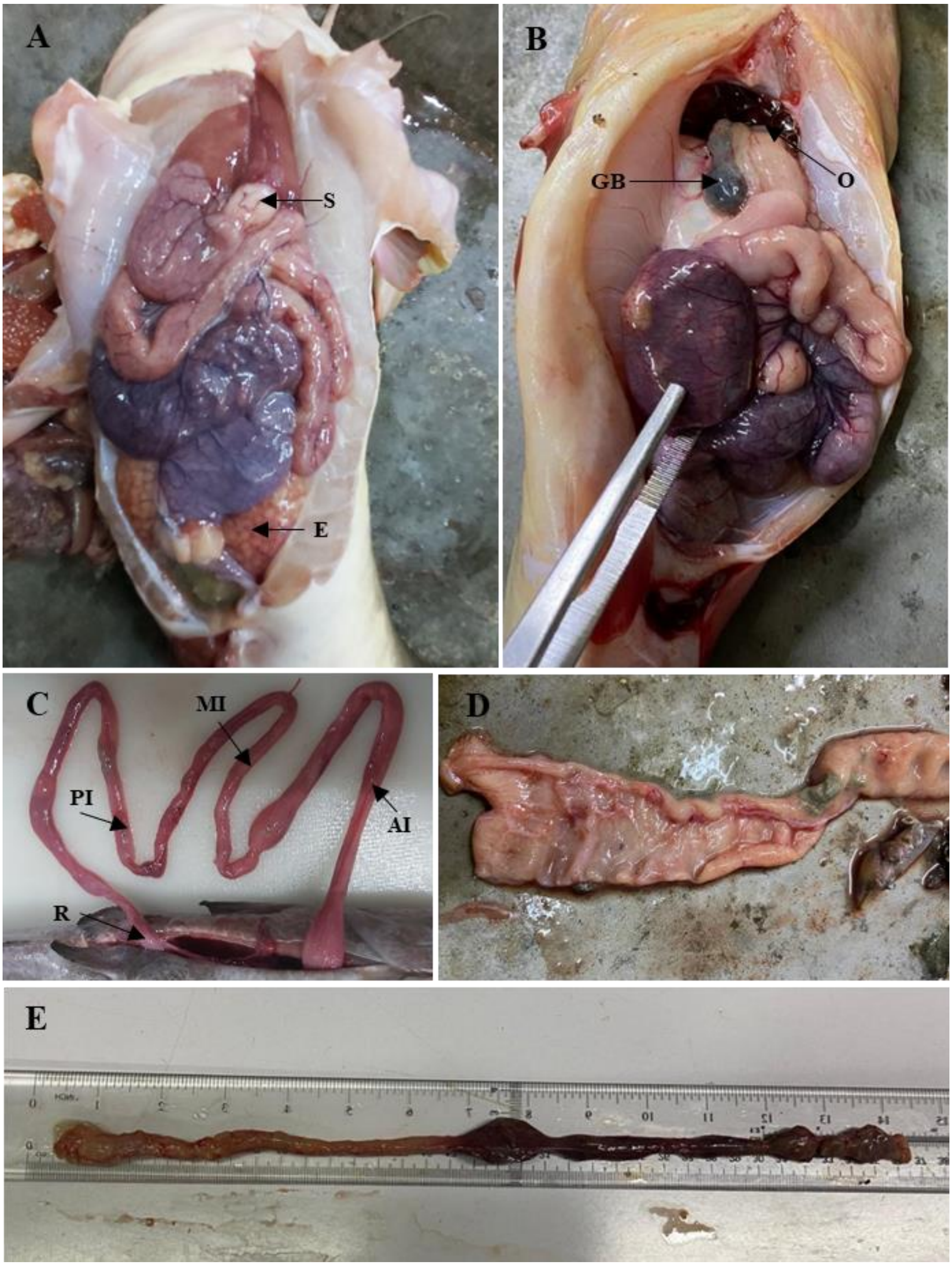

Figure 13: Intestinal morphology of *P. canius*- dissection of the abdominal region (A), separating intestine from other internal body parts (B), untangling the intestine (C), longitudinal dissection of the gut (D), and measuring the length of intestine (E). (S-

stomach, E- eggs, O- esophagus, GB- gallbladder, AI- anterior-intestine, MI- mid-intestine, PI- posterior-intestine and R- rectum).

The anterior mucosal layer is relatively thick and occasionally invaded by villi. The villi are longer and more branching in the anterior part of the gut than they are short and unbranched in the posterior part. The mucosal layer of the gut also contains mucus-producing goblet cells in addition to columnar cells. The back of the gut has a significantly higher density of them. The inner core of the intestinal folds and a thin layer surrounding the circular muscle fibers are both made of subepithelial connective tissue, which also creates the sub-mucosal layer. This is densely innervated and vascularized. An internal circular muscle layer and an exterior longitudinal muscle layer are created by placing them in that order. In this area, the longitudinal muscle fiber layer is noticeably thinner than the circular muscle fiber layer. The submucosal connective tissue layer occasionally passes through the circular muscle fiber layer. Even more so than in the pyloric stomach area, the muscularis-mucosa layer of the intestinal region is thick. In this area of the digestive system, the serosal layer is thickest and has a significantly denser composition (Figure 14).

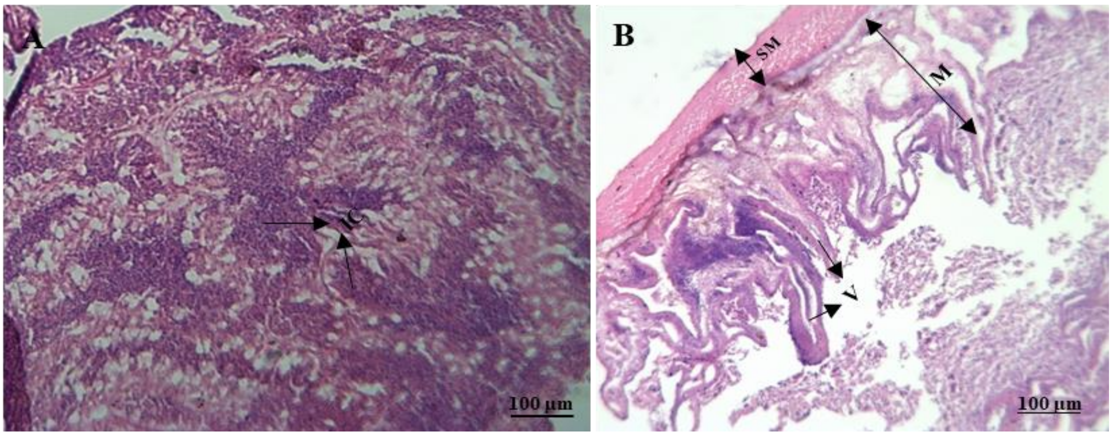

Figure 14: Histological images of the intestine of *Plotosus canius*- intestinal cells distribution pattern and structure (A) and internal structure of the intestine (B). (M-mucosa, V- villi, SM- submucosa, IC- intestine cell). Scale bar- 100 micro-meters.

4.5. Feeding behavior analysis of *Plotossus canius* in indoor conditions:

4.5.1. Feeding behavior of *Plotosus canius* in the aquarium

In the present study, the feeding behavior of *Plotosus canius* was observed in the aquarium and tank condition using different types of feed (crab, marcel, and trash fish). The fish could not see the feed despite its closeness in the tank conditions (even less than a few inches). The fish can only sense the feed if it touches the barbel or sometimes to the body. No social hierarchy was observed in the feeding behavior of this species. However, the young individual was more active in taking up food compared to the adult. Once after capturing the food, the young fishes engulf the whole particles if it is within their size. In the case of big food particles, they first bite into small pieces and share the rest of the pieces among their kin. The adult fish do not participate during the feeding time of the young. However, young individuals often move underneath the adults in search of food. In some cases, if the food particle touches the barbel of an adult, the adult takes the food. Usually, adults delay the uptake of food compared to the young. Once satisfied, the excess food has remained as it is in the tank.

4.5.2. Differences in the feed preference by *Plotosus canius* in the aquarium

In this study, we used three different feed types and observed the amount of food intake by *Plotosus canius*. Fish were fed at two-days intervals and 200 gm of feed was provided at a time to the aquarium. The average feed intake was (150 ± 18) gm when crab particles were used as feed by 5 fishes. A slightly higher (160 ± 22) gm feed intake was reported in the case of marcel. However, feed intake was 0 gm during the first day of feeding with trash fish (tilapia). On the subsequent days, a very small amount of feed (10 ± 7) gm was uptake by the 2-3 fishes. We found a statistically significant difference in the feed intake among the three different feed types (Figure 9).

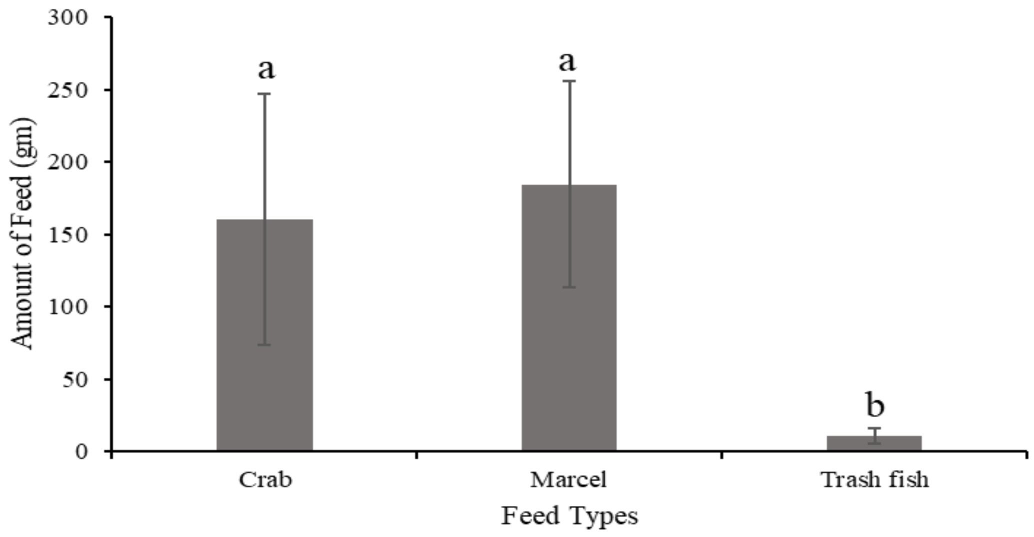

Figure 15: Feed consumption rate of *P. canius* in the indoor conditions. Values are presented as mean ± standard error of the mean (SD). Different subscripts of alphabets are statistically significant at $p < 0.05$ (ANOVA, Tukey's HSD post-hoc test). n = 7 for each treatment groups.

CHAPTER-5: DISCUSSION

The present study focused on the analysis of the food and feeding behavior of *P. canius*, a commercially important estuarine-marine fish species from the Bay of Bengal. This study analyzed the gastrointestinal indices, gut contents, and feeding behavior of *P. canius* from two different regions of Bangladesh and found a significant variation in the gastrointestinal indices, gut contents, and feeding behavior. The findings of the present study may serve as a baseline information for domestication, and successful conservation and management of this species.

5.1. Length-weight distribution of *P. canius*

In this study, recorded data indicates that the fish of the Chattogram region have higher body weight than the Cox's Bazar region for a similar body length. *P. canius* is distributed along the coast of Bangladesh and is also found in the Sundarbans area (Hoq, 2007). Fish fitness varies depending on environmental, physiological, and dietary factors in any given habitat, and quantitative description is a fundamental and critical tool for natural populations for successful conservation and management in a particular habitat (Tsoumani et al., 2006; Britton et al., 2007). Exogenous environmental influences are recognized to play a significant role in regulating species dynamics and can have a direct impact on fish physiology, metabolism, or reproductive success. Additionally, environmental factors indirectly can influence the abundance of zooplankton and other key food sources by altering their predators and prey (Ottersen et al., 2010). Fluctuations in the quantity, as well as the quality of available food, can have an impact on energy receipt and distribution and hence on individual fitness and, eventually, on the entire population and ecosystem structure (McBride et al., 2015; Queiros et al., 2019). Thus, for this species, it can happen on account of having available food along with suitable environmental conditions in the Chattogram region compared to the Cox's Bazar region. Further studies about the relationships between environmental variability, abundance of food items, and water quality need to be assessed which will further elucidate to determine the best management action plan.

5.2. Gut content analysis and feeding habits of *P. canius*

Investigating the nutritional state, state of energy, differential physical condition, and overall health of individuals and populations can reveal insights into population dynamics. Based on the food found in the gut of *P. canius*, it appears that this particular species typically hunts for invertebrates by drawing water inwards into its mouth. This suggests that *P. canius* may be able to capture larger prey items that are up to 40-50% of their own body size. The size of a predator's mouth opening can provide insight into the maximum size of prey they can consume, and is directly related to the range of prey sizes they can capture. Differences in mouth size have been linked to variations in the types and sizes of prey that predatory fish consume, with larger gape sizes allowing for the capture of larger prey (Scharf, 2000).

This study also revealed that the adult diet of *P. canius* consists mainly of crabs, mollusks, aquatic insects, plant materials, and isopods. Crustaceans and Mollusks were their primary food source, with occasional debris that may be classified as "accidental inclusions". The diet of smaller-sized *P. canius* consists solely of prawns, plankton, and insects. In Bangladeshi coastal waters, *P. canius* primarily feeds on crabs, shrimp, and mollusks, with additional food items including fish, insects, algae, and miscellaneous items (Ahmed and Haque, 2007). Sinha (1984) reported the major diets of *P. canius* as mollusks, amphibian bugs, plants, and amphipods with small amounts of detritus. However, in this study, there were differences in the diet composition of *P. canius* from two different geographical regions. The composition of prospective invertebrate diets in nearshore locations fluctuates with ecosystem type, such as the extent to which they are exposed to oceanographic activity (Lloret-Lloret et al., 2022). Further studies on whether any such fluctuation is bigger or less than that caused by seasonal or individual influences need to be elucidated.

5.3. Gastrointestinal indices of *Plotosus canius*

5.3.1. VSI and HSI

The liver and viscera are important indicators of the nutritional health of fish, as they are storage sites for energy. This is demonstrated by the hepato and viscera somatic indices, (Cui and Wootton, 1988; Dawood, 2016). A healthy liver indicates good metabolic activity and a long, healthy life. The ratio of liver weight to body weight is used to determine the level of reserved energy in various species under different environmental conditions (Tyler and Dunn, 1976). The ability of fish to accumulate fatty acid compounds is measured by the hepatosomatic index, which varies based on dietary habits and reproductive differences (Henderson et al., 1984). *P. canius* collected from the Chattogram region have higher values for VSI and HSI value, indicating good availability and utilization of food. Variations in VSI and HSI may be due to differences in food availability and favorable environmental conditions in the Chattogram region compared to the Cox's Bazar region. High VSI values also indicate better digestion rates, metabolism rates, and synthesis and secretion of digestive enzymes (Rasmussen et al., 2000; Malami et al., 2004). This leads to faster growth in terms of length and weight during a given period (Begum et al., 2008).

5.3.2. Condition factor and Relative Condition Factor (Kn)

Based on the research, it appears that various physiological and environmental factors can influence the condition factor (K) values of fish. Factors such as maturity, spawning, and food availability all play a role (Brown, 1957). Additional factors, like those noted by Hickling (1945) and Qasim (1957), could also be at play. The studies conducted suggest that fish in the Chattogram region have a slightly better condition than those in Cox's Bazar, indicating a higher likelihood of physiological status and gonadal maturity (Kurup, 1982).

5.3.3. RLG

The gut length of a fish is an indicator of its feeding habits. The RLG values for carnivorous (0.5–2.4), omnivorous (1.3–4.3), and herbivorous (3.7–6.0) fishes were previously listed by Al Hussaini (1947). In the case of *P. canius*, the RLG value was observed to be 1.30 ± 0.20 for Chattogram and 1.55 ± 0.32 for Coxs Bazar region. Therefore, it can be concluded that the gray eel catfish in these regions are carnivores. According to De et al. (2013), a higher RLG value indicates the presence of slowly digestible food in the gut. This can also be confirmed by observing the presence of plant debris along with cutaneous shells of crustaceans in their intestines. Slow growth in terms of length can also be observed as a result (Karasov and Martínez, 2007).

5.3.4. ISI

Wootton (1998) suggests that the way fish feed and their diets have a significant impact on both their internal and external morphology. Evaluating a fish's ISI value can provide insight into its physiological condition and overall health. Fish with higher ISI values tend to have larger and more developed guts relative to their size, indicating improved nutritional digestion and absorption. This, in turn, is often associated with better physical and mental health. However, unbalanced nutrient content, anti-nutritional elements, and environmental contaminants in alternative feed materials can compromise the intestinal function and health of fish (Sunder et al., 1984). The recorded value shows that fish from Chattogram (3.21 ± 0.96) have better health conditions than those from Cox's Bazar region (2.64 ± 0.75), indicating their voracious feeding habits.

5.4. Morphological and histological analysis of the intestine of *P. canius*

In this study, the anterior intestine of the grey eel catfish (*P. canius*) was found as secondary folding and branching in the villi region. It is hypothesized by Khanna and Mehrotra (1971) that this folding may enhance the intestine's total surface area for absorption. Additionally, AI-Hussaini (1949) established that the shortness of a fish's gut can be compensated for by increasing the complexity of its mucosal folds, and the length of the gut is determined by its average mucosal area. It is widely believed that herbivores have the longest gut, while carnivores have the shortest, with omnivores falling in between (Das and Moitra, 1956; Sinha, 1972; Kapoor et al., 1975). In the case of *P. canius* fish, their intestines are relatively short, ranging from 0.78-1.19 (averaging at 1.08) in terms of the ratio of gut length to total length. Khanna (1962) suggests that the type of food a fish consumes has a direct impact on the length of its intestine. Barrington (1957) believes that additional factors are involved in determining the length of a fish's stomach relative to its total length. Interestingly, the mucosal folds of the anterior region of the *P. canius* intestine display complex reticular and longitudinal bands, which may have developed to compensate for its shorter length without compromising its absorptive area.

5.5. Observation of feeding behavior in indoor conditions

Understanding the food and feeding habits is crucial for domestication and successful aquaculture development. Kaliyamurthy and Rao (1972) and Sinha (1984) have characterized *P. canius* as a predatory carnivore and a benthic feeder. Moreover, the monthly variations in food composition can differ among different species due to seasonal oscillations and the occurrence of each diet component (Oribhabor and Ogbeibu, 2012; Usman et al., 2018). The findings of the current study also suggest that *P. canius* is a carnivorous benthic feeder, based on the food items found in their gut. In this study, the indoor feeding behavior of *P. canius* was observed by offering them different types of feed and observed different behavioral responses of the fish, including their vision, smell detection, and sound detection abilities. Out of the three feed types, crab muscle yielded the best response. When feed was provided to the crab into the water, the fish did not detect it immediately indicating poor vision and smelling ability of this species. However, *P. canius* supplemented with Marcel showed a quicker response but the water quality quickly degraded and the fish began to show abnormal swimming. In the case of chopped tilapia fish as feed, the fish did not show any significant response to it.

P. canius exhibit intriguing feeding behavior, as they cannot detect food until it physically touches their body. Our hypothesis is that this species has underdeveloped neuromasts due to the high-water pressure found at the bottom of the sea, as well as other factors like precipitation rate, turbid water, and a preference for habitat in the bank of the bottom (Higgs and Fuiman, 1996). Neuromasts, which can be either free-standing or submerged within the lateral line system, detect mechanical stimuli like touch and water movements. In addition, the brain must integrate peripheral and central signals to regulate appetite and digestion. Similar to mammals, fish seem to have a hypothalamic center controlling food intake and energy homeostasis (Leh et al., 2012). Future studies are required to understand the neuroanatomical characterization and hypothalamic regulation of feeding and appetite in this species.

CHAPTER-6: CONCLUSIONS

6.1. Conclusions

In the field of fisheries research, obtaining information on the length-weight distribution, feeding behavior, gut contents, and gastrointestinal indices is crucial. Sustainable and productive fisheries and aquaculture practices are necessary for improving livelihoods, boosting the economy, and ensuring the sustainable use of natural resources. As the dependency on the capture fisheries has increased over the years, adopting a sustainable approach to fisheries and aquaculture may help to manage fish populations for the future. In aquaculture, accurate knowledge of feeding practices is essential to increase the yield of cultured fish. Understanding various biological data can also influence the management of wild fish species in both captive and open areas. This paper presents the results of an investigation into the food and feeding habits of an economically important catfish species, *P. canius*, in its natural habitat. The experimental results on length-weight distribution, gut contents, feeding habits, condition factor, relative condition factor, and gastrointestinal indices strongly support existing data, and the findings can aid in improving the domestication and culture of this species in the future.

CHAPTER-7: RECOMMENDATIONS AND FUTURE PERSPECTIVES

7.1. Recommendations and future perspectives

In this study, we aim to analyze biological data on the grey eel-catfish (*Plotosus canius*) and explore the blue economy of the Bay of Bengal. The study's results will assist in developing culture techniques, and artificial diet formulations, and determining the probable time of spawning. This knowledge will benefit both the management and aquaculture authorities, ensuring the fish's availability and economic importance. Despite performing a qualitative approach to fulfill the objectives of this study, there were some limitations that can be omitted by following the recommendations:

- ✓ To carry out basic and adequate research for its development and coordinate fisheries research activities in Bangladesh.
- ✓ To identify breeding biology in captive conditions, we must focus on its physiological research.
- ✓ We need more time for its proper biological study by knowing every aspect of the cycle.
- ✓ To spread all the knowledge for its development to users through the training of extension workers, planners, fish farmers, and other relevant persons.
- ✓ To know the cost-effective feed for its growing we must apply different types of feed by feeding on captive conditions to know the exact low-cost.
- ✓ As the fresh and properly preserved sample gives a better histological diagram. So, samples should be collected and preserved immediately after catching if it is possible.

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APPENDICES

Appendix 1: Data of various gastro-intestinal indices of *Plotosus canius* on the basis of various size groups:

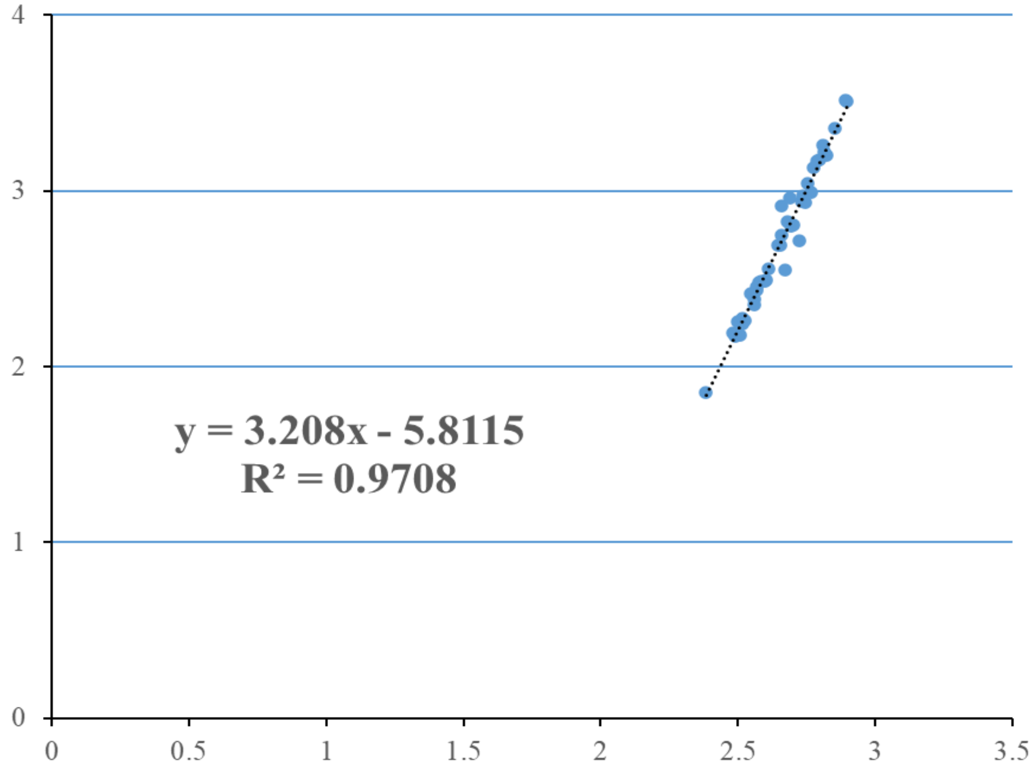
Region	Gastro-intestinal indices					
	Size	RLG	VSI	ISI	C.F.	Kn
Chattogram	300-400	1.35±0.21	8.36±0.25	3.09±1.29	0.53±0.03	0.85±0.06
	400-500	1.37±0.31	9.06±1.94	3.71±1.97	0.62±0.18	0.93±0.28
	500-600	1.26±0.16	8.55±1.21	3.72±1.26	0.43±0.10	0.75±0.14
	600-700	1.49±0.36	8.99±1.46	3.51±1.63	0.61±0.05	0.86±0.08
	700-800	1.38±0.21	9.93±2.52	4.42±2.33	0.66±0.03	0.90±0.03
Cox's Bazar	300-400	1.69±0.43	6.61±1.40	3.08±1.01	0.52±0.04	0.83±0.07
	400-500	1.33±0.33	9.40±2.12	2.30±0.69	0.55±0.03	0.83±0.03

Appendix 2: Independent samples t-test for the gastro-intestinal indices:

Index name	Df	t-value	p	Relationship
RLG	33	-2.94	0.0060	Significant
VSI	33	3.12	0.0030	Significant
ISI	32	1.86	0.0730	Non-significant
HSI	33	3.96	0.0003	Significant
Condition factor	35	4.36	0.0260	Non-significant
Relative condition factor	36	0.70	0.1724	Non-significant

Appendix 3: The marked data values in the results section:

Crab	Marcel	Trash fish
160.1875	184.29	10.31
86.91162	71.381	2.874741



Appendix 4: Logarithmic relation between the standard length and weight of pooled data of *Plotosus canius* collected from the two different regions of Bangladesh.

Brief Biography of the Author

Arpan Mallick Bijoy, son of Sasti Charan Mallick and Anju Rani Aich, is from Sitakundo thana under Chattogram district of Bangladesh. He has completed her B.Sc. in Fisheries (Hons.) Degree in 2020 from Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh obtaining 3.61 out of 4.00. Now, he is a candidate for the degree of M.Sc. under the Department of Fish Biology and Biotechnology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. He has research interest in the field of histology, management and fish reproductive biology.