Chapter One

Introduction

1.1 Background

Estuaries serve as an intermediary between sea and river, where a variety of freshwater and marine species, as well as juveniles, cohabit. These ecosystems provide habitat for a wide variety of marine and freshwater animals that move there at various stages of their life cycle (Rashed-Un-Nabi et al., 2011). Estuarine fish assemblages are known for their high diversity and richness, particularly among juveniles (Whitfield, 1999). Fishes use estuaries for food, reproduction, development, and protection and play an essential role in these environments as permanent and transient ecological components (Rez-Guzaman and Huidobro, 2002). Larval stages are uncertain, and it is assumed that marine fish larvae and juveniles move to estuaries to take advantage of abundant food and protection from predators in order to optimize their chances of survival (Van der Veer et al., 2001). As a result, a detailed understanding of fish larvae dynamics in estuaries would support the formulation of predictions regarding the role of estuarine nurseries. In terms of species composition and migration patterns, estuarine larval fish assemblages are diverse (Harris et al., 1999). These assemblages fluctuate through time and space, depending on the species reproductive seasons as well as ecological factors (Harris and Cyrus, 1995; Arshad et al., 2012).

The size of marine larval fish ranges between 2.5 and 3.0 mm right after hatching to 10–30 mm during transformation, which can take anywhere from a few days to several months. After hatching, the larvae float around in the water like plankton. In order to access planktonic food resources, pelagic larvae must maintain their location on the surface of the water (Webb, 1999). Most marine fish species produce pelagic eggs and drift with the currents. Closely related species are identified using standard morphometric measures.

Larval fish study is essential for a variety of reasons. Information on the abundance and distribution of fish eggs and larvae, for example, may reveal information on spawning seasons and locations, as well as the environmental needs of important fish species. Larval fish give significant information on species richness and reproductive activities, as well as assisting in the identification of nursery regions for larvae. More frequently, larval samples have been determined based on morphometric, meristic, and colour attributes (Rathnasuriya et al., 2021). Furthermore, the knowledge of larval fish is one of the major parts of the pelagic food web (Raymond, 1983), and it can serve as a crucial connection among tiny planktonic and bigger nektonic animals. In general, the larval stage is the most sensitive to environmental changes; any variation in the quality or quantity of ecological factors will be damaging to larval life and may reflect the recruitment potential for the future (Leis and Rennis, 1983). Fish eggs and larvae have contributed a lot to fisheries management and are expected to play a vital role in the future supplementing and conservation of fish stocks. Over the last 40 years, knowledge on fish early life has grown at an incredible rate (Rutherford, 2002; Pattira et al., 2012). It is now obvious that findings received from fish eggs and larvae offer a variety of distinctive contributions to fishery sciences that are critical for accurate fish population evaluation and monitoring (Fuiman and Werner, 2002).

The coastal and marine regions of Bangladesh are endowed with a warm tropical environment and abundant rainfall, all of which are enhanced with nutrients from the land, resulting in one of the world's most productive ecosystems (Hossain, 2001; Islam, 2003). Investigation, excavation, and management of the Bay of Bengal's living and non-living resources have the potential to support Bangladesh's economy. Especially after the recent actions of the International Tribunal for the Law of the Sea (ITLOS) on the Bangladesh-Myanmar maritime boundary in 2012 and the UNCLOS Arbitral Tribunal on the India-Bangladesh maritime boundary in 2014, where it established the sovereign rights of Bangladesh on more than 118,813 km^2 of territorial waters and 200 nautical miles (NM) of Exclusive Economic Zone (EEZ), along with all kinds of living and non-living resources under the continental shelf up to 354 nautical miles from the coast of Chittagong (MoFA, 2014). A broad range of biodiversity, such as fishes, crustaceans, mollusks, animals, seaweed, and other species, inhabit the Bay of Bengal's coastal and marine environments. Within the Bangladeshi seas, there are around 511 marine species, including shrimp (Murshed-E-Jahan et al., 2014). The yield of marine fisheries contributes to just 16.28% of total fish production. The coastal fishing sector's potential has not been effectively utilized. Rather, the resources were over-exploited, resulting in a decline in fish stocks (Shamsuzzaman et al., 2017).

The Bakkhali river originates from the south-eastern highlands of Mizoram in India, runs through Bangladesh's Naikhongchhari and Ramu districts, and ultimately falls into the Bay of Bengal's Moheshkhali channel in Cox's Bazar. This river is rather wide in comparison to other rivers in the Cox's Bazar area, and it runs for around 67 kilometers. The tidal regime in the Bakkhali river estuary is semidiurnal. Monsoon wind has a significant impact on its hydrology. Salt intrusion may be found up to 6 kilometers upstream, when a rubber dam was built for irrigation (Rashed-Un-Nabi et al., 2011). Anthropogenic and industrial activities, including fish harbours, fish processing factories, and a huge number of fish and shrimp farms, have a significant impact on the bottom portion of this estuary. The enormous volume of organic and inorganic pollution in the water body alters the chemical features of the water body by releasing harmful compounds, which has a negative impact on biodiversity. In the coastal area of Bangladesh, there are around 490 species of fishes (Hossain, 1971) and 19 species of shrimp or prawn (Chowdhury and Sanaullah, 1991) are found. However, thorough scientific research is an important function for the long-term sustainability of this fisheries resource. This research looks at the seasonal abundance of fish larvae at the Bakkhali river estuary over the course of a year. It provides an estimation of the number and families in the estuary throughout the year and groups the families into temporal assemblages and their spawning season.

1.2 Significance of the study

A number of studies were performed in Cox's Bazar and the Bay of Bengal on the abundance and distribution of marine fishes, their life cycles, and spawning season. However, there has been no study of fish larval assemblages in this area. This research presents a thorough overview of the abundance of larval groups in the Bakkhali river estuary on the Cox's Bazar coast. This study also effectively detects fish larvae and presents clear images of each larva. Furthermore, this study detects the spawning season of the identified fish families and observes temporal variations of diversity indices in the Bakkhali river estuary, which would be helpful in Cox's Bazar coast fisheries management approaches.

1.3 Objectives

- To identify larval fish family by their morphological characteristics
- To identify the quantity and distribution of fish larvae, as well as their biodiversity indices
- To compare fish larvae assemblages of different seasons and detect major spawning season of the identified fish families

Chapter Two

Review of Literature

2.1 Estuaries as nursing ground

The ability of larvae to access coastal nursery grounds that offer security and food for their survival and growth is vital to the development of early developmental stages of marine fish (Bailey et al., 2008). Estuaries and other nearshore coastal habitats are frequently referred to as major fish larval and juvenile habitats (Amara, 2003; Baptista et al., 2019). As a result, larval dispersion from breeding grounds to nursery sites is a crucial aspect of life history and fish population dynamics. The capability to regulate movement towards nursery areas strengthens with larval growth (Helfman et al., 2009), and larvae must respond to stimuli that signal their closeness (e.g., geomagnetic, scent, auditory, visually and chemical cues, river plumes) (Teod'osio et al., 2016). Many biological and environmental variables will impact survival and distribution of larvae throughout the dispersion process, which make this a highly vulnerable stage of the fish life cycle, when death rates ranging between 5% and 40% daily (Bailey et al., 2008; Houde, 2008). Fish, especially marine species, use estuaries and associated wetlands as a nursery and refuge location (McLusky and Elliot, 2004). Spawning takes place in the river, the estuary, or offshore, based on the fish species (Elliott et al., 2007). Some fish species release pelagic eggs, and these eggs, as well as the larvae which will hatch, will scatter at sea for several days to weeks. Other fish species deposit demersal eggs, which are bigger and heavier eggs that land on the substratum, possibly as a defense against being swept out of the estuary (Wolanski and Elliot, 2015). Estuarine and coastal environments are vital habitats and nursery grounds for a variety of species, providing enormous benefits to the people and entire environment (Gillanders et al., 2003).

2.2 Fish larvae and its importance

Due to the significant transformations that most species experience from early larval stages to adulthood, identifying larval fish has been a major morphological challenge in marine biology. Ichthyoplankton, the larvae of fishes, are usually pelagic, drifting in the sea and associating with predators and zooplankton. Even in species that become herbivores as adolescents or adults, most fish larvae are largely carnivorous during their larval stages, preying on small planktonic animals. In turn, larger nektonic and planktonic animals feed on larval fishes. To get out of this vulnerable stage, larvae undergo growth and ontogeny. During planktonic life, usually a few individuals out of thousands of newly hatched larvae survive the constant danger of hunger and predation. Fine-mesh plankton nets or specifically designed traps are used to collect eggs and larvae of marine fishes. 'Ichthyoplankton' communities are studied at sea to determine their locations, abundance, richness, and structure, as well as the relationships between larvae and their competitors and prey. These surveys are frequently utilized as part of stock assessments for fisheries management (Steele et al., 2001). In addition to specifying essential features of life history, studying the first stage of the fish life cycle gives significant information on breeding times, spawning sites, nurseries, and development, along with understanding population dynamics. It also allows for the estimation of migratory patterns, the possibilities for renewal (effective recruitment), the assessment of the species' conservation status, and the support of fishery stock maintenance (Cruz et al., 2016), that can be used to direct management and conservation actions.

2.3 Larval family

In a study at Pandas River estuary, Peninsular Malaysia, Clupeidae was the most frequent family, accounting for 41.07% of all families. The list was followed by Blenniidae (24.45 %), Teraponidae (8.80 %), Gobiidae (5.40 %), Sillaginidae (3.22 %), Nemipteridae (1.72 %), and Mullidae (1.28%). The most common family was clupeid larvae, which had the highest numbers in February–March that matched with Peninsular Malaysia's northeast monsoon season. The clupeid population peaked during the monsoon season (February–March), indicating seasonal spawning. Teraponidae, the second most abundant family, was found all year, with the maximum peak number in February, probably showing the family's reproductive season. These larvae were considerably more common in other months as well. Gobiid larvae were found all year, with the largest concentrations in January–March in the northeast monsoon (Arshad et al., 2012). Janekarn and Boonruang (1986) discovered that density of clupeid larvae was maximum in February in Western

Peninsular, Thailand. Two species of gobiid fish were discovered on the seagrass bed of Merchang Lagoon in Peninsular Malaysia, according to Aziz et al. (2006). Many studies have found that the Gobiidae family is highly dispersed in coastal regions, regardless of climate or other parameters such as seagrass diversity, temperature, or biological factors (Kwak and David, 2003). The high prevalence of the Gobiidae family can be linked to clumps of extremely varied recruitment in schooling species, which apparently originated from aggregative settling (Anand and Pillai, 2005). In a large scale study conducted by Lirdwitayaprasit et al. (2008) at three different regions of Bay of Bengal, much more information was found regarding fish larvae. Depending on larval frequency of occurrence, Photichthyidae, Myctophidae, Bregmacerotidae, Carangidae, and Callionymidae were observed as the most frequent families at the upper part, at the western part the constants families were Gonostomatidae, Photichthyidae, Myctophidae, Bregmacerotidae and in the Andaman sea region 14 families were constantly found. According to Chamchang (2006), the Myctophidae family was the most abundant, contributing almost 30.41 % of larvae, followed by the Stomiidae family. The percentage of fish families such as Scombridae, Mugilidae, Clupeidae, Carangidae, Engraulidae, Leiognathidae, Tetradontidae, Lutjanidae, Pomacentridae, Sciaenidae and Chirocentridae were studied during the research at Andaman sea. The proximate composition of larvae at both locations, however, varied. The Scombridae family was the most common (Station-I 10.12% and Station-II 10.14%), while the Chirocentridae family was the least common (2.61% and 2.72%) (Azhagar et al., 2009). In all three biogeographical areas of temperate South Africa, the clupeidae was the most common fish family in larval captures. This family was associated with a single species (*Gilchristella aestuaria*), which made up 77.00 % of cold temperate estuaries, 63.38 % of warm temperate estuaries, and 73.60 % of border estuaries on average. Gobiidae and blenniidae family made the second and third largest contribution in total catch. The remaining fish families contributed less than 1% of the overall catch.

2.4 Abundance and distribution

Seasonal patterns of fish larval abundance are connected to adult population reproductive behaviors and life cycles, which are typically connected to oceanic and climatic factors

(Hernandez-Miranda et al., 2003). Biotic variables are linked to food availability, and zooplankton density is sometimes linked to larval fish abundance; for example, the seasonality of larval fish abundance can be significantly connected with the abundance of copepod nauplii (Mateo et al. 2006). Arshad et al. (2012) yielded total 2687 larvae from the estuary of Pendas River, Malaysia with a mean abundance of 28.29 larvae per $100m^3$. The larval fish composition of Peninsular Malaysia comprised 19 families, with 17 in the middle estuary, 16 in the lower estuary and 14 found in the upper estuary. The cumulative number of fish families and densities in the estuary notably declined from the lower estuary to the higher estuary, according to the distribution pattern of overall fish larvae. This suggests that the estuary's fish larvae migrated from the sea. The dominating families (Clupeidae, Blenniidae, Gobiidae, and Teraponidae) migrated to the estuary between January–March (Northeast monsoon) and June–August (Southwest monsoon). The monsoonal effect on the abundance of fish larvae in the Pendas River estuary is a distinctive aspect of the larval assemblage. A study from Bay of Bengal collected a total of 6170 larval specimen which belong to total 18 families. Among them, 07 families were economically important. They were Hemirhamphidae, Carangidae, Sphyraenidae, Gempylidae, Scombridae, Bothidae and Cynoglossidae. All of them contributed about 5.64% to the total fish larvae. The most dominant family of that area was Carangidae followed by Scombridae and Gempylidae (Lirdwitayaprasit et al., 2008). In the northwest Indian Ocean (Red Sea, Arabian Sea and Persian Gulf), Nellen (1973) identified a total of 102 fish larval families. Among them, 44 families were oceanic plus deep benthic and 58 were shelf fish larvae. Another research conducted in southeast Indian Ocean (NW continental shelf, Australia), in which 103 larvae families were found (Young et al., 1986). He discovered that 36 families were marine and 67 were shelf fish larvae. Janekarn (1988) documented the variety of 55 and 62 families of larval fish discovered along Thailand's west coast in 1982 and 1983. Based on his and other research, he calculated the total number of 123 fish larval families on Thailand's west coast (Janekarn, 1992). In terms of the composition and number of fish larvae, Chamchang (2006) observed a low number of constant families, indicating that the system was not reliable. From this reference, 62 families of larval fish were discovered in the Andaman Sea along the coastline of Thailand and Myanmar. In the southeast coast of India, Azhagar et al.

(2009) found that the monthly distribution of finfish larval density at Kodikkarai coast varied from 8 larvae/10m^3 to 76 larvae/10m^3, whereas it varied from 10 larvae/10m^3 to 65 larvae/10m^3 at Arkattuthurai. The highest larval density was found at both locations during the summer season. During the monsoon, the larval density was at its lowest. In South Africa, Melville-Smith and Baird (1980) detected 15 families and 17 species of larval fish at the Swartkops Estuary. The following year, they conducted another study in the Kromme River Estuary, with a somewhat lower total of 12 families and 15 species identified (Melville and Baird, 1981). In the Swartkops Estuary's mouth region, Beckley (1985) discovered 17 species. In the Gamtoos Estuary, Strydom (1998) found 28 species within 15 fish families and nine unidentified species. In warm-temperate estuaries of the Eastern Cape, Strydom et al. (2003) reported 23 fish families with 63 species, including four unknown species, while Montoya-Maya and Strydom (2009) identified 17 families with 33 species in cool-temperate environments. The number of families and species increases substantially in subtropical estuaries on South Africa's eastern coastlines. Strydom (2015) conducted research on patterns in larval fish diversity, abundance, and distribution in 25 South African estuaries and found a total of 29 larvae family and 89 species. There were 24 families and 46 species in the cool-temperate estuaries, 23 families and 68 species in the warm temperate estuaries, and 18 families and 40 species in the boundary estuaries.

2.5 Diversity indices

In a large scale study at temperate South African estuaries by Strydom (2015), the Euhaline salinity zones indicating the accumulation impact of marine and estuarine species, had the highest alpha diversity, which represents intra-estuary trends. The warm temperate, continuously open Kromme Estuary in the Eastern Cape has the most diversity (1.77) at the Beta diversity level, followed by the cold temperate, permanently open Olifants Estuary (1.66) in the Western Cape. The small cold temperate, periodically available closed Diep Estuary in the Western Cape has the lowest diversity (0.15). Warm temperate estuaries (1.67) had more mean diversity of fish larvae than cold temperate (1.50) or boundary estuaries (0.96) at the Gamma diversity level. The differences across regions, on the other hand, were minimal. Despite the fact that there was no statistically significant variation in species diversity between estuaries, the permanently open estuaries (1.11) had a greater mean diversity than the seasonally open-closed estuaries (0.68) and estuarine lake (0.83) systems.

The changes in density of fish larvae among the stations were not significant ($p>0.05$) in the estuary of Pendas river, Malaysia. The middle estuary had the maximum average Shannon Winner diversity index (1.48), whereas the upper estuary had the lowest (1.18). The middle estuary had the highest evenness (0.77), and there was no distinction in evenness between the upper estuary and lower. The middle estuary had the maximum family richness (1.72) and the upper estuary had the lowest richness (1.34). The total larval fish density differed significantly ($p<0.05$) between months. Within monsoon and intermonsoon seasons, the Shannon Wiener index showed significant variance, which peaked in the months of December to January and May to August. Family richness also showed two distinct peaks over the course of a year. One peak occurred in January-March, and the other occurred in May–August (Arshad et al., 2012).

2.6 Spawning season

The majority of commercial species of Red sea spawn from May through August. They made about 61% of all commercially available species. The commercial species were made up of 44% of the 13 summer spawners. Winter and spring contributed six and five species, respectively, accounting for 19% and 16% of commercial species. Two species, Clupeidae and Engraulidae, have been discovered to reproduce at different times of the year and with no discernible pattern (El-Regal, 2013). In a descriptive study at Bay of Bengal of South East coast of India, Azhagar et al. (2009) depicted that, during all four seasons, larvae belonging to the Teraponide family were the most abundant (26.09%) in post-monsoon season, while Tetrodentridae were the least common (1.30%). On the other hand, Clupeidae was the most abundant family (17.22%) in summer, while Teraponidae was the least common (0.99%). The dominant group during the premonsoon season was Leiognathidae (25.58 %), and the minimum was Latidae (1.16%). During the monsoon season, Pomadasydae (35.26%) was the most abundant family, while Carrangidae was the least (1.55%). During the post-monsoon season, the most bony fishes of various taxa were present, followed by the summer season. Rajaseker et al. (2005) confirmed that rainfall data indicates negative correlations with the distribution and abundance of finfish larvae. Marichamy and Siraimeetan (1984) discovered two peak in the dispersion of fish larvae, the first in January-February and the second in June-July, both of which coincided with low temperatures and salinity in the Tuticorin area. The breeding season for frigate tuna in the Indian Ocean, according to Yoshida (1979), spanned from January to April. Stequert and Marsac (1989) found the highest abundance of skipjack tuna larvae in the eastern Indian Ocean in February. Females of *Sardinella gibbosa* and *S. fimbriata*, both gravid and ripe, were found in most months. Peak occurrences for *S. gibbosa* was in February-March in the southern region and March-April in the northern region, and for *S. fimbriata* May-July over the north west Bay of Bengal (Ghosh et al., 2013). Araújo et al. (2008) studied the majority of the 330 ovaries of *Anchoa tricolor* in a tropical bay in southeastern Brazil, which exhibited advanced maturation phases, which were prevalent from August to March; recovering stages were widespread from April to July. In September, roughly 40% of the females were in the early stages of spawning. From August through February, average GSI values climbed, culminating in September. From April to June, lower levels were recorded. From September to February, the Fulton fish condition was at its peak, and from June to August, it was at its lowest. Such differences suggest that *Stolephorus commersonnii* has the ability to regulate its reproductive period in response to environmental factors, with nutrient availability, photoperiod, and temperature being the most important factors affecting engraulid reproduction in coastal regions (Silva et al., 2003; Arajo et al., 2008). Several studies, notably Kim et al. (2013) and Andamari et al. (2002), have documented distinct spawning periods in anchovies (engraulidae) in tropical and sub-tropical waters.

Chapter Three

Methodology

3.1 Study Area

The Bakkhali river estuary is situated at the Bay of Bengal's southeastern coast in Bangladesh. This research was conducted in Bakkhali river estuary (N 21.471501, E 91.950445) with monthly sampling from March 2020 to February 2021 (Fig. 1). Study area was selected based on convenience and its ecological importance to coastal fisheries. Coordinates were taken through GPS meter and mapping was done by QGIS (version 3.4.5).

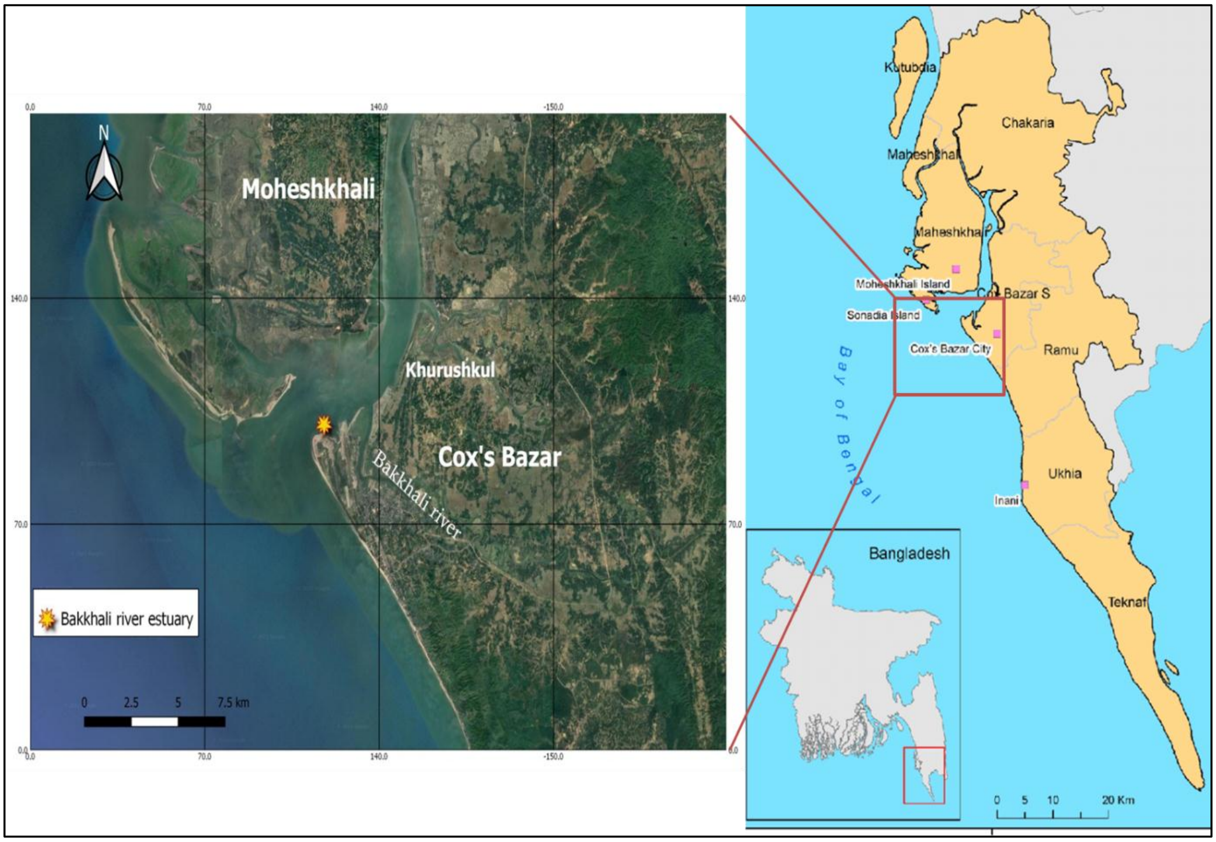

Fig. (1) Map of Cox's Bazar region and study site

3.2 Sampling Procedure

Fish larvae were collected from the Bakkhali river estuary by Bongo Net (0.50m mouth diameter, 1.3m long, and 500µm mesh at the body). A flow meter (KC Denmark A/S

23.090-23.091) was mounted to the net's mouth to quantify the amount of seawater filtered during each tow. The sampling period was about 10 minute's surface tow. The collected specimen was preserved in 90% ethanol in plastic bottle, transported to the aquatic ecology laboratory of CVASU for sorting out based on morphology and other attributes, and freshly preserved in 90% ethanol again.

3.3 Fish larvae sorting

For taxonomic identification, larvae were sorted from the whole sample. The first step of sorting was to discard ethanol from the sample. To do this, samples were thoroughly washed with distilled water so that sand particles, plastics, leaves, and other unwanted matters could easily be removed. Washed larvae were again placed into a jar with fresh ethanol and each sample was placed in a petri dish one by one to be analysed under a stereo microscope (OPTIKA Microscope Italy C-B3) at low magnification (10x) where several pictures were captured. Identified sample and picture was given a specific code so that it can be easily found later.

3.4 Morphological identification of fish larvae

Fish larvae were identified up to family level under the stereo microscope using the descriptions of related taxa given by Leis and Rennis (1983), Leis and Carson-Edwart (2000) and Rodriguez et al. (2017). The body structure, coloring pattern, and meristic and morphometric traits are the most significant charactrstics to identify larval fish. The different length measurements of a larva are referred to as morphmetric characteristics, such as total length (TL), standard length (SL), pre-anus length, head length, or the distance between the tip of the snout and the cleitrum's border, and the maximum dimension of the eye (Fig. 2) (Rodriguez et al., 2017). Considering larval growth is allometric, the development phases of the larvae were taken into consideration while measuring the various body areas for identification purposes.

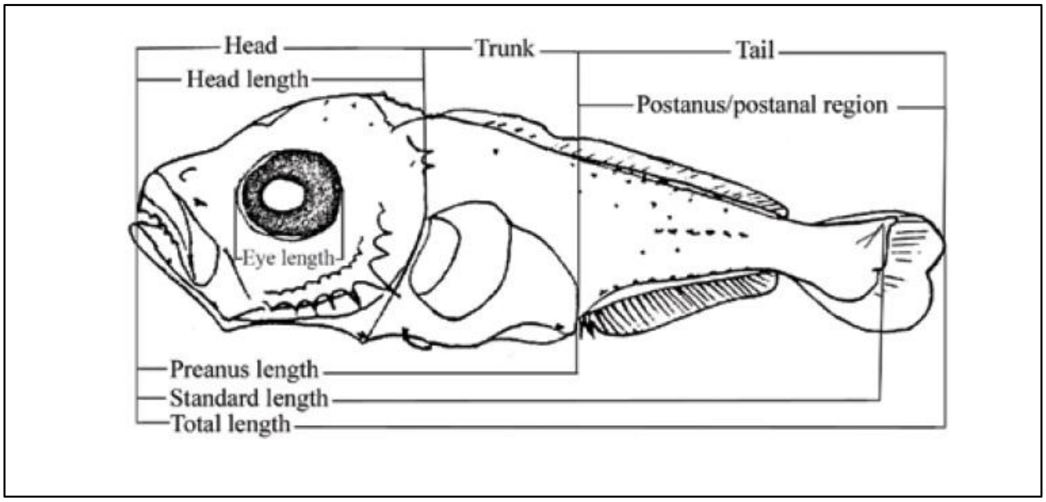

Fig. (2) Morphometric characteristics of a fish larva

Meristic features, such as the number of miomeres, vertebrae, or fin rays, are countable structures that appear in a series. They do have the limitation some of them, such as fin rays, are only fully formed in older larvae, which are unusual in plankton samples. Others, like miomeres or vertebrae, are challenging to see, even using staining techniques or other techniques like X–rays (Pothoff, 1984). Fish larvae have a wide range of pigmentary cells, often known as chromatophores. Melanophores are pigments that are either black or brown in color; those with yellow pigments are called xanthophores and erythrophores are red pigments (Russell, 1976). However, only black pigmented cells or melanophores remain in ethanol–preserved specimens. As a result, in larvae identification, the latter were typically the only ones considered. Melanophores are pigmentation compounds that are found in various regions of the body and define a species-specific pigmentation pattern (Rodriguez et al., 2017). Unidentified larvae samples were categorized as "unidentified" because the samples were too small to be identified or ruptured during collection and transportation.

3.5 Determination of number of larvae, diversity indices and constancy of occurrence

Numbers of fish larvae according to specific family were counted through naked eye. This number was simplified into per 1000m^3 for further analysis. Temporal variation of fish larvae were determined by number of larvae along with diversity indices. Diversity of the larval fish assemblage was measured by the Shannon-Wiener index (Shannon and Weaver, 1949) and equitability or evenness was expressed by Pielou's evenness index (Pielou, 1966). Family richness was calculated following Margalef (1958). At its most basic level, diversity refers to the number of different species that exist. The number of species (richness) and relative abundance of these species (evenness) differ among biological communities (Anon, 2021). Species richness and evenness are two independent components of species diversity, which is a vital factor in ecology. To generate diversity indices based on proportionate relative abundance of species, richness and evenness have been mathematically linked in various ways (Magurran, 2004). Shannon–Wiener (Shannon index) is a popular species biodiversity indicator. The Shannon index, which evaluates species richness and percentage of each species within a community, is one of the most well-known diversity indices (Arzamani et al., 2018). In this study, Shannon index was used to analyse family richness and evenness to calculate biodiversity.

3.5.1 Number of larvae individual per 1000 m^3

Bongo net diameter, d= 0.50m

So, net radius, r= 0.25m

Net opening area= πr^2

$= 3.1416 \times 0.25^2$

$= 0.19635 \times 2$; as each net has two openings

$= 0.3927$

- Volume of water passed in each sampling=

Indicated number of revolutions× Pitch of the impeller (0.3) ×Net opening area (m^2) ×1000

- Number of larvae per 1000m^3 = (Number of larvae in sample×1000) ÷Volume of water passed

3.5.2 Measurement of diversity

The type of diversity used here is E- diversity which is the diversity of species within a community or habitat. The diversity index was calculated by using the Shannon – Weaver diversity index (1949).

- Diversity index, $H = -\sum Pi \ In \ Pi$

where Pi = S / N

S = number of individuals of one species

N = total number of all individuals in the sample

In = logarithm to base e

The higher the value of *H*, the higher the diversity of species in a particular community. The lower the value of *H*, the lower the diversity. A value of *H* = 0 indicates a community that only has one species.

3.5.3 Measurement of species richness

Margalef's index was used as a simple measure of species richness (Margalef, 1958).

- Margalef's index = (S – 1) / In N

Here, S = total number of species

N = total number of individuals in the sample

3.5.4 Measurement of evenness

For calculating the evenness of species, the Pielou's Evenness Index (e) was used (Pielou, 1966).

- e = H / In S

Here, H = Shannon – Wiener diversity index

S = total number of species in the sample

3.5.5 Determination of the constancy of occurrence

The constancy of occurrence was determined based on the ecological index by Schifino et al. (2004), and the following formula was used for the calculation;

- Formula: C = P/Q x100

Where, C = Constancy of Occurrence of the family (%), P = number of samples where the family occurred, Q = total number of samples. The identified families were further divided into three categories based on the value of constancy of occurrence, which was; (1) constants (when $C>50\%$), (2) accessories (when $25\%\leq C\leq 50\%$), and (3) accidental (when $C<25\%$).

3.6 Determination of the spawning season

The spawning season was determined by considering the month before the month in which larvae began to be found in the selected station. Based on the monthly larval abundance, the spawning season of the identified families was categorized as the summer, winter and rainy monsoon. The winter season continues from November to February, while the summer season lasts from March to June. Monsoon months cover July, August, September and October.

3.7 Statistical Analysis

All the data were being entered into Microsoft Office Excel (version 2010, USA). Data management and data analysis done by SPSS (version 25). SPSS is a software package used for logical batched and non-batched statistical analysis.

Chapter Four

Results

4.1 Fish larval composition and abundance

The study area yielded a total of 883 larvae, with a mean abundance of 73.58±154.04 per 1000m^3 (Table 1). The larval fish assemblage included 11 families and unidentified samples were tagged as “Unidentified” (Fig. 3). The families are- Clupeidae, Ambassidae, Engraulidae, Gobiidae, Sillaginidae, Blenniidae, Terapontidae, Mugilidae, Megalopidae, Sparidae and Gerreidae. Among them, Clupeidae was the most abundant family, which contributes about 56.41% followed by Ambassidae (34.05%), Engraulidae (2.95%), Gobiidae (1.7%), Sillaginidae (0.79%), and others (4.1%) (Fig. 4). Two dominant larval families are Clupeidae and Ambassidae, which used Bakkhali river estuary as their nursing ground.

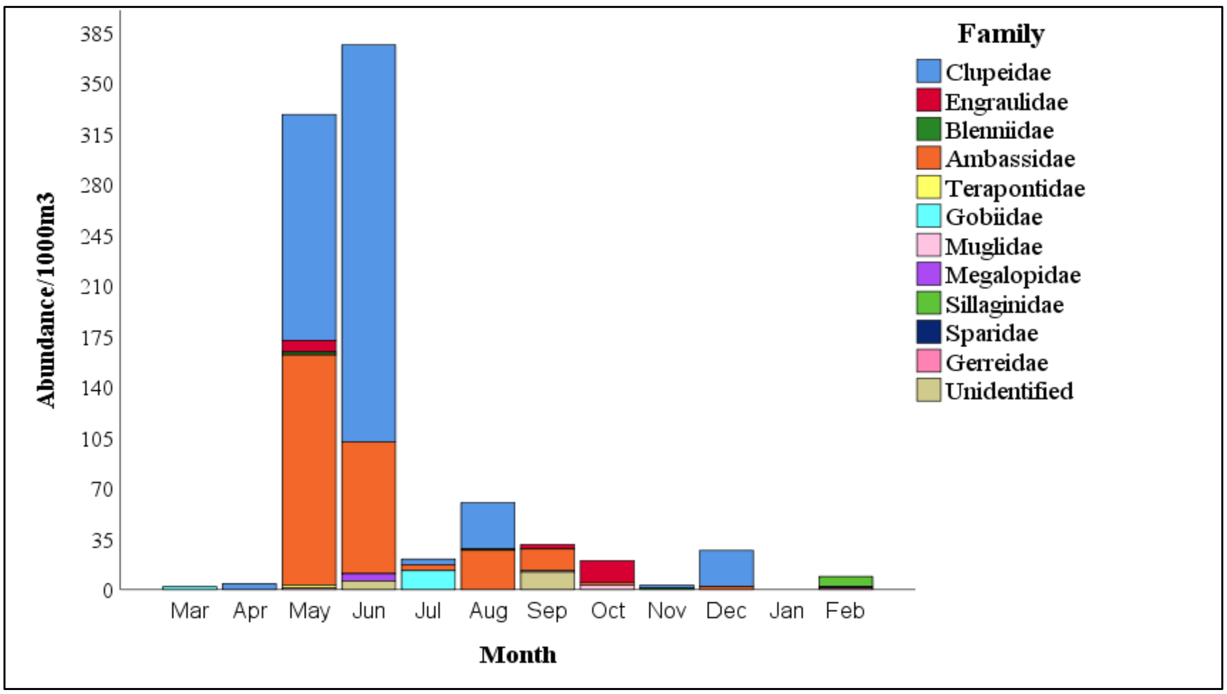

Fig. (3) Temporal variation of composition and abundance of larval family

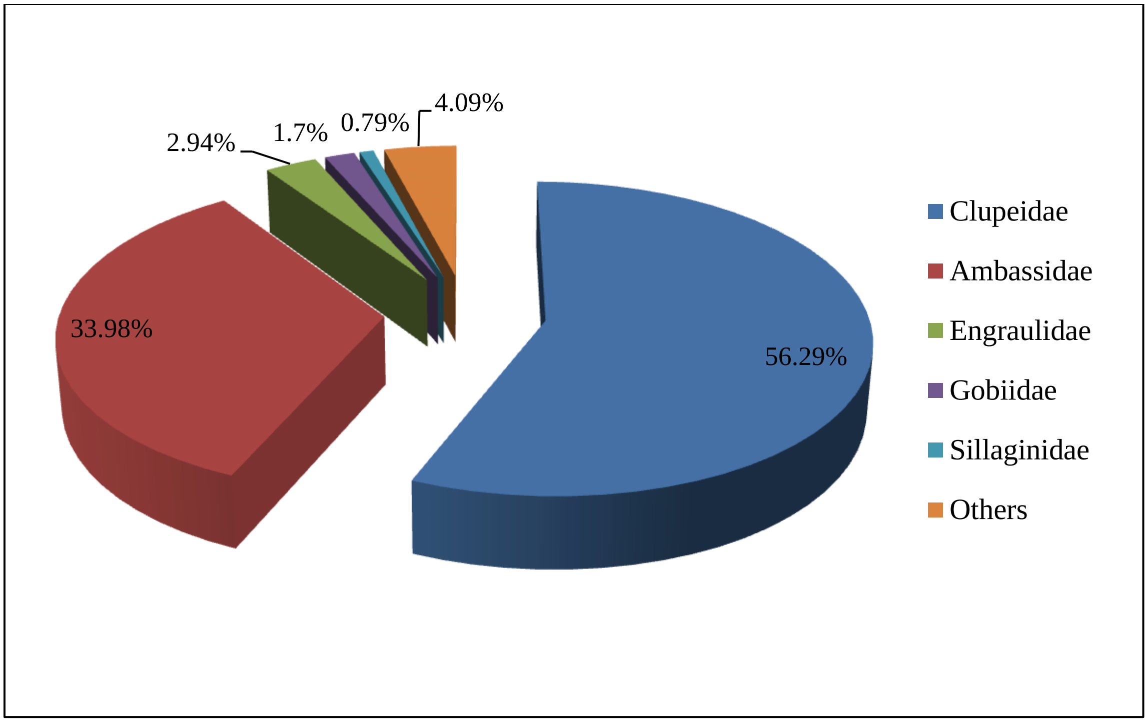

Fig. (4) Percentage of the families found

4.2 Constance of Occurrence

Depending on the frequency of occurrence, among the 11 families, the percentages by families of constant:accessory:accidental families were 18:27:55 (Table 1). It suggests that just 18% of families are discovered at a constant rate throughout the year in the Bakkhali river estuary, and they commonly use it as a nursing ground. The majority of the families were not found frequently in this region and were considered to be accidental.

Table. (1) Total number of fish larvae/1000 m^3 and constancy of occurrence

Family	Total number of larvae (larvae/ 1000m^3)	Mean number of larvae	SD	SE	Percentage of total catch	Rank	Frequency of Occurrence	Classification according to Constance of occurrence		
								1	2	3
Clupeidae	497	41.42	85.55	24.70	56.29	1	58.33	*		
Ambassidae	300	25.00	49.58	14.31	33.98	2	58.33	*		
Engraulidae	26	2.17	4.69	1.35	2.94	3	25.00		*	
Gobiidae	15	1.25	3.74	1.08	1.70	4	16.67			*
Sillaginidae	7	0.58	2.02	0.58	0.79	5	8.33			*
Mugilidae	5	0.42	0.90	0.26	0.57	6	25.00		*	
Megalopidae	5	0.42	1.44	0.42	0.57	7	8.33			*
Blenniidae	4	0.33	0.65	0.19	0.45	8	25.00		*	
Terapontidae	2	0.17	0.58	0.17	0.23	9	8.33			*
Sparidae	2	0.17	0.58	0.17	0.23	10	8.33			*
Gerreidae	2	0.17	0.58	0.17	0.23	11	8.33			*
Unidentified	18	1.50	3.73	1.08						
Total	883	73.58	154.04							

*(1) constants; (2) accessories; (3) accidental

4.3 Top two abundant families

4.3.1 Clupeidae

Clupeidae were the most abundant larvae and contributed more than half of the total abundance (56.29%) of the Bakkhali river estuary. This family was found in seven months of a year-from April to August, November and December (Fig. 5). The mean density of this family was 41.42±85.55 (Table 1) and the highest abundance was 274 individuals/1000m^3 in June (Fig. 5). Several commercially important species under the Clupeidae family are found in Cox's Bazar, such as *Tenualosa ilisha, T. toli, Hilsha kelee, Escualosa thoracata*.

4.3.2 Ambassidae

Ambassidae family was the second abundant family, which contributed 33.98% of the total abundance. This family was found in six consecutive months from May to October and December (Fig. 5). The mean density of the larvae was 25±49.58 (Table 1) and the highest abundance was observed in May, at 159 individuals/1000m^3 (Fig. 5). *Ambassis dussumieri* is the only species of Ambassidae that has been found on the Cox's Bazar coasts.

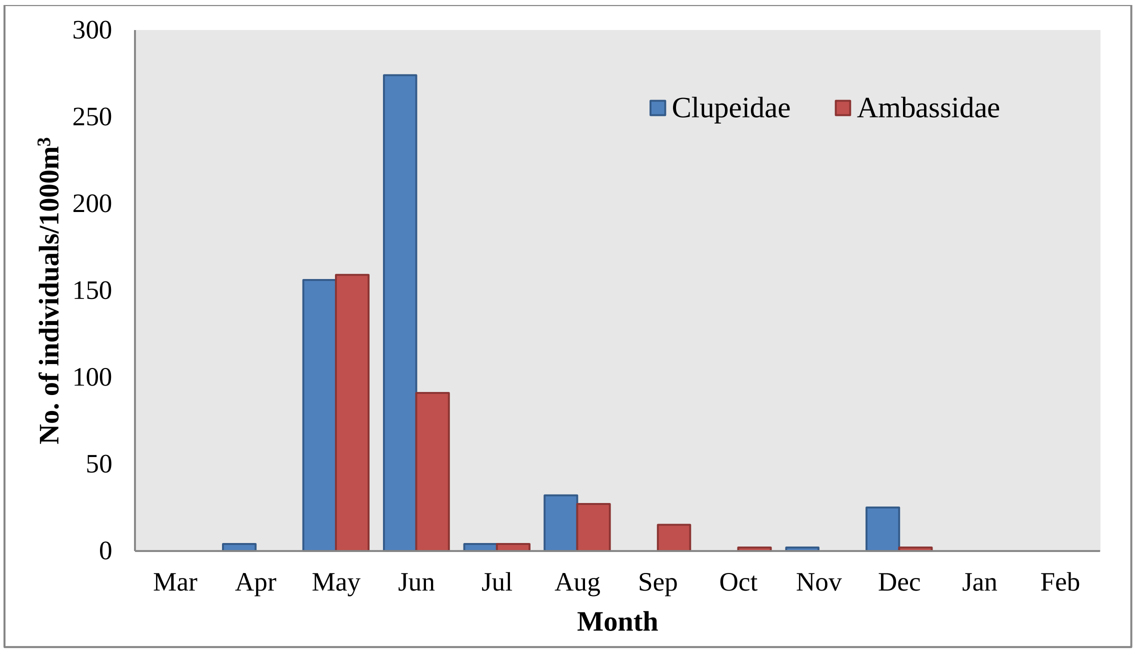

Fig. (5) Temporal abundance of top two families

4.4 Temporal variation and biodiversity indices

The highest mean total density of larvae was 94±126.59 that was observed in the month of June, while in January no fish larvae were found. However, in May, the highest number of families was observed (Table 2). Diversity indices showed significant variation within months. The highest Shannon-Wiener index was found in September (1.055) and July (0.929), respectively (Fig. 6). Margalef's richness index also clearly showed two significant peaks, one in November (0.910) and the next one in September

(0.874) (Fig. 7). In terms of Pieulo's evenness, the highest was 0.918 (November) and the second highest was 0.845 (July) (Fig. 8).

Table. (2) Temporal variation of larval abundance at Bakkhali river estuary

Month	Mean number of larvae	Number of family	Std. Deviation	Std. Error	Min	Max
March	2.00	1	-	-	2	2
April	4.00	1	-	-	4	4
May	54.67	6	79.670	32.54	1	159
June	94.00	4	126.59	63.30	5	274
July	7.00	3	5.20	3.00	4	13
August	20.00	3	16.64	9.61	1	32
September	7.75	4	6.80	3.40	1	15
October	6.67	3	7.23	4.18	2	15
November	1.50	2	0.71	0.50	1	2
December	13.50	2	16.26	11.50	2	25
January	-	-	-	-	-	-
February	3.00	3	3.46	2.00	1	7

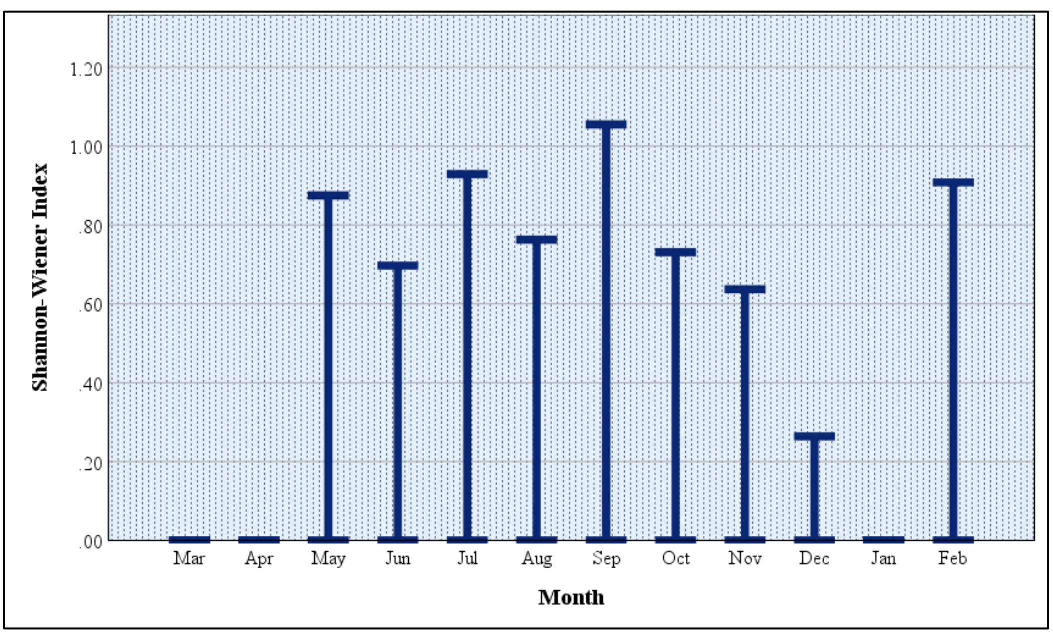

Fig. (6) Shannon-Wiener index of diversity of each month

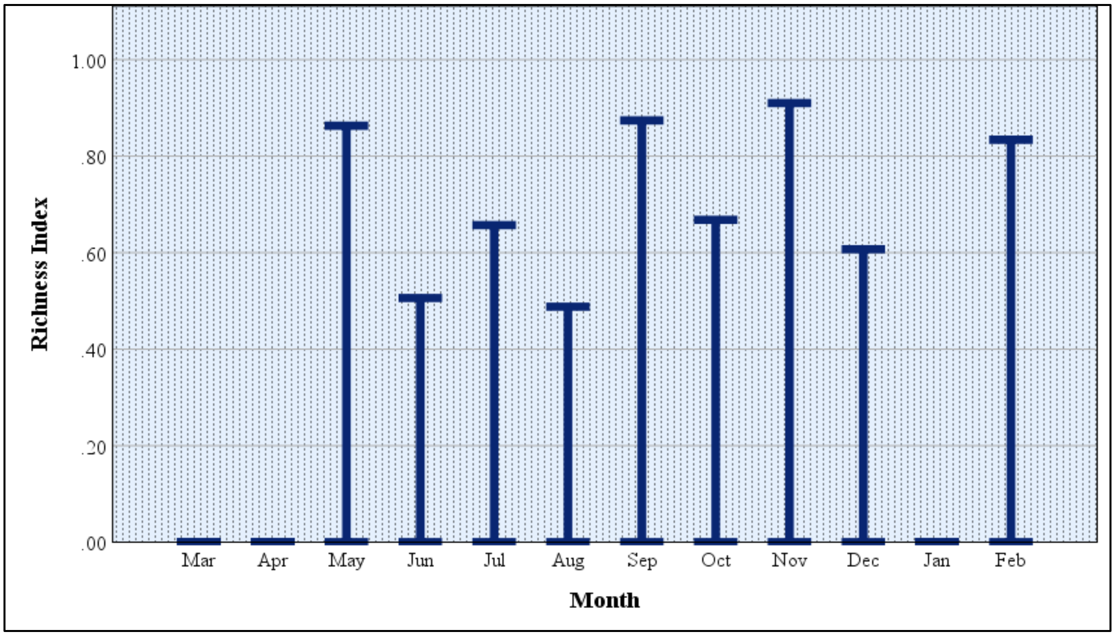

Fig. (7) Margalef's richness index of each month

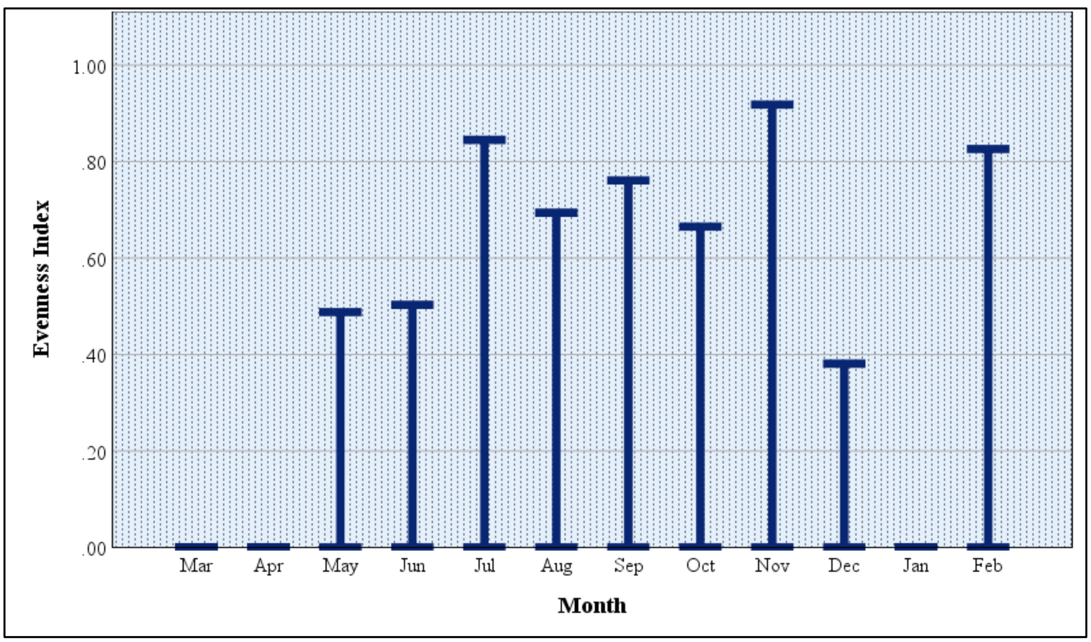

Fig. (8) Pieulo's evenness index of each month

4.5 Spawning season

Larvae of 11 fish families were collected and their time of occurrence was used as indication of their spawning season. The spawning seasons of the families were classified as Summer, Winter and Monsoon. Most of the families spawn in the warmer months of the year (March to June) based on the availability of their larvae (Fig. 9). Among the families, Clupeidae and Ambassidae were confirmed as spawner of summer, monsoon and winter, as their larvae were found in all three seasons. Engraulidae family were recorded to spawn in mid summer and mid monsoon, Blenniidae were documented to reproduce in mid summer, early monsoon and late monsoon, Mugilidae as mid summer and mid monsoon spawners, and Gobiidae as late winter and late summer. Five (05) families are classified as single spawners since they reproduce once a year. They are-Sillaginidae, Megalopidae, Terapontidae, Sparidae and Gerreidae (Table. 3).

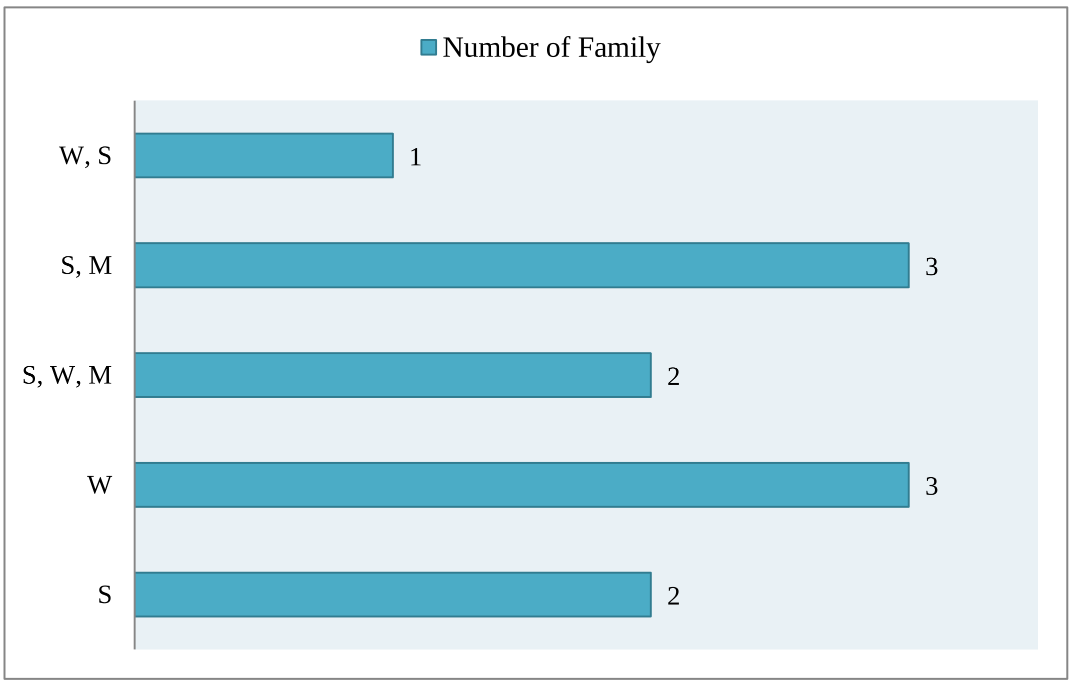

Fig. (9) Number of families in different spawning season

(W= Winter, S= Summer, M= Monsoon)

Table. (3) Spawning season of identified fish larvae with their frequency of occurrence and spawning month

Family	**Month**	**Spawning month**	**Spawning season**
Clupeidae	Apr- Aug, Nov, Dec	Mar-Jul, Oct, Nov	Summer, Early monsoon, Late monsoon, Early winter
Ambassidae	May-Oct, Dec	Apr-Sep, Nov	Summer, Monsoon, Early winter
Engraulidae	May, Sep, Oct	Apr, Aug, Sep	Mid summer, Mid monsoon
Gobiidae	Mar, Jul	Feb, Jun	Late winter, Late summer
Sillaginidae	Feb	Jan	Mid winter
Mugilidae	May, Sep, Oct	Apr, Aug, Sep	Mid summer, Mid monsoon
Megalopidae	Jun	May	Mid summer
Blenniidae	May, Aug, Nov	Apr, Jul, Oct	Mid summer, Early monsoon, Late monsoon
Terapontidae	May	Apr	Mid summer
Sparidae	Feb	Jan	Mid winter
Gerreidae	Feb	Jan	Mid winter

Chapter Five

Discussion

5.1 Fish larval composition and abundance

Because most fish larvae are tiny, they are readily neglected, and they have been classified as a big subcategory of zooplankton. However, because ichthyoplankton play a significant role in fish life cycles, they are crucial in assessing environmental effects, fisheries resources, and climate change reactions. Ichthyoplankton are also an essential food supply and link in marine food cycles, thus their assessment is necessary in fisheries resource management. This study has discovered 883 individuals and 11 families of fish larvae. Based on the morphological analysis of larvae, the families' are- Clupeidae, Ambassidae, Engraulidae, Gobiidae, Mugilidae, Megalopidae, Sillaginidae, Blenniidae, Terapontidae, Sparidae and Gerreidae (Fig. 2). This area appears to have less diversity than the previous work in the various parts of the Bay of Bengal and Indian Ocean. Nellen (1973) found 102 larval families in the Indian Ocean's north-eastern region, including the Arabian Sea, Red Sea, and the Persian Gulf. Youngs et al. (1986) discovered 103 larvae fish families in the southeast Indian Ocean, whereas Lirdwitayaprasit et al. (2008) found 52 families in the Bay of Bengal. Janekarn (1988) identified 62 families of fish larvae on the west coast of Thailand. Chamchang (2006) observed 62 families of fish larvae in the Andaman Sea along the coastline of Thailand and Myanmar. In the Tropical Eastern Indian Ocean, Beckley (2019) found 92 neritic and 21 mesopelagic teleost families. In the Indian Ocean, Rathnasuriya et al. (2021) identified 80 species belonging to 69 larval families using morphological and molecular methods. Lower larval diversity could be associated with low productivity along the Cox's Bazar-Teknaf coasts, as nutrients carried by rivers are assumed to be lost in deeper seas due to the narrow shelf (Qasim, 1977). Rashed-Un-Nabi et al. (2011) reported to have 49 fish and shrimp species by char jal in Bakkhali river estuary. Hossain et al. (2007), on the contrary, reported collecting 161 species using various types of nets from the Naaf river estuary, which is about 50 kilometers south from the current research site. Reason behind this fluctuation is that the rubber dam has created a controlled environment in the Bakkhali estuary, reducing the number of species. Changes in water properties caused by the dam might also have a big impact on the river's species population. (McAllister et al., 2001). Based on the percentage of the larvae, two (02) dominant families were identified as Clupeidae and Ambassidae. Lirdwitayaprasit et al. (2008) recognized Photichthyidae, Myctophidae, Bregmacerotidae, Gonostomatidae, Callionymidae, and Carangidae as abundant families in the Bay of Bengal. Another research by Tzeng et al. (1997) at the estuarine stations of Yenliao Bay confirmed Pomacentridae, Apogonidae, and Tripterygiidae as dominant families at the rocky stations, and Gobiidae was abundant. Chesalina et al. (2013) identified the four most common families: Sparidae, Scombridae, Clupeidae, and Nemipteridae. Arshad et al. (2012) studied Clupeidae as the most abundant followed by Blenniidae, Teraponidae, Gobiidae, Sillaginidae, Nemipteridae, and Mullidae in Pendas river estuary, Peninsular Malaysia.

5.2 Constance of Occurrence

Based on the constancy of occurrence among the 11 families, the percentages of constant: accessory: accidental families were 18:27:55. Only Clupeidae and Ambassidae family were found throughout the year in the Bakkhali river estuary, and they commonly used it as a nursing ground, which made them constant families. Engraulidae, Mugilidae, and Blenniidae were considered accessory families. The majority of the families were not found frequently in this region and were considered to be accidental (Table 2). The result of the study showed a low number of constant families in the Bay of Bengal. This was consistent with the findings of Lirdwitayaprasit et al. (2008), who observed a low number of constant (28) and accessory (22) families in the Bay of Bengal, with 50 families classified as accidental.

5.3 Temporal density and diversity indices

The abundance of fish larvae was greatest in June, during the summer season. However, the majority of the fish families were recorded in May, indicating that summer is the most prolific month. The highest Shannon-Wiener index (H) was observed 1.055 in September, which is in monsoon season. The lowest value, zero (0), was found in January, March and April. The greater the value of H, the greater the diversity of families in a given sample. The lower the value of H, the less diverse the population. A sample with a H value of 0 has only one family. In terms of richness and evenness, both were highest in November. The highest Margalef's richness was 0.91 and Pielou's evenness index was 0.918. Margalef's richness index has no range. Pielou's evenness is an indicator that shows both diversity and species richness. While family richness refers to the number of different family present in a particular region, evenness refers to the number of individuals of each family present. Pielou's evenness has a predicted number ranging from 0 (no evenness) to 1 (complete evenness). Pielou's evenness is connected to the Shannon-Wiener index since it is determined by dividing the Shannon-Wiener index by the total number of families. In this study, the evenness index is zero (0) for January, March, and April, indicating that there is no evenness.

The obtained Shannon-Wiener index in this study matched with Arshad et al. (2012) that indicated significant variation within monsoon and intermonsoon season at Pendas river estuary. However, family richness peaked in January-March at this estuary.

This study also exhibited similarity to Brinda et al. (2010) where Margalef's richness varied from 0.71 (May) to 0.91 (March). Pielou's evenness index ranged between 0.71 (May) and 0.93 (April). The study was conducted in three distinct stations at Vellar estuary, which is situated at the Southeast coast of India.

Again, a brief research performed by Zhang et al. (2021) from September to October in 44 different stations in Eastern Indian Ocean showed quite similar result to this study. The average Shannon-Wiener index was 0.83, where highest was 1.52 and lowest was zero (0). Mean richness index was 1.01 and evenness index was 0.79.

5.4 Spawning season

Fish spawning season is responsible for the presence of fish larvae. Fish planktonic phases often extend a few weeks to a few months (Victor, 1986; Brothers et al., 1983). The study of temporal and geographical patterns, as well as the availability of fish larvae in connection to oceanographic circumstances, can give insight into the adaptability and influence of spawning patterns on yearly strength for the entire physical and biological processes. (Somarakis et al., 2002). However, the majority of studies on fish spawning season focused on histological analysis. In this study, the spawning seasons are classified into three (03) distinct groups: summer, winter, and monsoon. Further these groups were subdivided into early, mid and late based on the availability in different months. Majority of the families (07) spawn in summer season, which means they reproduce from March to June and their larvae were found between April and May in this estuary. Two families, Clupeidae and Ambassidae, were confirmed as spawner of all three seasons. Engraulidae, Mugilidae and Blenniidae family spawn in summer and rainy monsoon. Sillaginidae, Sparidae and Gerreidae spawn in winter and Gobiidae was reported to spawn in winter and summer.

El-Regal (2013) reported that Clupeidae and Engraulidae tended to reproduce at irregular intervals throughout the year with no apparent pattern. Most of the commercially available species were summer spawners in Red Sea, which matched this study. Clupeoids grow quicker in tropical waters and have a shorter life cycle than in temperate environments (Araújo et al. 2008). Rajaseker et al. (2005) demonstrated that rainfall data shows significant negative relationship with the distribution and frequency of finfish larvae.

Chapter Six

Conclusion

In the Bakkhali river estuary, diversity of larval family was distinct in relation to month. A total of 883 larvae were collected and 11 families were identified based on their morphometric-meristic characterstics. The month of May had the highest number of larvae, whereas the month of June had the most families. The highest Shannon-Wiener index was observed in monsoon season while family richness and evenness were highest in winter. It was revealed that this estuary did not have as many families as anticipated in comparison to other estuaries. Clupeidae was the most frequent family followed by Ambassidae and Engraulidae. These three families comprise of some commercially important fish species such as *Tenualosa ilisha*, *T. toli*, *Ambassis dussumieri*, *Stolephorus indicus*, *Stolephorus insularis.* These fish families may spawn because of suitable environmental conditions, particularly salinity. As a result, many families use the Bakkhali river estuary as a nursing ground. In addition, spawning season was also identified based on the larval frequency. Clupeidae and Ambassidae larvae have been recorded in seven distinct months, indicating that their spawning season is summer, winter and monsoon. Overall, eight (08) families were identified as summer spawners, implying that summer (March to June) is the most productive month in the Bakkhali river estuary. This research will lay the foundation for improvement of fisheries resource management strategies in the Cox's Bazar region.

Chapter Seven

Recommendations

This study contains yearlong valuable data of larval fish abundance, diversity indices and their spawning season at Bakkhali river estuary of Cox's bazar coast of Bangladesh. According to this research work, the following recommendations maybe done:

- It will be possible to determine which fish larvae are using this estuary as nursing ground from this research.
- In addition, the breeding season of identified fishes in that region might be stated, as well as their ban period, in order to achieve sustainable fisheries management.
- It will serve as a foundation for future decisions about the management and conservation of the fishes of the Bakkhali river estuary.
- Responsible authority should monitor physical, chemical and biological characteristics of this region to serve as nursing ground for freshwater and marine fish larvae.

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PHOTO GALLERY

Plate 1. Sampling by bongo net

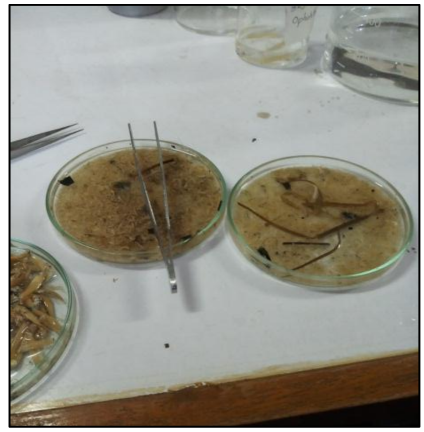

Plate 2. Fish larvae sorting from sample

Plate 3. Larvae identification under stereo microscope

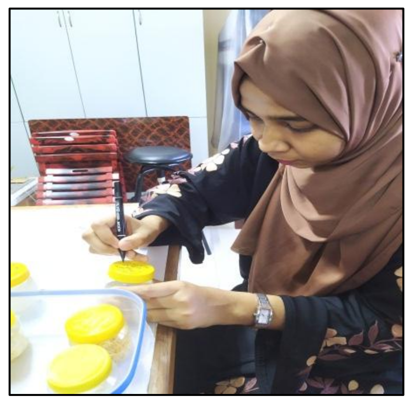

Plate 4. Larvae labeling and storage

Plate 5. Clupeidae larvae

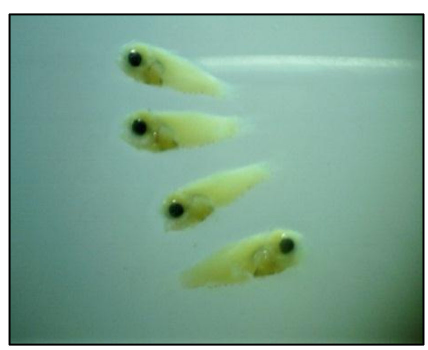

Plate 6. Ambassidae larvae

Plate 7. Engraulidae larvae

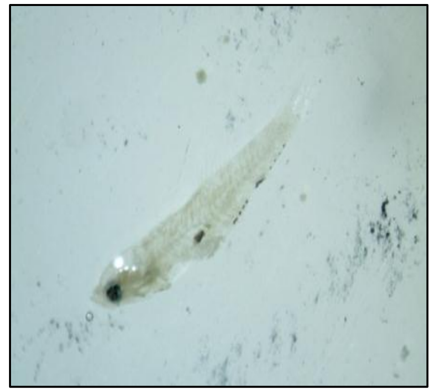

Plate 8. Gobiidae larvae

Plate 9. Sillaginidae larvae

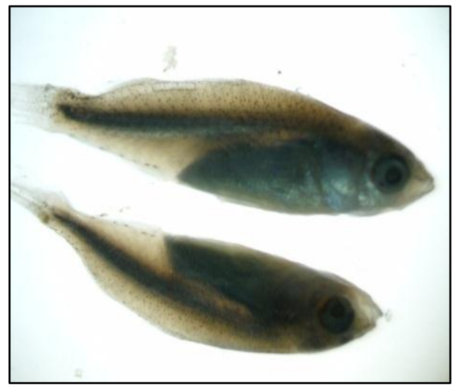

Plate 10. Mugilidae larvae

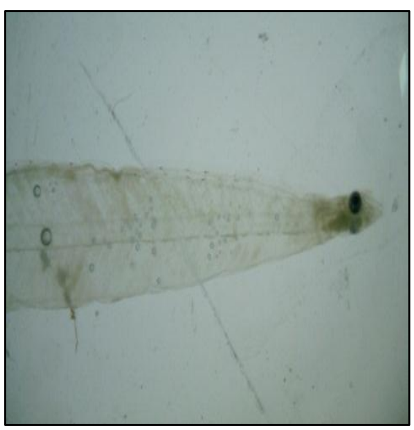

Plate 11. Megalopidae larvae

Plate 12. Blenniidae larvae


Plate 13. Terapontidae larvae


Plate 14. Sparidae larvae


Plate 15. Gerreidae larvae

APPENDICES

Appendix-1

Operation of Fish larvae sampling in Bakkhali river estuary, Cox's Bazar coasts

Month	Time		Flowmeter reading	Interval between flowmeter reading	Volume of water passed (m^3)
	Start	Finish			
Mar, 20	11.31	11.41	R1 (80405-81809)	1404	165
			R2 (81809-83902)	2093	247
			R3 (83964-87240)	3276	386
Apr, 20	12.15	12.27	R1(24966-26906)	1940	229
			R2(26906-29052)	2146	253
			R3(29053-31252)	2199	259
May, 20	11.00	11.10	R1 (30219-31221)	1102	130
			R2 (31322-33647)	2325	274
			R3 (33648- 36581)	2933	346
Jun, 20	11.15	11.25	R1(77696-80003)	2307	272
			R2(80003-81981)	1978	233
			R3(81981-83590)	1609	190
Jul, 20	10.46	10.56	R1(03508-06515)	3007	354
			R2(06515-09523)	3008	354
			R3(09523-13564)	4041	476
Aug, 20	10.24	10.34	R1(14091-14882)	791	93
			R2(14882-17552)	2670	315
			R3(17552-21324)	3772	444
Sep, 20	11.16	11.26	R1(56621-60428)	3807	449
			R2(60428-64820)	4392	517
			R3(64820-67983)	3163	373
Oct, 20	12.14	12.24	R1(69972-73070)	3098	365
			R2(73070-76992)	3922	462

			R3(76992-81241)	4249	501
Nov, 20	12.04	12.14	R1(03008-06317)	3309	390
			R2(06317-09125)	2808	331
			R3(09125-12609)	3484	410
Dec, 20	11.30	11.40	R1(56001-58275)	2274	268
			R2(53775-56001)	2226	262
			R3(58275-61123)	2848	336
Jan, 21	11.22	11.32	R1(61757-64146)	2389	281
			R2(64147-66449)	2303	271
			R3(66450-68625)	2175	256
Feb, 21	12.07	12.17	R1(07351-08846)	1495	176
			R2(08849-10049)	1200	141
			R3(10050-11925)	1875	221

Appendix-2

Monthly Abundance of fish larvae and their biodiversity indices

Month	**Family**	**Number of individual per 1000m^3**	**Shannon-Wiener index**	**Margalef's index**	**Pielou's index**
March	Gobiidae	2	0	0	0
April	Clupeidae	4	0	0	0
May	Engraulidae	8	0.091	0.863	0.051
May	Clupeidae	156	0.353		0.197
May	Terapontidae	2	0.031		0.017
May	Ambassidae	159	0.351		0.196
May	Blenniidae	2	0.031		0.017
May	Mugilidae	1	0.018		0.01
June	Clupeidae	274	0.384	0.506	0.277
June	Ambassidae	91	0.878		0.633
June	Megalopidae	5	0.057		0.041

June	Unidentified	6	0.066		0.048
July	Ambassidae	4	0.316	0.657	0.288
July	Clupeidae	4	0.316		0.288
July	Gobiidae	13	0.297		0.27
August	Ambassidae	27	0.359	0.488	0.327
August	Clupeidae	32	0.335		0.305
August	Blenniidae	1	0.068		0.062
September	Engraulidae	3	0.226	0.874	0.163
September	Mugilidae	1	0.111		0.08
September	Ambassidae	15	0.351		0.253
September	Unidentified	12	0.367		0.265
October	Engraulidae	15	0.216	0.668	0.196
October	Ambassidae	2	0.23		0.21
October	Mugilidae	3	0.285		0.259
November	Blenniidae	1	0.366	0.910	0.528
November	Clupeidae	2	0.27		0.39
December	Clupeidae	25	0.071	0.607	0.103
December	Ambassidae	2	0.193		0.278
January	-	0	0	0.000	0
February	Sparidae	2	0.31	0.834	0.282
February	Sillaginidae	7	0.288		0.262
February	Gerreidae	2	0.31		0.282

Appendix-3

Temporal variation of biodiversity indices at Bakkhali river estuary

Month	Shannon-Wiener index	Margalef's index	Pielou's index
Mar, 20	0.000	0.000	0.000
Apr, 20	0.000	0.000	0.000
May, 20	0.875	0.863	0.488

Jun, 20	0.697	0.506	0.503
Jul,20	0.929	0.657	0.845
Aug, 20	0.763	0.488	0.694
Sep, 20	1.055	0.874	0.761
Oct, 20	0.731	0.668	0.665
Nov, 20	0.637	0.910	0.918
Dec, 20	0.264	0.607	0.381
Jan, 21	0.000	0.000	0.000
Feb, 21	0.908	0.834	0.826

Brief biography of the author

Jannatul Mawa is the only daughter of Md. Ismail Hossain and Kamrun Nahar, was born in 10th March, 1997 in Chandpur, Bangladesh. She has achieved Secondary School Certificate from Dr. Khastagir Govt. Girls' High School and Higher Secondary Certificate from Chattogram City College. She has also achieved B.Sc. Fisheries (Hons.) from Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University. She is now pursuing Master of Science in Fisheries Resource Management under the Department of Fisheries Resource Management, Chattogram Veterinary and Animal Sciences University. She was employed as a research assistant for a period of March 2020 to February 2022 in the project entitled "Validation of the Identified Marine Fish Larvae of the Cox's Bazar-Teknaf Coastal Water Using DNA Bar-Coding Method" funded by WorldFish under direct supervision of Dr. Sk. Ahmad Al Nahid. She is passionate to qualify herself as a competent researcher, and thus to develop the fisheries sector of Bangladesh.