

Chapter-1: Introduction

1.1 Background

Estuaries are close-to-land coastal environments where freshwater dilutes the ocean (Dyer, 1997). They perform crucial economic tasks in the areas of transportation, manufacturing, and tourism as well as the drainage of waste from commercial, industrial, and agricultural operations (Heip and Herman, 1995; Raz-Guzman and Huidobro, 2002). These ecosystems simultaneously provide protection for a variety of marine taxa in addition to resident species (Weinstein, 1985; Weisberg et al., 1996; Cowley and Whitfield, 2002; McLusky and Elliott, 2004) which offer significant economic potential and excellent fishery yields (Houde and Rutherford, 1993).

The ability of larvae to reach coastal nursery grounds, which offer refuge and food availability for their growth and survival, is crucial for the success of the early life stages of marine fish (Bailey et al., 2008). Estuaries are included in the nearshore coastal habitats that are frequently referred to as crucial fish larvae and juvenile nurseries (Amara, 2003; Strydom et al., 2003; Borges et al., 2007; Cabral et al., 2007; Primo et al., 2013; Baptista et al., 2019). 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). Therefore, larval dispersion from spawning grounds to nursery sites is a key component of fish population dynamics and life history. With larval growth, the capacity to mediate movement to nursery regions grows (Helfman et al., 2009). The quality and availability of the habitat, as well as biotic (such as the amount of prey and predators) and abiotic factors, appear to have a significant impact on juvenile abundance and distribution (e.g. temperature, salinity, freshwater runoff) (Able et al., 2005; Bento et al., 2016; Martinho et al., 2007a). These assemblages fluctuate through time and space, depending on the species reproductive seasons as well as ecological factors (Arshad et al., 2012).

The size of marine larval fish ranges from 2.5 to 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. Most

marine fish species produced pelagic eggs and drift with the currents. Closely related species are identified using standard morphometric measures.

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.

In estuaries and coastal locations, Ichthyoplankton and juvenile communities have been extensively researched, but few studies have included both phases of the fish life cycle (Strydom et al., 2003; Able et al., 2006; Ramos et al., 2010; Primo et al., 2013). Fish eggs and larvae have made major contributions to fisheries management and are anticipated to continue to do so as fish stocks are augmented and conserved in the future. The amount of knowledge about the development of fish has increased dramatically during the past 40 years (Rutherford, 2002; Pattira et al., 2012). It is now obvious that findings received from fish eggs and larvae offers 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 region of Bangladesh is 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). A broad range of biodiversity such as fishes, crustaceans, mollusks, animals, seaweeds, and other species inhabit the Bay of Bengal's coastal and marine environments. Within Bangladeshi seas, there are around 511 marine species, including shrimp (Murshed-E-Jahan et al., 2014). The yield of marine fisheries contributes for 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).

1.2 Significance of the Study

Fish spawning season has a direct impact on the presence of fish larvae. Fish often go through a planktonic stage that lasts from a few weeks to a few months (Victor, 1986;

Brothers et al., 1983). The dispersal of spawning products is governed by adults. Even yet, the distribution, quantity, growth, and survival of larvae are directly influenced by a mix of physical and biological factors (such as water flow and temperature, location, and the abundance of prey and predators) (Heath, 1992).

The Naf River is a transboundary river that divides Myanmar and Bangladesh. It is an extended estuary that separates Cox's Bazaar from Arakan in the district's far southeast (Myanmar). On the district's southern borders, it rises in the Arakan Hills and empties into the Bay of Bengal. Between 1.61 and 3.22 kilometers make up its width. The Naf River has a maximum depth of 400 feet and an average depth of 128 feet (Banglapedia). The Naf River is a sizable body of water that is regarded as an estuary with abundant fishery resources and provides for the needs of the nearby fishing community. The feasibility study of the Naf River's water will help the locals' ability to fish, cultivate, and irrigate their land.

1.3 Objectives

- To identify fish larval family composition in the Naf River Estuary;
- To identify the most numerous families of fish larvae as well as their distribution and overall abundance; and
- To identify spawning season of fishes at Naf River Estuary.

Chapter-2: Review of literature

2.1 Fish larval Composition and 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 marine fish, 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.

Larvae go through growth and ontogeny to emerge from this delicate stage. Out of thousands of newly formed larvae, only a handful typically survives the continual threat of starvation and predation during planktonic life. The eggs and larvae of marine fish are captured using fine-mesh plankton nets or specially made traps.

In order to ascertain their distribution, richness, structure, and connections between larvae and their rivals and prey, "Ichthyoplankton" communities are investigated at sea. These surveys are commonly used in fisheries management stock assessments (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 (Nakatani et al., 2001; Cruz et al., 2016), that can be used to direct management and conservation actions.

2.2 Activities around Estuaries

Early stages of marine fish development depend on larvae's capacity to find coastal nursery grounds that provide safety and nourishment for their survival and growth (Bailey et al., 2008). Major fish larval and juvenile habitats are typically referred to as estuaries and other nearshore coastal environments (Amara, 2003; Baptista et al., 2019). Therefore, larval dispersal from breeding grounds to nursery sites is an essential component of fish population dynamics and life history. With larval growth,

the capacity to control migration toward nursery locations becomes more developed (Helfman et al., 2009), and Larvae must react to signs that indicate their proximity (e.g., geomagnetic, scent, auditory, visually and chemical cues, river plumes) (Teod'osio et al., 2016). Larval survival and dispersal will be impacted by a variety of biological and environmental factors during the dispersion process, making this a particularly sensitive stage of the fish life cycle with mortality rates ranging from 05 to 40 percent every day (Bailey et al., 2008; Houde, 2008). Estuaries and related wetlands provide as a habitat for spawning and shelter for fish, particularly marine species (McLusky et al., 2004). Depending on the kind of fish, spawning occurs in rivers, estuaries, or offshore areas (Elliott et al., 2007b). Several fishes defend their eggs by attaching them to this substrate material, which keeps the eggs and larvae from being washed out to sea (Elliott et al., 2002). Pelagic eggs, along with the larvae they will hatch into, are released by some fish species and float around the ocean for days or weeks. Perhaps as a defense mechanism against being swept out of the estuary, other fish species lay demersal eggs, which are larger and heavier eggs that fall on the substrate (Wolanski, 2015). The people and the surrounding environment greatly benefit from estuarine and coastal areas, which are important habitats and breeding grounds for a range of species (Beck et al., 2001; Gillanders et al., 2003).

2.3 Larval Abundance and distribution

The life cycles and reproductive patterns of the adult population, which are often correlated with climatic and oceanic conditions, are connected to seasonal patterns of fish larval abundance (Hernandez-Miranda et al., 2003). Food availability is correlated with biotic variables, and zooplankton density is occasionally correlated with larval fish abundance. For instance, the seasonality of larval fish abundance can be highly correlated with the quantity of copepod nauplii (Mateo et al. 2006). In all, 2687 larvae were collected by Arshad et al. (2012) from the Malaysian estuary of the Pendas River, with a mean abundance of 28.29 larvae per 100 m³. In Peninsular Malaysia, there were 19 families of larval fish, including 17 in the middle estuary, 16 in the lower estuary, and 14 in the upper estuary. The head of the estuary had the highest concentration of freshwater larvae, and the station with the highest salinity had the highest concentration of marine larvae. According to the length frequency distribution, the estuary is not doing a good job of retaining marine larvae. According to the distribution pattern of all fish larvae, there were significantly fewer fish families

and densities overall in the higher estuary than in the lower estuary. This indicates that fish larvae in the estuary may have moved there from the sea. Between January-March (Northeast monsoon) and June-August, the dominant families (Clupeidae, Blenniidae, Gobiidae, and Teraponidae) migrated to the estuary (Southwest monsoon). One particular feature of the larval assemblage is the monsoonal impact on the quantity of fish larvae in the Pendas River estuary. 6170 larval specimens from a total of 18 families were gathered for a study from the Bay of Bengal. Seven families were significant from an economic standpoint. Hemirhamphidae, Carangidae, Sphyrnaeidae, Gempylidae, Scombridae, Bothidae, and Cynoglossidae were among them. They all contributed roughly 5.64 percent of the total number of fish larvae. Carangidae, Scombridae, and Gempylidae were the three most prevalent families in that region (Lirdwitayaprasit et al., 2008). Nellen (1973) found 102 fish larval families in the Red Sea, Arabian Sea, and Persian Gulf in the northwest Indian Ocean. Among them, 58 were shelf fish larvae and 44 families were oceanic plus deep benthic. 103 larvae families were discovered in a different study in the southeast Indian Ocean (Australia's NW continental shelf) (Young et al., 1986). He found that, 67 families of shelf fish larvae and 36 families of marine larvae. Fish larvae from 55 and 62 different families were found along Thailand's west coast in 1982 and 1983, according to Janekarn (1988). Based on his and other research, he calculated the total number of 123 fish larval families on Thailand's west coast (Janekarn, 1992). Chamchang (2006) reported a low number of stable families in the composition and quantity of fish larvae, demonstrating the unreliability of the system; In the Andaman Sea off the coasts of Thailand and Myanmar, between 6°44.47'N and 12°40.80'N and 95°51.20'E and 96°45.30'E, 62 families of fish larvae were found. Azhagar et al. (2009) discovered that the monthly distribution of finfish larval density ranged between 8 and 76 larvae per cubic meter of water along the Kodikkarai shoreline and between 10 and 65 larvae per cubic meter at Arkattuthurai. At both locations, the summertime was when the larvae density was maximum. Larval density was at its lowest during the monsoon. Melville-Smith and Baird (1980) found 17 larval fish species across 15 families in the Swartkops Estuary in South Africa. The following year, they carried out a similar investigation in the Kromme River Estuary and identified 12 groups and 15 species, a somewhat lower total. Beckley (1985) found 17 species in the Swartkops Estuary mouth area. Strydom (1998) discovered 15 fish families, 28 species, and nine undetermined species in the Gamtoos Estuary. Strydom

et al. (2003) recorded 23 fish families with 63 species in warm-temperate estuaries of the Eastern Cape, whereas Montoya-Maya and Strydom (2009) found 17 families with 33 species in cool-temperate settings. In subtropical estuaries along South Africa's eastern coastlines, the number of groups and species significantly rises. Strydom (2015) studied the patterns of variety, abundance, and distribution of fish larvae in 25 South African estuaries and discovered a total of 89 species and 29 larvae families. The warm temperate estuaries had 23 families and 68 species, the cold temperate estuaries had 24 families and 46 species, and the boundary estuaries had 18 families and 40 species.

2.4 Larval Family

Clupeidae made up 41.07 percent of all families in a survey conducted at the Pandas River estuary in Peninsular Malaysia. Blenniidae (24.45%), Teraponidae (8.80%), Gobiidae (5.40%), Sillaginidae (3.22%), Nemipteridae (1.72%), and Mullidae were the next on the list (1.28%). Clupeid larvae, the most prevalent family, peaked in abundance during Peninsular Malaysia's northeast monsoon season in February and March. The monsoon season (February-March) produced the highest clupeid population, indicating seasonal spawning. The largest peak number of Teraponidae, the second most numerous families, was recorded in February, perhaps indicating the family's reproductive season. Even in other months, these larvae were substantially more prevalent. The biggest populations of gobiid larvae were seen in the northeast monsoon from January to March (Arshad et al., 2012). According to Janekarn and Boonruang (1986), the density of clupeid larvae was at its highest in February in Thailand's Western Peninsula. According to (Aziz et al., 2006), two species of gobiid fish were found in Peninsular Malaysia's Merchang Lagoon's seagrass substrate. No matter the climate or other characteristics like seagrass diversity, temperature, or biological factors, numerous studies have indicated that the Gobiidae family is widely scattered in coastal areas (Blaber et al., 1997, Kwak and David, 2003). Clumps of unusually diverse recruitment in schooling species, which appear to have developed from aggregative settlement, are associated with the high predominance of the Gobiidae family (Anand and Pillai, 2005). There was a lot more information discovered about fish larvae in a thorough study carried out by Lirdwitayaprasit et al. (2008) in three separate Bay of Bengal locations. Photichthyidae, Myctophidae, Bregmacerotidae, Carangidae, and Callionymidae were seen in relation to the

frequency of larval presence Gonostomatidae, Photichthyidae, Myctophidae, and Bregmacerotidae were permanent families in the upper part; in the western part; and in the Andaman sea region, 14 families were consistently found. The Myctophidae family, which contributed over 30.41 percent of the larvae was the most numerous, according to Chamchang (2007), followed by the Stomiidae family. During the investigation at the Andaman Sea, the proportion of fish families including Scombridae, Mugilidae, Clupeidae, Carangidae, Engraulidae, Leiognathidae, Tetradontidae, Lutjanidae, Pomacentridae, Sciaenidae, and Chirocentridae was examined. However, the proximity of the larvae in the two places differed. The Scombridae family was the most prevalent (Station-I 10.12% and Station-II 10.14%), while the Chirocentridae family was the least prevalent (Station-I 2.61% and Station-II 2.72%) (Azhagar et al., 2009). The clupeidae fish family was the most frequently captured fish family in all three biogeographical regions of temperate South Africa. A single species (*G. aestuaria*) makes up the family, and on average, it made up 77.00 percent of cold-temperate estuaries, 63.38 percent of warm-temperate estuaries, and 73.60 percent of border estuaries. In terms of overall catch, the gobiidae and blenniidae families came in second and third, respectively. Less than 1% of the total catch came from the remaining fish groups.

2.5 Diversity indices

The Euhaline salinity zones, which indicate the accumulating impact of marine and estuarine species, showed the highest alpha diversity, which shows intra-estuary trends, in a large-scale research at temperate South African estuaries by Strydom (2015). The most diverse estuary at the Beta diversity level is the warm-temperate, permanently open Kromme Estuary in the Eastern Cape (diversity score: 1.77), followed by the cold-temperate, permanently open Olifants Estuary in the Western Cape (diversity score: 1.66). The Western Cape's narrow, closed Diep Estuary, which is chilly and only seldom accessible, has the least diversity (0.15). At the Gamma diversity level, warm temperate estuaries (1.67) had a higher mean diversity of fish larvae than cold temperate (1.50) or boundary estuaries (0.96) did. On the other hand, there weren't much variations between the regions. Although there was no statistically significant difference in the species diversity among the estuaries, the permanently open estuaries (1.11) had a higher 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). Between months, there were significant differences in the overall fish larval density ($p < 0.05$). The Shannon Wiener index demonstrated significant variation between monsoon and inter-monsoon seasons, with peaks in December to January and May to August. Additionally, throughout the course of a year, family wealth displayed two unique peaks. Both peaks occurred between January and March and May and August (Arshad et al., 2012).

2.6 Spawning season

The majority of Red sea species used for commerce spawn between May and August. Of all commercially accessible species, they produced around 61 percent. 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. Clupeidae and Engraulidae, two species, have been shown to reproduce at various times of the year and without any discernable 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 Teraponidae 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 Terponidae was the least common (0.99%). The dominant group during the pre-monsoon season was Leiognathidae (25.58 %), and the minimum was Latidae (1.16%). During the monsoon season, Pomadasidae (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 Siraimetan (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.

According to Yoshida (1979), the frigate tuna reproductive season in the Indian Ocean lasted from January to April. The eastern Indian Ocean's quantity of skipjack tuna larvae peaked in February, according to Stequert and Marsac (1989). Females of *S. gibbosa* and *S. fimbriata*, both gravid and ripe, were found in most months. Peak occurrences of *S. gibbosa* occurred in the southern region from February to March, in the northern region from March to April, and across the northwestern Bay of Bengal from May to July (Ghosh et al., 2013). Most of the 330 *Anchoa tricolor* ovaries were researched by Araujo et al. (2008) in a tropical bay in southeast Brazil. These ovaries showed advanced maturation phases, which were common from August to March; recovery stages were common from April to July. About 40% of the females were in the beginning stages of spawning in September. 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 variations imply that *S. commersonnii* has the capacity to adjust its reproductive cycle in response to environmental conditions, with temperature, nutrient availability, and photoperiod being the three main determinants of Engraulid reproduction in coastal areas (Silva et al., 2003; Araujo et al., 2008). Anchovies (Engraulidae) have different spawning seasons in tropical and subtropical waters, according to several studies, most notably Kim et al. (2013) and Andamari et al. (2002).

Chapter-3: Materials and Methods

3.1 Study site

The study was conducted at the Naf River, which is located on the southeast coast of Bangladesh. It is situated roughly between latitude 20.76830 N and longitude 92.30970 E at the tip of the Bay of Bengal (Figure-01). Sampling was conducted in the morning in the middle of each month from March 2020 to February 2021. Coordinates were taken through a GPS meter.



Figure-01: Map of the Naf River Estuary

3.2 Sampling Procedure

By using a bongo net with a 0.50 m mouth diameter, 1.3 m length, and 500 µm mesh at the body, fish larvae were collected from the chosen site. To measure the amount of seawater filtered during each tow, a flow meter (KC Denmark A/S 23.090-23.091) was fastened to the mouth of the net. The sample time was roughly 10 minutes of surface time during the day. The obtained specimen was preserved in 90% ethanol, transferred to the Aquatic Ecology Laboratory, CVASU and freshly preserved in 90% ethanol before being sorted according to morphology and other characteristics.

3.3 Fish larvae sorting

Larvae from the entire sample were typically sorted for taxonomic identification. The sample's ethanol content was removed as part of the initial sorting process. To do this, samples were thoroughly washed with distilled water and sieved through meshes of 0.1 mm to easily remove sand particles, plastics, leaves, and other undesired materials. 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 analyzed under a stereo microscope (OPTIKA Microscope Italy C-B3) at low magnification (10x) and several pictures were taken. Each picture was given a specific code so that it can be easily found later.

3.4 Morphological identification of fish larvae

Using the descriptions of related taxa provided in Leis and Rennis (1983), Leis and Carson-Edwart (2000), and Rodriguez, fish larvae were recognized under the stereo microscope up to the family level. The most important qualities for identifying larval fish are the body structure, coloration pattern, and meristic and morphometric traits. Due to their unique bodily composition, larvae can be classified into a wide range of broad groups (Russell, 1976). Typical fish-shaped larvae, bodies with unusual shapes or displaying specialized larval characteristics for the plankton life, cranial armatures, elongated fin rays, stalked eyes, or huge and early produced fins are a few examples. Because the samples were too small to identify, unidentified larvae samples were labeled as "unidentified".

3.5 Determination of Larval Abundance

Numbers of fish larvae according to specific family were counted through naked eye. This number is simplified into per 1000 m³ for further analysis. Temporal variation of fish larvae were determined by number of larvae along with diversity indices. Pielou's evenness index (Pielou, 1966) and the Shannon-Wiener index (Shannon and Weaver, 1949) were employed to evaluate the variety of the larval fish assemblage. Following (Margalef, 1958) family richness was calculated. Diversity can be defined most simply as the variety of species that exist. Different biological communities have different levels of species diversity (richness) and relative abundance of these species (evenness) (Anon, 2021). The two separate parts of species diversity, species richness (S) and evenness (E), a critical component of ecology (E). Popular species biodiversity indices include the Shannon-Wiener (Shannon index) and Simpson's index. The Simpson's index determines the likelihood that two people selected at random from a sample will be of the same species. One of the most well-known diversity indexes is the Shannon index, which measures species richness and the proportion of each species in a community (Arzamani, 2018). In this study, family richness and evenness were analyzed using the Shannon index, which also served to determine biodiversity.

3.5.1 Number of larvae individual per 1000 m³

Bongo net diameter, $d = 0.50$ m

So, net radius, $r = 0.25$ m

Net opening area = πr^2

$$= 3.1416 \times (0.25)^2$$

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

$$= 0.3927 \text{ m}^2$$

- Volume of water passed in each sampling = Indicated number of revolutions \times Pitch of the impeller (0.3) \times Net opening area (m²) \times 1000
- Number of larvae per 1000 m³ = (Number of larvae in sample \times 1000) \div Volume of water passed

3.5.2 Measurement of larval diversity

E-diversity, or the diversity of species inside a community or environment, is the form of diversity that is being discussed here. The Shannon-Wiener diversity index was used to generate the diversity index (Shannon and Weaver, 1949).

- Diversity index, $H = - \sum P_i \ln P_i$

where $P_i = S / N$

S = number of individuals of one species

N = total number of all individuals in the sample

In = logarithm to base e

The diversity of species in a given community increases with increasing H value. The diversity decreases as H value increases. A community with a value of $H = 0$ only contains one species.

3.5.3 Measurement of larval richness

As a straightforward indicator of species richness, Margalef's index was used (Margalef, 1958).

- Margalef's index = $(S - 1) / \ln N$

Here, S = total species

N = total no. of participants in the sample

3.5.4 Measurement of evenness

The Pielou's Evenness Index (e) was utilized to determine the evenness of species (Pielou, 1966).

- $e = H / \ln S$

Here, H = Shannon-wiener diversity index

S = total number of species in the sample

3.5.5 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 winter, summer and monsoon. Winter season comprises from November to February, summer is between March and June and monsoon states from July to October.

3.5.6 Statistical Analysis

Microsoft Office Excel (version 2010, USA) was used to enter all of the data, data management and data analysis. The results were expressed using graphs, tables and pie-chart.

3.5.7 Photo Gallery



Figure-02: Bongo net



Figure-03: Bongo net operation



Figure-04: Sample collection and initial sorting



Figure-05: Identification of larval family

Chapter-4: Results

4.1 Total fish larvae and dominant families

The study site yielded 1681 larvae in total, with a mean abundance of 140.08 per 1000 m³ (Table-01). The larval fish collection consisted of eight families and unidentified samples had been shown on Figure-06. Clupeidae, which contributed roughly 45.03 percent of the catch, was the most numerous family, followed by Engraulidae (32.89%), Ambassidae (19.15%), Blenniidae (0.83%), Sillaginidae (0.41%), and miscellaneous families (1.77%) according to figure-07. Clupeidae, Engraulidae, and Ambassidae were the three most prevalent larval families, and they use the Naf River Estuary as their nursing ground (Figure-07).

Table-01: Abundance of marine larvae at Naf River Estuary

Family	Total number of larvae (larvae/1000 m ³)	Mean number of larvae	SD	Percentage of total catch	Frequency of Occurrence
Clupeidae	757	63.08	112.04	45.03	58.33
Engraulidae	553	46.08	125.47	32.89	41.67
Ambassidae	322	26.83	95.05	19.15	41.67
Blenniidae	14	1.17	3.16	0.83	25.00
Sillaginidae	7	0.58	0.32	0.41	16.67
Gobiidae	1	0.08	0.32	0.06	8.33
Carangidae	1	0.08	0.32	0.06	8.33
Sciaenidae	1	0.08	0.32	0.059	8.33
Unidentified	25	2.08	7.91	1.487	

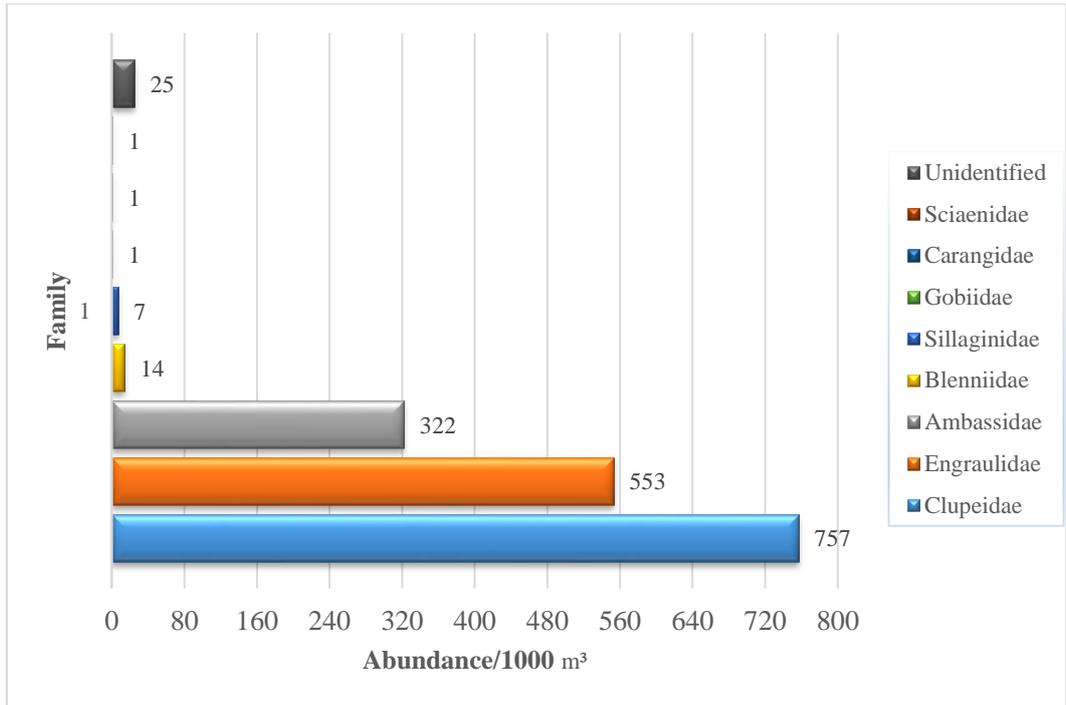


Figure-06: Abundance of Fish Larval Families

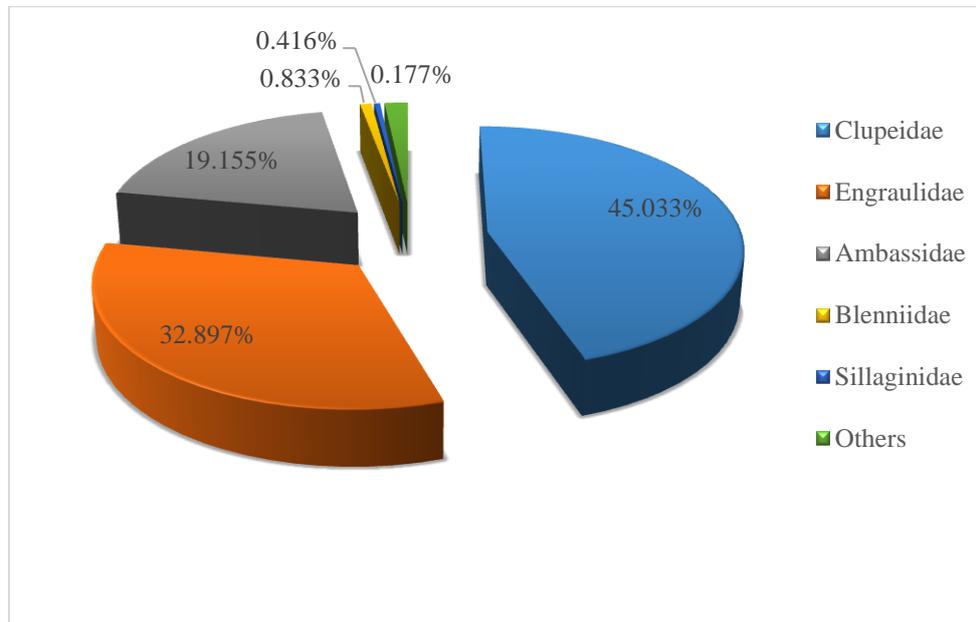


Figure-07: Percentage of Fish Larvae Abundance

4.2 Top three abundant families

Clupeidae, Engraulidae, and Ambassidae were the three most prevalent larval families, which was shown in figure-07. Temporal abundance of these 3 families had been shown on figure-08.

4.2.1 Clupeidae

Clupeidae were the most abundant larvae and contributed almost half of the total abundance (45.033%) of the Naf river estuary. This family was found in seven months of a year-from April to July, November, December and February (Figure-08). The mean density of this family was 63 individuals/1000 m³ and the highest abundance was 321 individuals/1000 m³ in May. Several commercially important species under the Clupeidae family were found in Cox's Bazar, such as *Tenuwalosa ilisha*, *T. toli*, *Hilsha kelee*, *Escualosa thoracata*.

4.2.2 Engraulidae

Engraulidae family was the second abundant family, which contributed 32.897% of the total abundance. This family was found in four consecutive months from August to November (Figure-08). The mean density of the larvae was 46.08 individuals/1000 m³ and the highest abundance was observed in August, at 402 individuals/1000 m³. *Thryssa hamiltonii*, *Stolephorus indicus* and *Stolephorus insularis* are the commercially important species under the Engraulidae family that has been found on the Cox's Bazar coasts.

4.2.3 Ambassidae

Ambassidae family was the third abundant family, which contributed 19.155% of the total abundance. This family was found in three months March, July and August (Figure-08). The mean density of the larvae was 26.83 individuals/1000 m³ and the highest abundance was observed in August, at 302 individuals/1000 m³. *Ambassis dussumieri* is the only species of Ambassidae that has been found on the Cox's Bazar coasts.

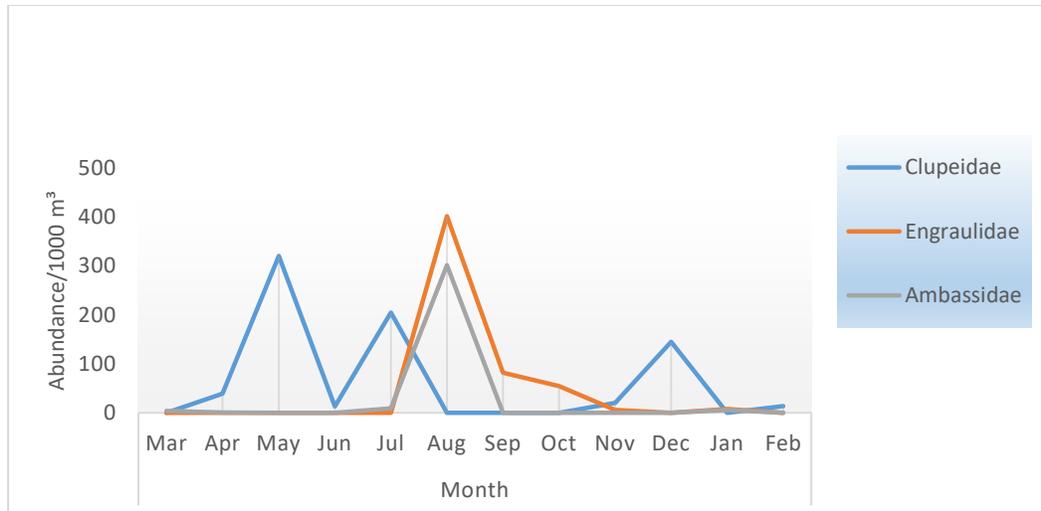


Figure-08: Temporal abundance of top three families

4.3 Temporal variation of larval abundance

The highest mean total density of larvae (704 individuals/1000 m³) was observed in the month of August, while in March, June and January; Relatively lower fish larvae were found (Figure-09). In May, July and December, Clupeidae larvae were most abundant. On the other hand Engraulidae and Ambassidae were most abundant in August (Figure-09).

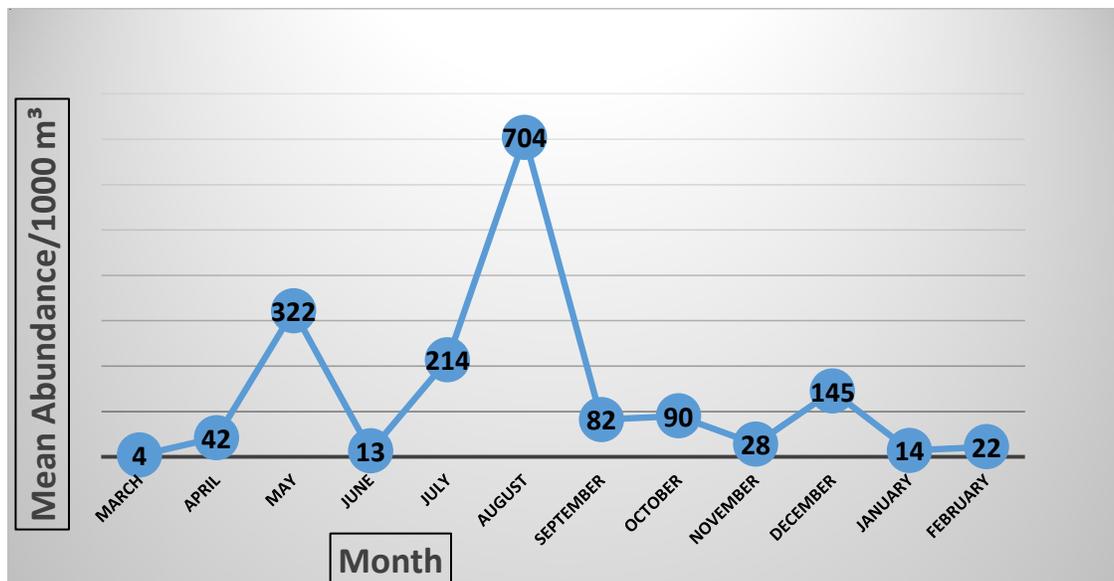


Figure-09: Mean Abundance of larvae in each month

4.4 Diversity index, Richness, and Evenness

In April, the highest number of families was observed. Diversity indices showed significant variation within months. The highest Shannon-Wiener index was found in October (0.901) and February (0.86) respectively (Figure-10). Margalef's richness index also clearly showed two significant peaks, one in April (0.806) and the next one in February (0.647) (Figure-12). In terms of Pieulo's evenness, the highest was 1.408 in January and the second highest was 0.985 in August (Figure-11).

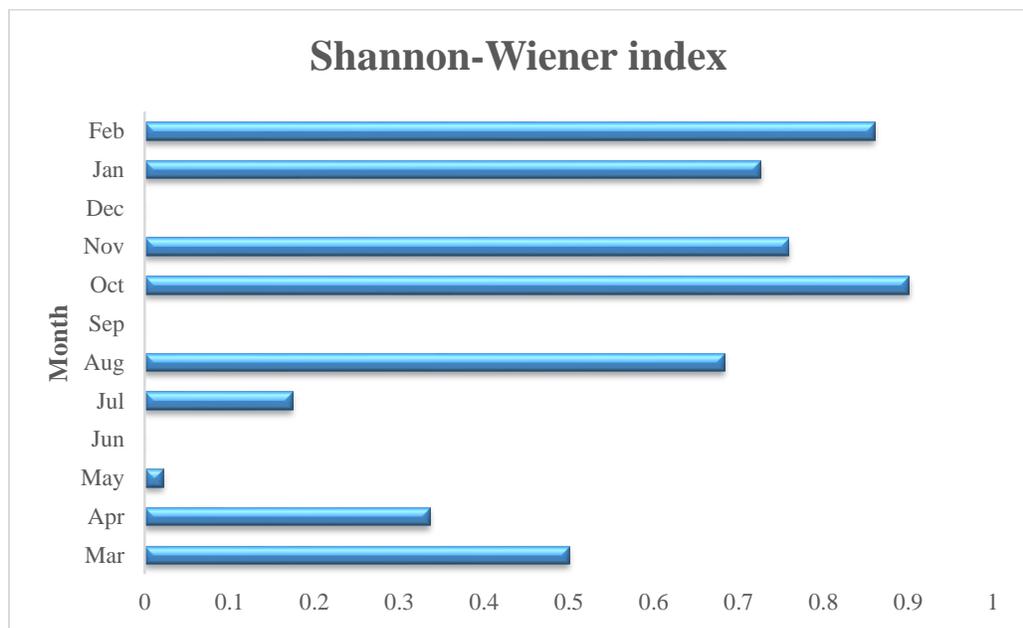


Figure-10: Shannon-Wiener index of diversity

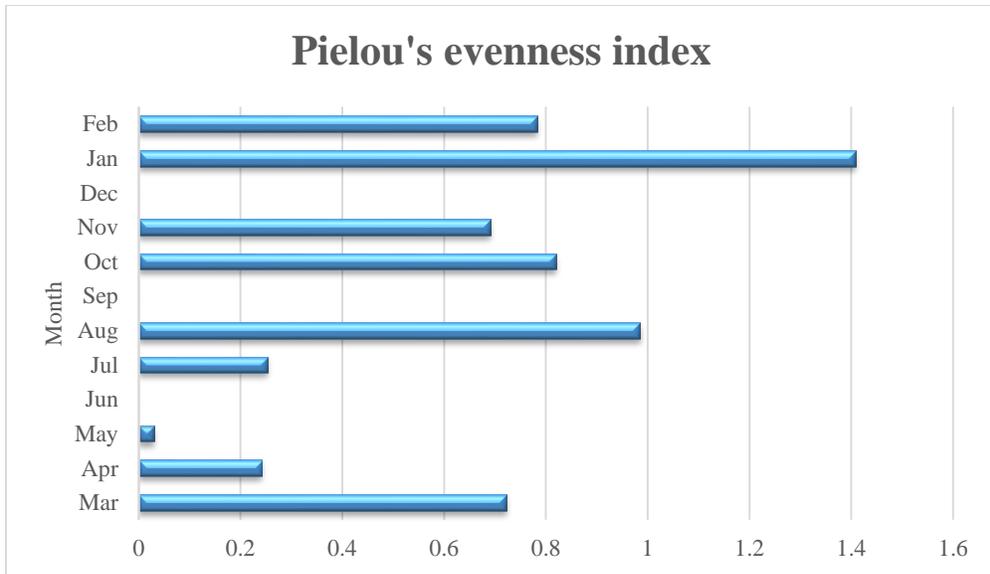


Figure-11: Pielou's evenness index

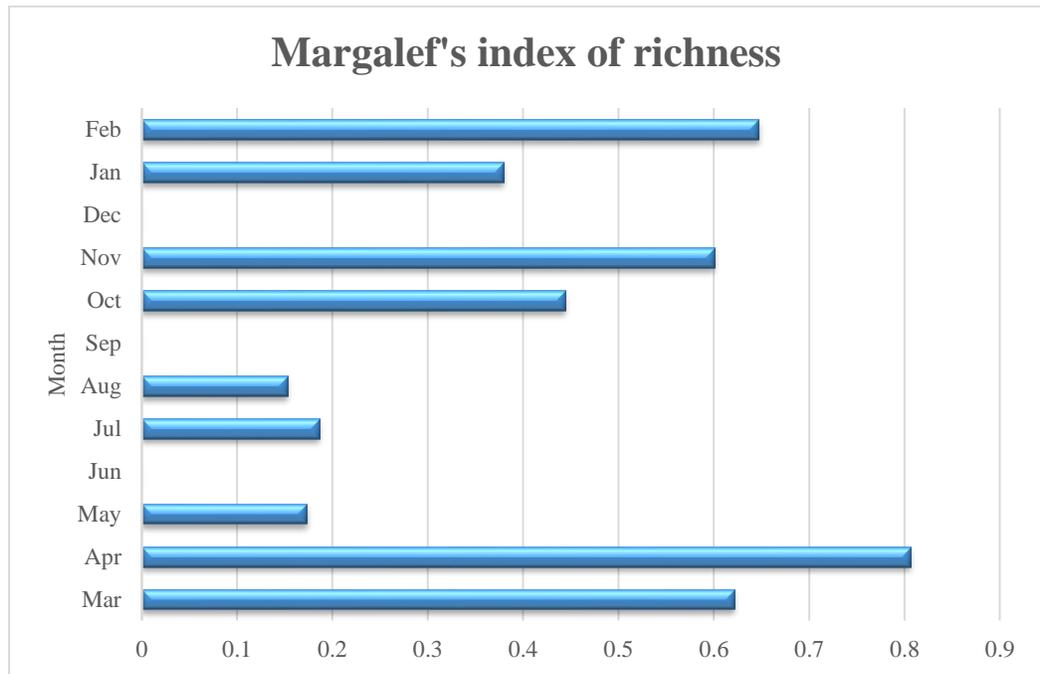


Figure-12: Margalef's Richness index of family

4.5 Spawning season

Larvae of 08 fish families were collected and their time of occurrence was used as indication of their spawning season. Most of the families spawn in the warmer months of the year (March to May) based on the availability of their larvae. Because their larvae were discovered in seven months of the year, the families Clupeidae and Ambassidae were identified as Summer, Pre-Monsoon, Monsoon, and Post-Monsoon spawners (Table-02). Summer and Monsoon were seen for spawning by the Engraulidae family, Summer, Pre-Monsoon and Post-Monsoon for spawning by the Blenniidae family, and Winter and Pre-Monsoon for spawning by the Gobiidae family. Due to their yearly reproduction, three (3) families are categorized as single spawners.

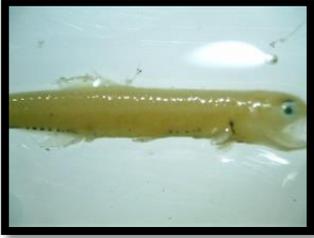
Table-02: Spawning season of fish larvae with their frequency of occurrence and spawning month

Family	Frequency of occurrence	Spawning month	Spawning season
Clupeidae	Feb, Apr to Jul, Nov, Dec	Jan, Mar-Jun, Oct, Nov	Mid-Winter, Summer, Late Monsoon, Early Winter
Engraulidae	Jan, Aug-Nov	Dec, Jul to Oct	Mid-Winter, Monsoon
Ambassidae	Mar, Apr, Jul, Aug	Feb, Mar, Jun, Jul	Late Winter, Early Summer, Late Summer, Early Monsoon
Blenniidae	Feb, Oct, Nov	Jan, Sep, Oct	Mid-Winter, Mid & Late Monsoon
Sillaginidae	Feb, Apr	Jan, Mar	Mid-Winter, Early Summer
Gobiidae	Apr	Mar	Early Summer
Carangidae	May	Feb	Late Winter
Sciaenidae	May	Apr	Mid-Summer

4.6 Identified Larval Families

According to Leis and Rennis (1983), Leis and Carson-Edwart (2000), and Rodriguez, total eight (08) families were identified under Stereo-microscope.

Table-03: Identified Larval Families

Serial	Family	Larvae stage
1.	Clupeidae	
2.	Engraulidae	
3.	Ambassidae	
4.	Blenniidae	
5.	Sillaginidae	

6.	Gobiidae	
7.	Carangidae	
8.	Sciaenidae	

Chapter-5: Discussion

5.1 Fish larval Family and Abundance

This study has discovered 1681 individuals and 08 families. Based on the morphological analysis of larvae, the families were- Clupeidae, Engraulidae, Ambassidae, Blenniidae, Sillaginidae, Gobiidae, Carangidae and Sciaenidae (Figure-06). Abundance of Naf River Estuary is little less than other areas of the Cox's bazar-Teknaf coast such as Bakkhali Estuary, Reju Khal & Moheshkhali para. 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. On Thailand's west coast, 62 families of fish larvae were identified by Janekarn (1988). In the Andaman Sea between Thailand and Myanmar, Chamchang (2006) found 62 kinds of fish larvae. 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). Hossain et al. (2007), on the contrary, reported collecting 161 species using various types of nets from the Naf river estuary.

Based on the percentage of the larvae, three (03) dominant families were identified as Clupeidae, Engraulidae and Ambassidae. Photichthyidae, Myctophidae, Bregmacerotidae, Gonostomatidae, Callionymidae, and Carangidae were identified as abundant families in the Bay of Bengal by Lirdwitayaprasit et al. (2008). The four most prevalent families were identified by Chesalina et al. (2013) as Sparidae, Scombridae, Clupeidae, and Nemipteridae. In the Pendas River Estuary, Peninsular Malaysia, Clupeidae was found to be the most numerous family, followed by Blenniidae, Teraponidae, Gobiidae, Sillaginidae, Nemipteridae, and Mullidae.

5.2 Temporal distribution and diversity indices

The abundance of fish larvae was greatest in May to August, during the mid-summer to mid-rainy monsoon season. However, the majority of the fish families were recorded in

April. In October, during the monsoon season, the highest Shannon-Wiener index (H) was recorded at 0.901. In June, September, and December, the lowest value, zero (0), was discovered. As the H value rises, so does the diversity of the families in a particular sample. The population is less diversified the lower the value of H. One family only exists in a sample with an H value of 0. Richness, on the other hand, peaked in April, while evenness peaked in January. Pielou's evenness index was 1.408 while Margalef's greatest richness was 0.806. The range of Margalef's richness index is zero. Pielou's evenness is a marker for both species richness and variety. Family evenness refers to the number of members of each family present, whereas family richness refers to the number of distinct families present in a given area. The projected value for Pielou's evenness ranges from 0 (no evenness) to 1. (Complete evenness). Since Pielou's evenness is calculated by dividing the Shannon-Wiener index by the total number of families, it is related to the Shannon-Wiener index. For the months of June, September, and December in this study, the evenness index is zero (zero), signifying that there is no evenness.

The Shannon-Wiener index found in this study was consistent with that found by Arshad et al. (2012) at the Pendas River Estuary, which showed significant change over the monsoon and intermonsoon seasons. But in this estuary, family wealth surged between March and May.

Margalef's richness ranged from 0.71 (May) to 0.91 in this study, which also showed similarities to Brinda et al. (2010) (March). The evenness index for Pielou ranged from 0.71 in May to 0.93 in April. At the Velar Estuary, which is located on India's Southeast coast, the study was carried out at three different locations.

5.3 Spawning season

Fish spawning season is responsible for the presence of fish larvae. Fish often go through a planktonic phase that lasts a few weeks to a few months (Victor, 1986; Brothers et al., 1983). The ability to adapt and the impact of spawning patterns on yearly strength for all physical and biological processes can be understood by examining temporal and geographic patterns, as well as the availability of fish larvae in relation to oceanographic conditions (Somarakis et al., 2002). However, histological examination was the main focus of the majority of studies on fish spawning season. The spawning seasons are

divided into five (05) separate groups in this study: Summer, Winter, Pre Monsoon, Monsoon and Post Monsoon. As the monsoon season in Bangladesh lengthens, it has been separated into three distinct sub-seasons: pre-monsoon, monsoon, and post-monsoon. Majority of the families (04) spawn in early summer season, which means they reproduce from March to May and their larvae were found between May-July in this estuary. In winter, pre monsoon and monsoon, three (03) families were found to use Naf river estuary as nursing ground. Only three (03) families were post monsoon spawners.

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-6: Conclusions

The Naf River Estuary's distribution and larval composition varied depending on the season. On the basis of their morpho-meristic traits, 08 families were found among the 1681 collected larvae. The most larvae and families were found in the month of August, which also had the most individuals. The post-monsoon season indicated the highest Shannon-Wiener index, whereas the winter indicated the highest family richness and evenness. It was discovered that in contrast to other estuaries, this one did not have as many families as was anticipated. The most frequent family was Clupeidae, followed by Engraulidae and Ambassidae. *Tenualosa ilisha*, *T. toli*, *Ambassis dussumieri*, *Stolephorus indicus*, and *S. insularis* are only a few of the commercially significant fish species that belong to these three families. Due to favorable environmental factors, particularly salinity, several fish groups may spawn. As a result, the Naf estuary serves as a nursing ground for numerous families. The frequency of larvae also allowed for the identification of the spawning season. The discovery of Clupeidae and Ambassidae larvae in seven separate months suggests that their spawning seasons are the Summer, Pre-Monsoon, Monsoon and Post-Monsoon. In total, four (04) families were found to be early summer spawners, suggesting that the Summer (April to August) is the Naf River Estuary's most fruitful season.

Chapter 7: Recommendations and Future perspectives

According to this research work, the following recommendations maybe done:

- The responsible authority should keep an eye on the physical, chemical, and biological aspects of the Naf River Estuary.
- The responsible authority should take the appropriate action to improve the ecological state in conjunction with the commercial and tourism activity in the area of Teknaf.
- To increase the productivity of the Naf River, more research should be done to identify various components of fishing.
- People should be made aware of the significance of the Naf River by responsible authorities.
- To maintain ecological equilibrium, the general public should be informed about the use of illegal fishing gear and overexploitation.
- The dumping of oil and trash by ships along the Naf River should be under the supervision of the appropriate authorities.

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Appendices

Month	Family	Count	Total
March	Ambassidae	4	5
	Carangidae	1	
April	Ambassidae	1	42
	Sillaginidae	1	
	Clupeidae	39	
	Gobiidae	1	
May	Clupeidae	321	322
	Sciaenidae	1	
June	Clupeidae	13	13
July	Clupeidae	205	214
	Ambassidae	9	
August	Ambassidae	302	704
	Engraulidae	402	
September	Engraulidae	82	82
October	Engraulidae	55	90
	Blenniidae	10	
	Unidentified	25	
November	Engraulidae	6	28
	Blenniidae	2	
	Clupeidae	20	
December	Clupeidae	145	145
January	Engraulidae	8	14
	Ambassidae	6	
February	Clupeidae	14	22
	Blenniidae	2	
	Sillaginidae	6	

Appendix A: Temporal Abundance of Fish Larvae at Naf River Estuary

Family	Individual
Clupeidae	757
Engraulidae	553
Ambassidae	322
Blenniidae	14
Sillaginidae	7
Gobiidae	1
Carangidae	1
Sciaenidae	1
Unidentified	25
Total	1681

Appendix B: Total Identified families and Individual of Naf River Estuary

Month	Diversity index	Richness	Evenness
Mar	0.5	0.621	0.722
Apr	0.336	0.806	0.242
May	0.021	0.173	0.03
Jun	0	0	0
Jul	0.174	0.186	0.252
Aug	0.683	0.153	0.985
Sep	0	0	0
Oct	0.901	0.444	0.82
Nov	0.759	0.6	0.691
Dec	0	0	0
Jan	0.726	0.379	1.408
Feb	0.86	0.647	0.783

Appendix C: Diversity indices of each month at Naf River Estuary

Family	Frequency of occurrence	Spawning month	Spawning season
Clupeidae	Feb, Apr-Jul, Nov, Dec	Jan, Mar-Jun, Oct, Nov	Mid-Winter, Summer, Late Rainy Monsoon, Early Winter
Engraulidae	Jan, Aug-Nov	Dec, Jul-Oct	Mid-Winter, Rainy Monsoon
Ambassidae	Mar, Apr, Jul, Aug	Feb, Mar, Jun, Jul	Late-Winter, Early Summer, Late Summer, Early Rainy Monsoon
Blenniidae	Feb, Oct, Nov	Jan, Sep, Oct	Mid-Winter, Mid & Late Rainy Monsoon
Sillaginidae	Feb, Apr	Jan, Mar	Mid-Winter, Early Summer
Gobiidae	Apr	Mar	Early Summer
Carangidae	Mar	Feb	Late Winter
Sciaenidae	May	Apr	Mid-Summer

Appendix D: Larval frequency and Spawning season of the identified families at Naf River Estuary

Family	Total number of larvae (larvae/1000 m ³)	Mean number of larvae	SD	Percentage of total catch	Frequency of Occurrence	Classification according to Constance of occurrence		
						1	2	3
Clupeidae	757	63.08	112.04	45.033	58.33	*		
Engraulidae	553	46.08	125.47	32.897	41.67		*	
Ambassidae	322	26.83	95.05	19.155	41.67		*	
Blenniidae	14	1.17	3.16	0.833	25.00		*	
Sillaginidae	7	0.58	0.32	0.416	16.67			*
Gobiidae	1	0.08	0.32	0.059	8.33			*
Carangidae	1	0.08	0.32	0.059	8.33			*
Sciaenidae	1	0.08	0.32	0.059	8.33			*
Unidentified	25	2.08	7.91	1.487				

1. Constants;
2. Accessories;
3. Accidental.

Appendix E: Statistical analysis of marine larvae at Naf River Estuary

Brief Biography of the Author

Faijabul Afridi Fahim was born in Cox's Bazar and grew up in Chattogram. He passed the Secondary School Certificate Examination in 2013 and Higher Secondary Certificate Examination in 2015. He graduated in 2020 from the Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram. Now, he is a candidate for the degree of MS in Fisheries Resource Management under the Department of Fisheries Resource Management, Faculty of Fisheries, CVASU. He wants to be a successful researcher based on what's going on inside Cox's Bazar-Teknaf coastline.