CHAPTER - 01

INTRODUCTION

Food is any component, substance or living thing that can be used by fish to support the needs of life. On the other hand, feeding is the process by which organisms typically animals obtain food (Sahney et al., 2010). The quantity and quality of the food that fish eat are known as their food habits while feeding habits are the place, time and way the food is obtained by fish. In contrast, feeding behavior includes several activities that takes place before and after the consumption of food item (Rønnestad et al., 2013). At the beginning of their lives, fish often eat food based on the size of their mouth opening. After maturing, the amount and types of food changed in both quantity and quality. Food preferences are highly influenced by the type of food present in the living habitat, environmental condition, fish size or sexual development and competition between and within species (Zacharia and Abdurahiman, 2004). Food acquisition is essential for heterotrophic organisms since it supplies energy for somatic growth, reproduction and life maintenance. It also plays a crucial role in ecology of food webs and species interaction including predation and resource competition. Fish diet and feeding patterns are important biological indicators of the interaction between fish and natural foods. Therefore, understanding the food and feeding behavior is important to consider the feed selection in commercial aquaculture and to ensure better health in sustainable management in the open water bodies.

The food and feeding habits of fishes have great importance in the operation of aquaculture. It is beneficial to choose fish species for aquaculture that will cooperatively utilize potential food sources in the waterbodies rather than competing with one another. In order to encourage optimal growth and output in aquaculture, proper and balanced feeding procedures are essential. Fish can grow faster to their full potential and reach marketable sizes when given sufficient and nourishing nutrition. Fish that are well-fed have improved feed conversion ratios (FCR) which indicates that they effectively convert food into body mass. Efficient feeding practices in aquaculture can minimize environmental impacts (Wilfart et al., 2023). Appropriate feeding techniques such as using feed with optimal nutrient levels and minimizing feed wastage promotes sustainable aquaculture by reducing nutrient pollution and maintaining water quality. To comprehend fish biology and

management, studies on food and feeding behavior is essential (Chakraborty et al., 2021). Fish diet adjustments are a normal part of environmental adaptation to prevent competition between young and adults (Gomathy and Vivekananadan, 2017). The time and extent of changes in food and feeding habit vary from one species to another species. The feeding intensity also changes with availability of preferred food, seasons and maturity stage. Depending on the species, the feeding intensity falls during the spawning season in comparison to the non-spawning period. (Khongngain et al., 2017; Sarkar et al., 2017). It is therefore important to understand the food and feeding habits, intensity and feed choice to understand the feeding dynamics of commercially important species for better aquaculture output.

To comprehend the dietary needs, ecological functions and overall ecosystem dynamics of fish it is crucial to evaluate their food and feeding habits. For preliminary analyses of food and feeding behavior, gut content analysis has been used for a long time (Hyslop, 1980; Cortes, 1997) while it was recorded qualitatively, quantitatively and as a percentage of abundance (Pelicice and Agostinho, 2006; Baker et al., 2014). Gut content analysis is a method that scientists use to learn about the food preferences and feeding preferences of various fish species. Direct gut content analysis, which involves dissecting or evacuating the gut and inspecting its contents is the most easily performed and widely used technique (Athira, 2022). The basic goal of fish gut content analysis is to determine the most often devoured prey or whether a specific food type is present in the stomach (Wala et al., 2023). Fish gut contents analysis may provide important details about its diet and trophic level and thus become a standard exercise in fisheries biology (Nishad et al., 2021). Assessment of the contents of the stomach and feeding patterns can be used to evaluate ontogenetic consequences, prey preferences and habitat preferences and to develop conservation plans (Mishra, 2020). Nansimole et al., (2014) claimed that the development, abundance and productivity of waterbodies are better understood by qualitative and quantitative dietary analysis of fish in their natural habitat which are also used to understand fish diet and feeding habits.

A study on general wellbeing is essential to determine the degree of fitness, gonadal maturation and reproductive success of a fish. Some quantitative analyses of fishes like indices including visceral somatic indices (VSI), hepato somatic indices (HSI), intestine

somatic indices (ISI), gonado somatic indices (GSI) and relative length of gut (RLG) analysis are important parameters to evaluate the fitness of the fish population. The study of HSI and VSI indices has an important role in the metabolism of fish related to digestion and absorption, synthesis and secretion of digestive enzymes and carbohydrate metabolism (McLaughlin et al., 1983). GSI and HSI are the factors involved in the activation of the brain pituitary gonad (BPG) axis (Akel and Moharram, 2007). These variables are essential for gaining a better understanding of some biological processes involved in estimating male and female fish reproductive success (Akel and Moharram, 2007).

ISI is another biological parameter used to determine the feeding intensity of fish. The monthly fluctuation of ISI indicates the variation in feeding intensity (Sangma et al., 2019). In addition, RLG is an adjuvant index that provides an idea of the nature of food consumed (Sarpanah et al., 2010). Intestine length or gut length (GL) is regarded as a diet indicator (Kramer and Bryant, 1995a) and can be used to compare the diets of different species, especially fishes (Al-Hussaini, 1947; Karachle and Stergiou, 2010a). Intestines of herbivorous species are longer than those of omnivorous species for a given body length and those of omnivorous species are longer than those of carnivorous species (Kapoor et al., 1976; Kramer and Bryant 1995b; Karachle and Stergiou 2010a, b). As a result, the following pattern of fish GL variation to species feeding habit are generally accepted: Carnivores < Omnivores < Herbivores (Kapoor et al., 1976; Ribble and Smith, 1983; Kramer and Bryant, 1995b). This kind of analysis provides values that can be used to determine the trophic level of a specific fish (trophic level > 4 = piscivores, for instance) (Hyslop, 1980). According to Seaburg (1957), the majority of fishes fall into one of the three main trophic types of carnivorous, omnivorous or herbivorous feeders. Trophic values for aquatic organism range from 2 for herbivores to 5.5 for specialist predators of marine mammals (Nishad et al., 2021). Therefore, determining the GL could provide valuable insights for choosing best suitable species for aquaculture. The length of the gut of an animal depends on the nature of the food they consume and increases with an increasing proportion of vegetable materials reported by Biswas (1993). Besides ISI and RLG both are linked with food and feeding conditions of fish. So, there may be interlinkage between them too. Therefore, studying the gastrointestinal indices will be helpful

for understanding the general well-being of fish that will serve successful conservation and management plan.

The Bay of Bengal (BoB) is a large marine aquatic ecosystem that is home to numerous fish and other organisms and is regarded as one of the most important parts of the blue economy of Bangladesh. The Bay of Bengal has been renowned for having abundant fisheries resources around the globe. The BoB is regarded as one of the world's most prolific regions because of its location and climate which creates an ideal setting for a variety of fish species (Islam, 2003). Being an adjacent country, Bangladesh is fortunate to have access to the rich and diversified fisheries resources of the BoB which contributes to the abundance and high quality of coastal fisheries resources. According to Barua et al., (2012), it has a 714 km long coastline and a 166,000 km² EEZ that is home to 1093 aquatic marine creatures. The croakers or jewfish belong to the largest family of Sciaenidae under Perciformes orders and are one of the important fish species of great economic importance in the BoB. Fishes of the Sciaenidae family can be found in shallow inshore areas to depths of more than 300 meters in tropical and subtropical waters all over the world (Talwar, 1995). Some species reach estuaries and even freshwater, but the majority of species are confined to continental shelves and slopes and live near the bottom.

The croakers are locally known as "Poa mach" and are highly valued food fish in the Bay of Bengal. According to Mohammed and Abood (2019), members of this family of fish are also known as drummers because of the sound they made using swim bladder-related muscles. In the Sciaenidae family, 32 species of marine, brackish and freshwater fishes belonging to 15 genera have been reported by Habib and Islam (2020). In addition, Barman et al., (2022) found 19 species under 11 genera from Bangladeshi maritime water, with *Johnius* being the most prevalent genus. The croakers are often found in shallow water, 70 % of biomass distribution by depth strata of this group is found in the shallow water (10m-40m) area of the BoB (Lamboeuf, 1987; Fanning et al., 2019). They are often identified by their large dorsal fin, which rarely separates into its spinous and soft-rayed sections. Furthermore, their lateral line extends all the way to the caudal fin. They are bottom dwelling carnivores, feeding on small crustaceans, fishes and benthic organisms.

The Bay of Bengal is enriched with a variety of croaker's species like belanger's croaker (Johnius belangerii), hammer croaker (Johnius borneensis), pama croakers (Otolithoides pama), tigertooth croaker (Otolithes ruber). According to Sasaki (2001) and Hoese et al., (2006) Johnius belangerii (belanger's croaker) is a demersal, schooling species that lives in shallow coastal, estuary and mangrove waters down to 100 m (most commonly at 7–40 m) on sand, mud, and rock bottoms. These fish appears to tolerate certain conditions and has a large distribution in the Indo- West Pacific, East of Southern China, West of Persian and Indonesia. According to Ramteke et al., (2020), this species is known for its carnivorous feeding habit which mainly consists of invertebrates, particularly stomatopods, mollusks and isopods. Otolithes ruber, commonly known as the red spot croaker have an elongated, slender body with a slightly compressed shape. Previous research has reported on several biological characteristics of O. ruber citing a maximum and common length of 90 cm (TL) and 40 cm (TL) respectively (Sousa and Dias, 1981). According to Froese and Pauly (2019), adults of O. ruber are mostly carnivores and eat fish, prawns, and other invertebrates. J. borneensis is a marine-estuarine-dependent fish (Solania and Seronay, 2017). Sharpnose hammer croakers has a rounded snout but not enlarged or protruding, it also has a large, oblique upper jaw that extends backward below the back half of the eye, no barbell on the chin, teeth that are well differentiated into large and small in both jaws, cycloid scales on the head and swim bladder that is shaped like a hammer (Sasaki et al., 2001). Due to high consumer acceptance and an increase in the human population and consequent increases in the demand for fish, the harvesting pressure of these fish is intensifying every year (Barman et al., 2022). Therefore, there is an increasing need to conserve and manage these commercial fishery resources by creating appropriate management strategies like culture technique and artificial breeding methods using biological information and time series data to ensure the fishes economic viability. As a result, a detailed investigation of the link between several indicators of croaker species is crucial for fisheries management and stock improvement in the BoB.

Thus, recognizing the economic importance of croaker, the present study focused on biological parameters to provide proper information regarding gonadal condition, food and feeding habits, energy level and overall fitness of fish health as well as to analyze whether there are any interrelationships among different biological indices of these croakers. Data obtained from this study will contribute to our knowledge of the reproductive biology of jewfish and provide some information to future biologists for more intensive research in advanced farming techniques and artificial breeding techniques and ultimately in the conservation and management of this fish.

1.1 Objectives of the study:

- 1. To determine the visceral somatic index (VSI), hepato somatic index (HSI), intestine somatic index (ISI), relative length of gut (RLG) of *J.belangerii*, *J. borneensis*, *O. ruber* and comparative analysis among these three croaker species.
- 2. To analyze the feeding habits and dietary preferences of the three croaker species in the Bay of Bengal.

CHAPTER - 02

REVIEW OF LITERATURE

Croakers are excellent food and sport fishes all over the world. Management and sustainable exploitation of present fish stocks highly relies on understanding the biological indices and interrelationships among them. Although several studies have been conducted on the biological index of jew fish worldwide relatively little is known about the croakers from the Bay of Bengal. Before conducting any research under a definite experimental procedure, it is important to have a look at previously conducted research activities on the related topics. A brief of the few important studies regarding Sciaenidae are presented below-

2.1 Food and feeding habit

Sciaenid fishes are extremely valuable both commercially and recreationally all around the world (Kirkley and Kerstetter et al., 1997). Sciaenid are mostly found in coastal marine environments, while certain species are found exclusively in freshwater (Chao, 1986; Nelson et al., 1994). Marine species of the family can be found on the temperate and tropical continental shelves of the Pacific, Atlantic and Indian seas, but not on the Pacific and Indian oceanic islands (Nelson et al., 1994). Many marine species prefer estuaries as nursery grounds for their larvae and juveniles (Chao, 1986; Flores-Coto, 1992). The majority of species are linked with sandy and muddy bottoms near river mouths, some can be found on coral reefs and in surf zones (Sangeetha et al., 2022). There are 584 nominal species listed globally, with 289 valid species and 69 genera (Parenti, 2020). Thirty percent of sciaenid species are found in four genera (Johnius with 32 species, Cynoscion with 25, Stellifer with 24 and Umbrina with 17) while 43 % of the genera (31) are monotypic (Parenti, 2020). Lal Mohan (1981) described 36 species from 17 genera in the Indian seas. Nelson et al., (2016) included 283 species in 67 genera in his book "Fishes of the World". Fish Base reports 18 species from the Arabian Sea and 27 species from the Bay of Bengal. Scianidae family is primarily carnivorous, preying on small fish and benthic invertebrates (Parenti, 2020). According to Salarpouri et al., (2021) the Atrobucca nibe, black mouth croakers is carnivorous and abstemious in nature with high vacuity index (VI = 86.5 ± 16.8 %). This species preferred foods were skinny cheek lanternfish (65 %), Japanese threadfin bream (13 %), and deep – sea shrimp (11 %). Manojkumar (2011) observed that *Johnieops sina* is carnivorous that largely feeds on fish, crabs and mollusks. According to Nair (1979), the juveniles graze mostly on zooplankton, particularly copepods and amphipods, in addition to prawns. According to Chakraborty et al., (2000), the primary dietary items of *J. sina* were prawns and fish. Bezerra et al., (2014) conducted study on the Maranhão coast of the Northeast Brazil on the feeding habit of *Macrodon ancylodon* (Actinopterygii, Sciaenidae) and revealed that the diet was dominated by fish, crustaceans and occasionally cephalopods. Male and female diets were similar and the percentage of prey items varied across juveniles, subadults, and adults (p > 0.05). Chaves and Vendel (1998) worked on the feeding habit of *Stellifer rastrifer* (Perciformes, Sciaenidae) in the Guaratuba mangrove and observed that the diet consisted primarily of invertibrates, specifically decapoda non brachyura and polychaeta. Plants, copepod, gammaridea and mollusca made up a lower share. The contribution of each dietary item varied according to season and individual size.

2.2 Visceral somatic index (VSI)

VSI is a measure of relative size and weight of the visceral organs (such as liver, stomach and intestines) compared to their body size. It is closely monitors the net amount of fillet produced by a fish. A substantial gross weight can result from an increase in the mass of viscera, yet these pats of the fish are not valued as fillets (Igejongbo and Esther, 2022). The VSI increases with increasing dietary lipid therefore, viscera resulting from digestible feed calories are usually discarded (Rasmussen et al., 2000; Chaiyapechara et al., 2003). VSI is a crucial biological index since its measurements is required to determine the nutritional value of food (Igejongbo and Esther, 2022). A higher VSI may suggest increased energy reserves or an enlarged organ due to stress or disease. 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. To date, there is no information regarding the visceral somatic indices of croakers, especially from the Bay of Bengal. This study will focus on the VSI of croaker species from the Bay of Bengal which will serve as a baseline for future research on other croaker species in the world.

2.3 Hepato somatic index (HSI)

The hepato somatic 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 hepato somatic 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 well- being of the 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. Worldwide few research worked on the HSI value of croakers. Sylla et al., (2016) studied the monthly variation of HSI of cassava croaker indicating two periods of reproduction from March to June and September to November. The hepatic reserves seem to be mobilized to ensure the energetic cost of reproduction.

Olopade and Dienye (2023) worked on the health status of the three most abundant and economically valuable croaker (Sciaenidae) species in Nigeria's coastal waters and the HSI values recorded ranges from 0.64 ± 0.04 to 1.93 ± 0.03 for *Pseudotolithus typus*, 0.73 ± 0.03 to 1.61 ± 0.01 for *P. Elongtatus* and 0.74 ± 0.04 to 1.85 ± 0.05 for *P. senegalensis*. The results revealed that there were no significant variations in HSI values between May and June (p > 0.05). From March to July, the monthly HSI values observed in this study for the three species were relatively low. This could be due to the fact that most spawning occurs during the rainy season.

A study conducted on *Pseudotolithus elongates* by Ekanem et al., (2004) revealed that the GSI and HSI values have an inverse connection. They observed that in May, the mean GSI

value reached its lowest point (0.9) but the HSI mean value was quite high (1.66) indicating low reproductive activity and a significant energy in the liver.

In a study conducted by Olapade and Tarawallie (2014) on *Pseudotolithus senegalensis* from Tombo, a coastal fishing community in the Western rural district of Sierra Leone, revealed that similar to the gonad somatic index, the highest hepato somatic index was reported in March (2.80). The study shows that *P. senegalensis* does not have an obligatory spawning month and seems to display multiple spawning behaviors. However very little is known about the HSI values of croaker species from the Bay of Bengal. Therefore, this study will focus on the HSI of three croaker species from the Bay of Bengal.

2.4 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. 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 to understand the potential impact of various factors on their intestinal physiology. Till now, no study has been reported on the ISI value of croakers. So, this study will focus on the ISI value of three jew fishes from the Bay of Bengal, Bangladesh.

2.5 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, 2014).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 (<1.0), omnivorous (1.0-3.0) and herbivorous (>3.0) fishes were previously listed by Al Hussaini (1949).Bhakta et al., (2019) conducted

a study on the food and feeding habits of *Otolithoides pama* in Hooghly-Matlah estuary of West Bengal, India and recorded the monthly mean RLG value varied from 0.42 ± 0.05 to 0.56 ± 0.10 for both sexes which denotes *O. pama* is carnivorous in nature. However there is no information on the RLG value of other croaker species from the Bay of Bengal, Bangladesh. So this study will focus on the RLG value of croakers.

2.6 Gonado somatic index (GSI)

A relationship between the weights of the gonad and the body weight is expressed by the gonado somatic index (GSI) (Devlaming et al., 1982). The gonado somatic index value provides a precise indication of the spawning season for any species as well as the essential measures to take to improve fisheries in the targeted areas (Vahneichong et al., 2017). The higher GSI levels suggest that the organism is in a good state and ready for spawning (Pope et al., 2010).

Several research has been conducted on the reproductive biology of croakers. Sylla et al., (2016) carried out a study regarding the reproductive biology of the cassava croaker, *Pseudotolithus senegalensis* from the Ivory Coast continental shelf and recorded GSI value ranging from 0.07 to 0.76 suggesting that there are two spawning periods March to June and September to November.

Tuuli et al., (2011) studied the reproductive biology of the greyfin croaker *Pennahia anea* in the Northern South China Sea and suggest that spawning activity occurred from March to April to June, with a peak in May based on GSI and gonad histology.

Solania and Seronay (2017) worked on the reproductive features of the *Johnius borneensis* from the Agusan River estuary between the months of February – April, 2017. While analyzing the reproductive features, the highest GSI value was recorded in stage VI (5.56%) in females and in males (0.77%). Female had higher GSI compared to males because of the heavy weight of ovaries which contain eggs (Chao and Musick, 1997).

Studies conducted by Ekanem et al., (2004) in the Cross river estuary to figure out the spawning season of *Pseudotolithus elongates* from January to December 1998. High mean values of GSI were 3.75 to 1.34 in December- March and 2.05 to 2.18 in July –September

suggesting that these were the species peak spawning times. The least spawning activity was seen in May with the lowest GSI value of 0.9.

Several studies on other biological parameters including condition factor (K), length – weight relationship (LWR), gonado somatic index (GSI) of *Pseudotolithus senegalensis* from Tombo, a coastal fishing community in the Western district of Sierra Leone was conducted by Olapade and Tarawallie (2014). The result of this study revealed that *P*. *Senegalensis* does not have an obligatory spawning month but exhibit multiple spawning behavior. Gonado somatic index, length- weight relationship, condition factor of *O. ruber* were recorded previously from different areas (Farkhondeh et al., 2018; Al-Zaidy, 2020; Qasim, 2022).

Though there is little information available about some species of croaker from different parts of the world, information about the biological parameters is inadequate in the literature especially from the Bay of Bengal. These study will provide necessary information on the biological parameter of this croaker species which will help in its successful understanding of the life story data as well as the conservation and management of croaker species in the Bay of Bengal.

CHAPTER - 03

MATERIALS AND METHODS

3.1 Sampling site

The study was conducted at the Coastal Biodiversity, Marine Fisheries and Wildlife Research Center of Chattogram Veterinary and Animal Sciences University (CVASU), Dorianogor, Cox's Bazar.

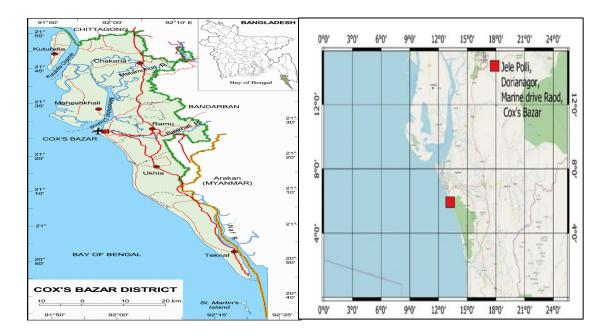
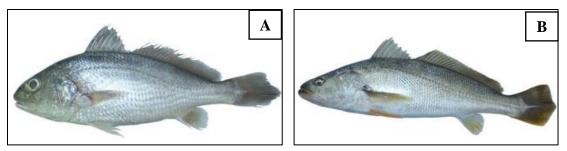


Figure 1: Map of sampling site area

3.2 Collection of samples

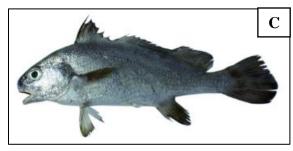
In this study, a total of 105 fresh samples of *Johnius borneensis* (Sada poa), *Otolithes ruber* (Lal poa) and *Johnius belangerii* (Rupali poa) (Figure 2) were collected from the coast of Dorianogor jele polli of Cox's Bazar, Bangladesh during September-October 2021 and September-October 2022.

The collected samples were then transported in an ice box to the laboratory of Coastal Biodiversity, Marine Fisheries and Wildlife Research Center, Cox's Bazar for further analysis.



Johnius borneensis

Otolithes ruber



Johnius belangerii

Figure 2: Experimental fish: (A) Johnius borneensis; (B) Otolithes ruber and (C) Johnius belangerii

3.3 Morphometric measurements

At the time of measurement, the total length (TL) and standard length (SL) of each collected fish were measured by using a measuring scale and recorded in centimeters (cm). The weight of the fish was measured by using an electric balance (KD-300KC balance) and recorded in grams (g). Total length (TL) was measured from the tip of the snout to the tip of the longer lobe of the caudal fin and standard length (SL) was measured from the tip of the snout to the caudal peduncle (Figure 3).

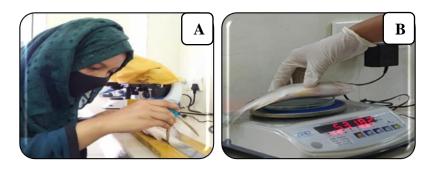


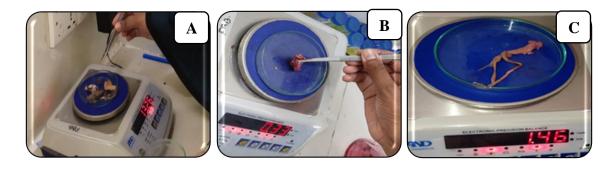
Figure 3: Measurement of (A) Length and (B) Weight of fish

3.4 Collection of internal organs

At first, the collected fishes were dissected to collect the internal organs including the liver, gonad, intestine, and viscera (Figure 4). Then, the weight of the individual organs was recorded separately in gram (g) to determine the visceral somatic index (VSI), hepato somatic index (HSI), intestine somatic index (ISI) and gonado somatic index (GSI) respectively (Figure 5). 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 (Figure 5).



Figure 4: Collection of internal organ



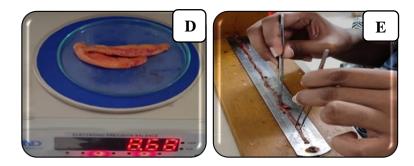


Figure 5: Measurement of (A) Viscera weight; (B) Liver weight; (C) Intestine weight;(D) Gonad weight and (E) Intestine length of fish

3.5 Sex identification

Sex of the fishes were determined by acetocarmine gonad squashing method (Guerrero and Shelton, 1974).



Figure 6: Microscopic view of prepared slide (female)

3.6 Food particles identification

The stomach and intestine were dissected and then debris and mud were removed from the intestine of the fish. After that food found in the intestine were collected and washed. The individual food item as well as the total weight of the food was recorded and weighted. Gut analysis has been carried out to detect the food items present in the gut to ascertain eating habits and to identify the most favored food category for the fish species.



Figure 7: Identification of food particles

3.7 Calculation of gastrointestinal indices

3.7.1 Visceral somatic index (VSI)

VSI is the ratio of visceral weight to total body weight of fish prior to removal of the viscera.VSI was estimated by using the following equation (Ighwela et al., 2014).

 $VSI = \frac{Total weight of all viscera}{Total body weight of fish prior to removal of the viscera} \times 100$

3.7.2 Hepato somatic index (HSI)

HSI is the ratio of liver weight to the body weight. HSI was calculated according to the method of Rajaguru (1992) by determining the weight of the liver as a percentage of the total live weight of the fish.

$$HSI = \frac{Liver \ Weight}{Total \ body \ weight \ of \ fish} \times 100$$

3.7.3 Intestine somatic index (ISI)

ISI is the relationship between weights of gut to total body weight of fish. ISI was estimated by using the following equation (Zhang et al., 2013).

$$ISI = \frac{\text{Weight of gut}}{\text{Total Body weight of fish})} \times 100$$

3.7.4 Relative length of gut (RLG)

RLG is the ratio of full unstretched gut length to the total length of the fish. It was calculated by using the following equation (Al-Hussaini, 1949).

$$RLG = \frac{\text{Length of the gut}}{\text{Length of the body}} \times 100$$

3.7.5 Gonado somatic index (GSI)

GSI is the ratio of gonad weight to body weight. GSI is widely used as an index of gonadal activity and as an index for spawning preparedness's. It was calculated by using the following formula (Afonso-Dias et al., 2005).

$$GSI = \frac{\text{Weight of gonad}}{\text{Total body weight of fish}} \times 100$$

3.8 Statistical analysis

Values were presented as mean \pm standard deviation of the mean (SD). All statistical analysis were performed in Microsoft Excel and SPSS 26.0 for Windows (SPSS Inc.). Statistically significant differences were analyzed using one way analysis of variance (ANOVA) or student t-test depending on the quality and size of the data. Statistically significant variations were set as p <0.05 unless described anywhere in the text.

CHAPTER - 04

RESULTS

4.1 Difference in the VSI values among three croaker species (*J. borneensis*, *O. ruber* and *J. belangerii*) from the Bay of Bengal

In the present study, the visceral somatic index (VSI) was measured from three croaker species (*J. borneensis*, *O. ruber* and *J. belangerii*) collected from the Bay of Bengal. The highest mean VSI value was recorded in *J. borneensis* (7.44 \pm 2.53). The mean VSI was recorded as 5.92 \pm 1.99 and 5.69 \pm 1.61 for *O. ruber* and *J. belangerii*, respectively (Figure 8). A statistically significant difference (p < 0.05) was observed in the VSI values among the three species (Figure 8).

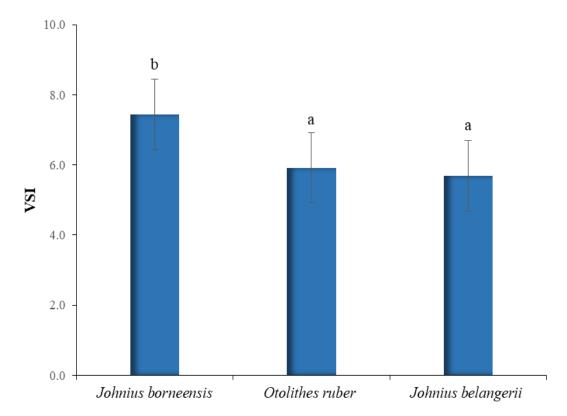


Figure 8: Visceral somatic index (VSI) values of *J. borneensis, O. ruber* and *J. belangerii* collected from the Bay of Bengal coast of Bangladesh. Values are presented as mean \pm standard deviation (SD) of the mean (n=35 for each species). Different subscript of alphabets is statistically significant at p <0.05 (ANOVA, Tukey's HSD post-hoc test).

4.2 Difference in the HSI values among three croaker species (*J. borneensis*, *O. ruber* and *J. belangerii*) from the Bay of Bengal

The HSI value was also recorded from the *J. borneensis*, *O. ruber* and *J. belangerii* and found a statistically significant difference (p < 0.05) among the three croaker species (Figure 9). The mean HSI value was 0.65 ± 0.26 , 0.52 ± 0.16 and 0.85 ± 0.25 for *J. borneensis O. ruber J. belangerii, respectively.*

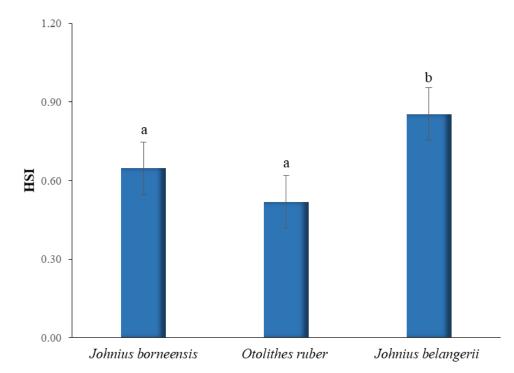


Figure 9: Hepato somatic index (HSI) values of *J. borneensis, O. ruber* and *J. belangerii* collected from the Bay of Bengal coast of Bangladesh. Values are presented as mean \pm standard deviation (SD) of the mean (n=35 for each species). Different subscript of alphabets is statistically significant at p <0.05 (ANOVA, Tukey's HSD post-hoc test).

4.3 Difference in the ISI values among three croaker species (*J. borneensis, O. ruber* and *J. belangerii*) from the Bay of Bengal

The mean ISI value was 0.50 ± 0.15 , 0.51 ± 0.17 and 0.79 ± 0.26 for *J. borneensis, O. ruber* and *J. belangerii* respectively. The recorded ISI value was closely related between *J. borneensis* and *O. ruber* and significantly lower than *J. belangerii* (Figure 10).

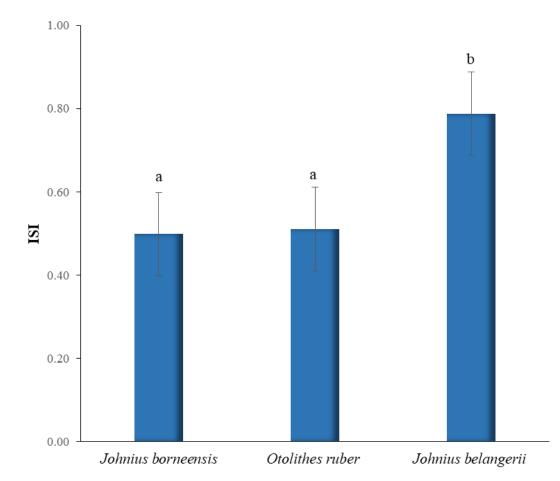
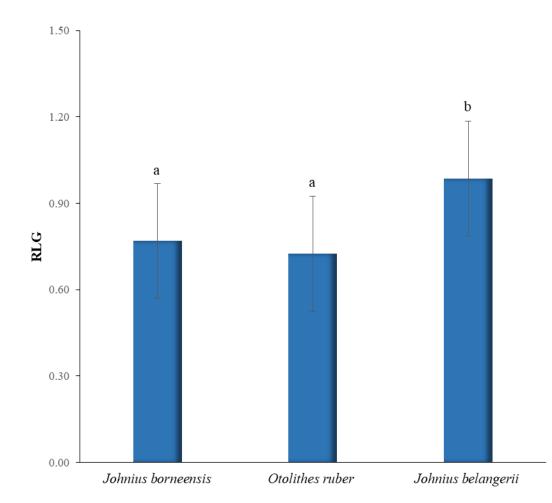
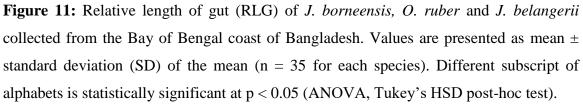


Figure 10: Intestine somatic index (ISI) values of *J. borneensis, O. ruber* and *J. belangerii* collected from the Bay of Bengal coast of Bangladesh. Values are presented as mean \pm standard deviation (SD) of the mean (n=35 for each species). Different subscript of alphabets is statistically significant at p <0.05 (ANOVA, Tukey's HSD post-hoc test).

4.4 Difference in the RLG values among three croaker species (*J. borneensis*, *O. ruber* and *J. belangerii*) from the Bay of Bengal

In this study, the relative length of gut (RLG) value was measured from *J. borneensis, O. ruber* and *J. belangerii*. The highest RLG value (0.99 ± 0.10) was recorded in *J. belangerii*. The RLG value was recorded as 0.77 ± 0.15 and 0.72 ± 0.10 in *J. borneensis* and *O. ruber*, respectively. A statistically significant differences (p < 0.05) was found in the RLG values among the three species (Figure 11).





4.5 Difference in the GSI values among three croaker species (*J. borneensis*, *O. ruber* and *J. belangerii*) from the Bay of Bengal

The mean GSI (male) values were 0.33 ± 0.19 , 0.21 ± 0.07 , and 0.16 for *J. borneensis*, *O. ruber* and *J. belangerii*, respectively for males (Figure 12A). While for females, the mean GSI value for *J. borneensis*, *O. ruber* and *J. belangerii* were 3.15 ± 1.98 , 0.92 ± 0.92 and 1.89 ± 1.73 respectively. A statistically significant difference (p < 0.05) in the GSI values among the three species for females (Figure 12B).

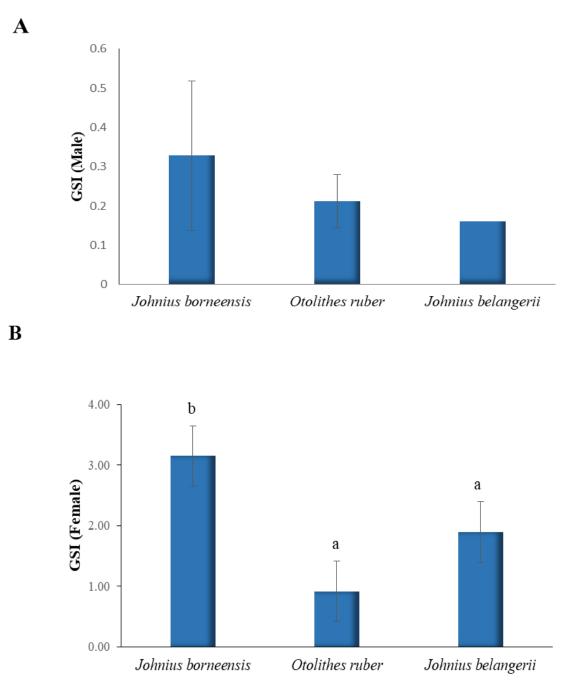


Figure 12: Gonado-somatic index (GSI) of *J. borneensis, O. ruber* and *J. belangerii* collected from the Bay of Bengal coast of Bangladesh in the month of August to September. Values are presented as mean \pm standard deviation (SD) of the mean (n=35 for each species). Different subscript of alphabets is statistically significant at p < 0.05 (ANOVA, Tukey's HSD post-hoc test). For graph A, we found only one male for *J. belangerii*. So, we couldn't perform any ANOVA for figure 12A.

4.6 Abundance of food items found in three croaker species (*J. borneensis*, *O. ruber* and *J. belangerii*) from the Bay of Bengal

In the present study, different food items were collected from the intestine and stomach of three croaker species (Figure 13). The qualitative percentage of various food items is provided in figure 14–16. Based on the available food items and mouth patterns, it is obvious that all three species are carnivores in nature.

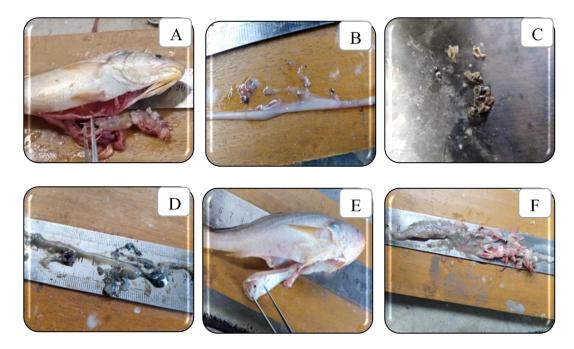
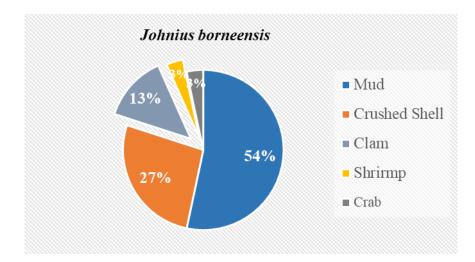
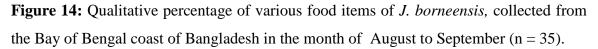


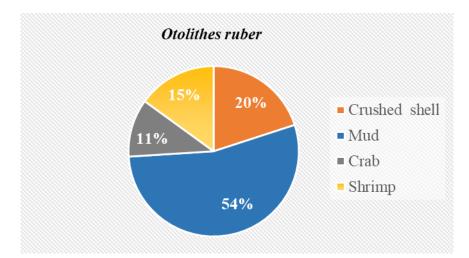
Figure 13: Food found in the intestine (A) Shrimp; (B) Fish Larvae; (C) Crushed Shell;(D) Mud; (E) *Setipinna phasa* and (F) Tong sole and Shrimp

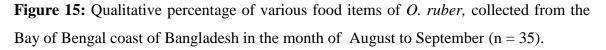
In *J. borneensis*, the food types observed during the gut content analysis were categorized into mud/debris, crushed shell, clam and crab. Gut content analysis revealed that mud/debris were the most abundant feed types among the food classes with an index of preponderance value was 54 %. Crushed shell was observed as the second most abundant food class with an index of preponderance value of 27 %. Clam (13%) was observed as the next abundant food class followed by shrimp body parts (3%) and crab (3%) (Figure 14).





Gut content analysis of *O. ruber* revealed that the food item of *O. ruber* were comprised of the members of shrimp, crab, mud, and crushed shell which is comparatively less diverse than the *J. borneensis* and *J. belangerii*. Mud and the crushed shell composed the maximum percentage of diet of *O. ruber* which was 54% and 20% respectively. The percentage of shrimp and crab was 15% and 11% respectively (Figure 15).





On the other hand, the stomach and intestine of *J. belangerii* were comprised of fish, fish larvae, and shrimp and crushed shells. Fish and mud composed the maximum percentage

of the diet of *J. belangerii* which was 40% and 33% respectively. The other food component was shrimp (14%), fish larvae (10%) and crushed shell (3%) (Figure 16).

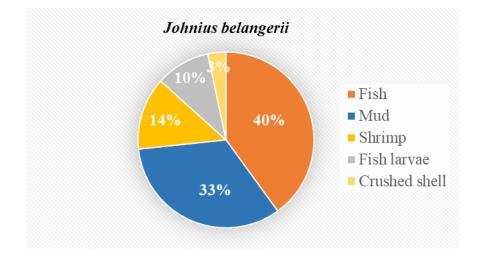


Figure 16: Qualitative percentage of various food items of *J. belangerii*, collected from the Bay of Bengal coast of Bangladesh in the month of August to September (n = 35).

CHAPTER - 05

DISCUSSION

In the present study, significant differences in the biological indices were found among *J. borneensis, O. ruber* and *J.belangerii* from the Bay of Bengal, Bangladesh. In addition, the gut content was also found to vary among these three closely related species in the Bay of Bengal. This study is the first record regarding the gastrointestinal indices and feeding habits of croakers from the Bay of Bengal. The result of the present study indicates the potential differences in life history characteristics among the three croaker species in the Bay of Bengal.

For successful conservation and management in fisheries research, quantitative description is a fundamental tool for studying natural populations (Ricker, 1975; Froese, 2006; Tsoumani et al., 2006; Britton and Davis, 2007). Fish health can vary depending on environmental, physiological and dietary aspects in any given habitat. The collected data in the present study showed that the overall condition of fish health in the Bay of Bengal is in a good state. In addition, the comparative assessment of gastrointestinal indices and feeding habits among three species of jew fish species indicates the differences in the food and feeding habit among those closely related species in the Bay of Bengal.

5.1 Gastrointestinal indices of J. borneensis, O. ruber and J. belangerii

5.1.1 VSI and HSI values of J. borneensis, O. ruber and J. belangerii

Since the liver and viscera are organs that store energy, hepato and viscera somatic indices can be employed as indicators of the nutritional health of fish (Cui and Wootton, 1998; Dawood, 2016). The condition of the liver is a sign of metabolic activity and long and healthy existence. The ratio of liver weight to body weight indicates reserved energy levels in selected species in various environmental condition (Tyler and Dunn, 1976). The ability of fish to accumulate fatty acid compounds is depicted by the hepato somatic index. Hepato somatic index vary depending on dietary habits and reproductive differences (Henderson et al., 1984).

A study on *Johnieops sina* from Ratangiri coast of India represented high HSI value from September to December for both sexes after the spawning season (Kumar et al., 2014). An investigation on yellow giant croaker *Nibea japonica* species shows maximum growth when dietary lipid content was 8.2% and the VSI value was 5.12 and at the same time, the HSI value was 2.08 (Han et al., 2014). The VSI value in the present study was 7.44 ± 2.53 , 5.92 ± 1.99 and 5.69 ± 1.61 for *J. borneensis*, *O. ruber* and *J. belangerii* respectively which has similarity to the study on the yellow giant croaker indicating that the fat content of the three studied species was in good condition. The HSI value for *J. borneensis*, *O. ruber* and *J. belangerii* were 0.65 ± 0.26 , 0.52 ± 0.16 and 0.85 ± 0.25 which is slightly lower than the yellow giant croaker. Variations in the VSI and HSI may be due to the availability of food and favorable environmental conditions in the particular habitat.

5.1.2 ISI value of J. borneensis, O. ruber and J. belangerii

Internal and external morphology (e.g.: body form, mouth shape, fin and tooth shape and positioning, gut length etc.) is significantly connected with its way of feeding (Wotton, 1998). The condition and health of fish are evaluated using the ISI, a statistic used in fisheries and aquatic biology. In general, fish with a higher ISI value are thought to have an expanded and developed gut in comparison to their size. This may be a sign of improved nutritional digestion and absorption which is frequently linked to enhanced physical and mental health. The intestinal function and health of fish may be hampered by the unbalanced nutrient content, anti-nutritional elements and environmental contaminants in alternative feed materials. De Figueiredo and Vieira (2005), found that the stomach of the white mouth croaker remains almost full during most of the day. In the current study, the ISI value was found 0.50 ± 0.15 , 0.51 ± 0.17 and 0.79 ± 0.26 for *J. borneensis, O. ruber*, and *J. belangerii* respectively which indicates the voracious feeding habit of the species. These investigation suggest that ingestion is continuous, followed by rapid digestion.

5.1.3 RLG value of J. borneensis, O. ruber and J. belangerii

The length of the gut is used to determine the feeding nature of the fish. Different RLG value indicates the difference in the feeding habit of the fishes. The length of the gut in herbivorous fish is longer than that of carnivorous fish (Ribble and Smith, 1983). Fish that have an RLG below 1 are carnivorous, while fish that have an RLG over 1 are omnivores or herbivores (Yamagishi et al., 2005).

In the case of *Otolithoides pama*, RLG value was observed in Hoogly- Matlah estuary of West Bengal and values ranged from 0.42 ± 0.05 to 0.51 ± 0.05 in males. The monthly mean $(\pm \text{SD})$ RLG values ranged from 0.43 ± 0.04 to 0.56 ± 0.10 in females. Throughout the years, the results for both sexes were less than one indicating that the animal is a carnivore in nature (Bhakta et al., 2019). In this study the relative length of gut value were found 0.77 ± 0.15 , 0.72 ± 0.10 and 0.99 ± 0.10 for *J. borneensis, O. ruber* and *J. belangerii* respectively which indicates that all three species are carnivores in nature.

5.1.4 GSI value of J. borneensis, O. ruber and J. belangerii

The gonado somatic index (GSI) has been frequently used since it rises as fish mature, reaching its maximum during the peak time and falling shortly after spawning (Volkoff and London, 2018). In general, female fish have greater GSI values than males. This implies that body weight and gonad weight are inversely related. In the present study, higher GSI was also recorded in females compared to males (Figure 12).

Analysis of the reproductive biology of *J. borneensis* from the Agusan River estuary reflects the predominance of the female population in the stock with a sex ratio of 1:3 in males and females (Solania and Seronay, 2017). The predominance of females in large groups could be due to maturation and breeding (Shamsul and Ajazuddin, 1992). Another study on GSI from lower the Agusan River basin revealed mean ovarian GSI value of *J. borneensis* was 2.80 and 1.32 respectively in the month of September and October and the testicular GSI value measured 0.9 and 0.4 in the month of September and October respectively and is similar to the Bay of Bengal. However, it is obligatory to conduct year-round study to further confirm the spawning season which is currently under investigation.

In case of *O. ruber*, the GSI value was found (0.21 ± 0.70) and (0.92 ± 0.92) in males and females respectively in the present study during the month of September and October. Farkhondeh et al., (2018) studied the highest GSI (7.1 ± 0.6) in April indicating an upward trend from January to June. The observed outcome demonstrated that the *O. ruber* may reproduce year-round, with a spawning peak occurring from April to June (spring season). While Qasim (2022) concluded that the breeding season of *O. ruber* from March to April, only produces once a year. Further investigation with year-round histological analysis of

gonads is necessary to confirm the spawning season of *O. ruber* in the Bay of Bengal, Bangladesh.

Very little is known about the GSI and spawning season of *J. belangerii* from different parts of the world. Wang et al., (2012) reported that June to September, was the reproductive season from the China waters. Detailed investigation with year-round histological analysis of gonads is necessary to confirm the spawning season of *J. belangerii* in the Bay of Bengal, Bangladesh.

5.2 Feeding behavior of J. borneensis, O. ruber and J. belangerii

In *J. borneensis* collected from the Bay of Bengal, the result showed a major part of the intestine is composed of a carnivore diet. The gut was dominated by organic materials. The high concentration of organic matter particles in the stomach contents might be due to continuous food intake rather than direct absorption of particulate organic matter (Denadai et al., 2015). In general *J. borneensis* has a diverse diet that includes mollusks, crustacean and a variety of fish species. Some indigestible materials including shell pieces and crustacean fragments were identified in abundance in *J. borneensis* gut. It is possible that the organic components of these products may have been digested in the stomach leaving just the inorganic fraction in the intestine. The diet composition of *O. ruber* was dominated by crustaceans. Food and feeding habit of *O. ruber* have been reported for this species by other authors also.

Nair (1979), observed that the young *O. ruber* was found to be primarily feeding on zooplankton and pelagic creatures at the surface with a progressive shift to a predacious and carnivorous behavior, mostly eating on teleosts and prawns near the bottom as they grew in size. According to Vaidya (1960), the post-larvae and juveniles graze on surface plankton whereas the adults are carnivorous, eating crustaceans, teleosts and cephalopods. Suseelan and Nair (1969), described the fish as an active carnivore with prawns and teleost being the majority of its diet which indicates the similarity with the findings of the present study.

The diet compositions of *J.belangerii* changed along with month and body length. Juveniles mainly preyed on amphipoda and the adults mainly fed on fish and shrimps before reproduction and fed on amphipoda during the reproductive period (Wang et al., 2012). Mohamed and Abood (2019), observed that the main food items of *J. belangerii* were shrimps (52.9%), crab (37.9%) and fish (9.2%). The result of this study demonstrated that the population of croakers is considered carnivores with a preference for consuming benthic organisms such as crustacean and bivalves from the Bay of Bengal. Further studies with more number of samples and a year round study will further demonstrate the seasonal fluctuation in the feeding pattern of croakers in the Bay of Bengal.

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CONCLUSION

The study conducted on three croaker species - J. borneensis, O. ruber and J. belangerii from the Bay of Bengal, Bangladesh, has shed light on critical aspects of their biology, feeding habits and reproductive behaviors. The study investigated the visceral somatic index (VSI) of the croaker species. Among them, J. borneensis exhibited the highest mean VSI value, highlighting its potentially superior energy storage compared to O. ruber and J. belangerii. The hepato somatic index (HSI) was also considered revealing noteworthy differences among the species. Statistically significant differences in VSI and HSI values emphasize the distinct nutritional requirements and metabolic activities of these croaker species. The observed variation in these indices might be attributed to differences in dietary habits and reproductive strategies, emphasizing the role of environmental conditions. The intestine somatic index (ISI) values hinted at distinct feeding behaviors. J. belangerii showed significantly higher ISI values indicating a potentially more voracious feeding habit. These findings suggest that this species might have evolved efficient strategies for nutrient acquisition. The relative length of the gut (RLG) was examined revealing differences in feeding habits. The highest RLG value was recorded in J. belangerii, signifying its carnivorous nature. In contrast, J. borneensis and O. ruber exhibited lower RLG values, reaffirming their inclination towards carnivory. Gonado somatic index (GSI) values shed light on reproductive patterns. Female croakers displayed higher GSI values than males, indicating their investment in reproduction. This research contributes to our understanding of the ecosystem's dynamics and the intricate relationships between species and their environment. Overall, this study contributes essential data to the field of fisheries science, supporting effective conservation and management efforts. This study serves as a valuable foundation for future research and decision-making in the realm of marine conservation and management.

RECOMMENDATIONS AND FUTURE PERSPECTIVES

Although a quantitative technique was used to explore the objectives of the research, there are certain study constrains that can be minimized by following recommendation:

- The study focused on three croaker species from a specific region (Bay of Bengal, Bangladesh). A larger sample size, including more species from diverse locations, would enhance the study's generalizability and provide a more comprehensive understanding of croaker biology.
- The study might not capture seasonal fluctuations in feeding habits, reproductive behaviors, and gastrointestinal indices. Conducting the study over multiple seasons would provide a more complete picture of how these factors change over time.
- The study focuses on adult croakers, while including data on juvenile and larval stages could provide a holistic understanding of the species life history strategies.

Future perspectives of the present study may include:

- Knowledge of the feeding habits and dietary preferences of jewfish can aid fisheries managers in developing sustainable harvesting strategies.
- Understanding the dietary preferences and digestive physiology of jewfish can benefit aquaculture development.
- Research in this area could help assess how climate change and environmental shifts are affecting jewfish populations.
- This research can also contribute to the broader field of marine biology by providing data on the physiology and biology of jewfish. This information can be used for comparative studies with other fish species and enhance our understanding of fish biology in general.

Minimizing these limitations through the recommended, future perspectives can strengthen the study's findings, enhance its ecological significance and contribute to more effective conservation and management efforts for croaker species and their habitats.

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