EFFECT OF ACIDIFICATION ON THE ABUNDANCE OF FISH LARVAE IN REZU KHAL ESTUARY, COX'S BAZAR COASTS, BANGLADESH

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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The Author

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ABBREVIATION

OA	Ocean Acidification
CO_2	Carbon Dioxide
pCO_2	Partial Pressure of Carbon Dioxide
CO_3^{2-}	Carbonate Ion
$CaCO_3$	Calcium Carbonate
BOB	Bay of Bengal
DIC	Dissolved Inorganic Carbon
ppm	Parts per million
Yr-1	Per Year
A_T	Total Alkalinity
mg/l	Milligram per litter
mg	Milligram
kg	Kilogram
µm	Micrometer
µatm	Micro atmosphere
°C	Degree Celsius
DO	Dissolve oxygen
PSU	Practical salinity units
Ω	Omega
i.e	That is
et al	Associates

Abstract

These studies mainly focus on to understand how ocean acidification concise recently hatched larvae stocks, their growth, recruitment and mortality rate that influence larvae abundance. This study was carried out from January to December 2021, in the Rezu khal Estuary, Cox's Bazar which is one of the important estuarine systems in Bangladesh. Different fish larvae were collected by using Bongo net (500µm) which was being towed from bottom to surface. During sampling period, a total of 1,511 larvae were found, mean density was 125 /1000 m^3 of fish larvae observed in laboratory. The average range of hydrological parameter were found 27.65^0C for surface water temperature, 8.37 for pH, 104.17 mg/l for alkalinity, 23.98 psu for salinity, which were within ideal range during study period. Ocean acidification factor including pCO_2, HCO_3^-, CO_3^{2-}, DIC^-, $\Omega_{Aragonite}$, $\Omega_{Calcite}$ were also determined by using "Seacarb" package of R programming language and validated from CO_2SYS software for CO_2 system calculation. The mean value of ocean acidification factor such as $pCO_{2,}$ HCO_3^- ,CO_3^{2-},DIC^-, $\Omega_{Aragonite}$, $\Omega_{Calcite}$ was recorded 82.94 µatm,.00065 mol/kg, .000125 mol/kg, .000808 mol/kg, 2.3364 and 3.6593 respectively. The results showed an insignificant relationship among pCO_2, pH and larvae abundance in Rezu Khal. Larvae abundance was positively correlated with pH and negatively correlated with pCO_2. However, there was a strong negative correlation between pCO_2 and pH. Using these outputs, impact of ocean acidification on fish larval abundance can easily be identified. This research will help decision makers to take appropriate decisions on reducing the degree of acidification in Cox's Bazar coasts of the Bay of Bengal.

Keywords: ocean acidification; larvae abundance; pCO_2; pH; Rezu Khal estuary

Chapter 1

Introduction

1.1 Background

Ocean acidification generally occurs when acidity of seawater increase or pH become low due to breakage of elevated CO_2 in the atmosphere (Feely et al., 2008). Carbon dioxide from the atmosphere assimilate to the dissolve inorganic carbon (DIC^-) propagate from the filth of dissolved organic matter, primary producer (Mostofa et al., 2013), leekage from ocean floor (Taylor et al., 2014), volcanic vent situated in superficial submarine zones (Claessens et al., 2013), methane oxidize in anaerobic condition (Haroon et al., 2013) and oxidation of sulphide mixed with carbonate solution in sea water (Torres et al., 2014). Anthropogenic behavior likely burning of fossil fuel, land use for prolonged period (Le Quéré et al., 2009), deforestation because of increasing population (La pola et al., 2014) are mainly responsible for increasing of CO_2 in the atmosphere. In addition, there could be major contributions as of usual sources for example decaying of dead plant material (King et al., 2012), magmatic eruptions from earth crust (Hall-Spencer et al., 2008), respiration increase in organic matter of soil due to global warming (Knorr et al., 2005).

Acidification of ocean is to blame for variation into carbonate structure of ocean, with possessions of ongoing influence of CO_2, dissolved inorganic carbon, lower pH, alkalinity, and carbonate ions in diffusion condition (Beaufort et al., 2011). Hence, degree and outcome of acidification may enhanced with a number of several process, among them impacts of native environment as well as global or riverine runoff (Bauer et al., 2013), utilization of land practices through modification (Lapola et al., 2014), acid rain in atmospheric (Baker et al., 2007) which are consider some exciting factor. Another consequence can be described by increased mineralization of dissolved organic matter as well as Primary Producer such as phytoplankton due to global warming (Mostofa et al., 2013). Maximum mentioned impact were likely near basis of eutrophication or algal blooms which occur in coastal salt water, that has an effect on cycling of carbon as well as carbonate chemistry which generally control process of acidification (Bauer et al., 2013). Variation in carbonate structure of ocean water occur due to acidification(Beaufort et al., 2011) that has impacts on breathing organisms as well as associated process of ecosystem services (Mostofa et al., 2013). Bearing in mind the probable shocking impact of marine ecosystems, living organisms as well as ecosystem services, it is necessary to understand every possible reason of acidification and interrelation among them (Cooley et al., 2009).Ocean acidification is taking place all over the oceans globally due to liberate of terrestrial CO_2 in the environment, along with anthropogenic activity add around one-third of CO_2 in ocean water (Sabine et al., 2004). Addition of CO_2 in water lowers the pH concentration by reducing the available use of carbonate ions in sea water. Therefore, primary production and limited flow from fields determined CO_2 concentrations and pH in ocean water (Mc Elhany & Busch, 2013), seas which has elevated-latitude are more subjected to the addition of increasing temperatures and acidification (Fabry et al., 2009).

Acidification of ocean is documented an imperative driven factor to alter natural process (Doney et al., 2012). Most of the research focuses on the effect of ocean acidification on calcifying invertebrate specially Cnidaria and Mollusk (Heuer & Grosell, 2014) because they are highly dependent on calcium carbonate (Orr et al., 2005). The premature living stages have studied greatly because they are more vulnerable to rising pCO_2, because of their inadequate physiological system (Brauner, 2008). Outcome take into account instability of sensory organ as well as neurological function such as olfaction (Dixson et al., 2010), vision power (Chung et al., 2014), lateralization (Domenici et al., 2012), hearing (Simpson et al., 2011), learning (Ferrari et al., 2012), and activity levels (Munday et al., 2010). This instability has a considerable influence on interrelation between predator and prey (Ferrari et al., 2012) and can eventually bring about greater mortality rates than before (Munday et al., 2010). Swimming power boasts up survival rate because these help to find out food get off from predators as well as influence distribution (Leis, 2006). Survival rate of fish larvae greatly influence by variation in growth as well as maturity (Houde, 1997), which may have linkage to changed metabolic requirement or distribution of metabolic energy demanding situation (Matson et al., 2012). The distribution of metabolic resources to keep away inner stress or injury might be challenging for larvae resulting growth, activeness, maturity, survival that has influence on population, communities or ecosystem (Cunha et al., 2007).

Environment deviation is recognized to control productivity in a lot of marine fish larvae. Some of the researchers suggest that increased temperature for prolonged period may have influence on reduction of fish stock (Cheung et al., 2012). It is predicted that acidification can reduce the production of larvae in various organism (Cooley & Doney, 2009). Some evidence suggests that increasing CO_2, decreasing pH in ocean water can alter the growth maturity in various marine organisms (Fabry et al., 2008). Generally, calcifying invertebrates are less susceptible to ocean acidification than fishes (Melzner et al., 2009). Though, fish larvae are more vulnerable to atmospheric pH as they have tendency to remain in surface water as well as necessity of developed mechanisms for maintenance of acid base balance (Kikkawa et al., 2003). Study have acknowledged that increasing CO_2 is responsible for higher mortality rate (Baumann et al., 2012), enhance morphological deformity (Frommel et al., 2012), neurological disfunction of larvae (Devine et al., 2011). Since, survival rates during embryonic development are low and be likely to go for beside smaller addition of individuals in fish abundance or less variation in premature growth has gradual influence (Hurst et al., 2010) which drive the recruitment rates of individuals in a population stock (Houde & Hoyt, 1987).

Ocean acidification is measured as a key hazard to lots of marine species because it hampers many physiological functions such as alteration of acid-base balance, biosynthesis of protein or metabolic activity (Portner et al., 2004; Rosa et al., 2013). Therefore, increasing CO_2 is considered harmful for recruitment, development (Baumann et al., 2012), as well as ecological behavior of various marine organism (Munday et al., 2009). Introduction to elevated CO_2 in atmosphere has influence on marine calcifying species but availability of carbonate ions in water which is necessary for calcification process has been reduces (Fabry et al., 2008). To avoid hypercapnic situation, fish have evolve the power to build up bicarbonate and interchange bicarbonate ions through gills (Portner et al., 2005), osmoregulatory functions and balancing of internal pH has not been fully developed in newly hatched larvae (Baumann et al., 2012; Frommel et al., 2012). Premature fish larvae are more susceptible to present atmospheric condition as well as their ultimate failure to manage or adjust might comprise lower perseverance of marine species (Rosa et al., 2012).

The study area Rezu Khal estuary on the south eastern coast of BD represents typical subtropical estuaries which are regarded as an important estuarine system both ecologically and economically. The extreme and varying environmental gradients of estuary favour a variety of fish species through various physic-chemical features and tropic structures. These ecosystems offer protection not only for resident species but also for a wide range of marine and fresh water species which migrate to the estuaries at certain stage of their life cycle. The distinct characters of estuary is the low fish diversity associated with high abundance of individual species, the majority of which show broad tolerance limits for hydrobiological factors.

1.2 Significance of the research

From ecological point of view, Rezu khal is a major river of Ukhia under Cox's Bazar district, contributed significantly as nursery grounds, providing numerous economically emerging species, plenty of food and safe areas. Figuring out how acidification influences marine fish larvae is necessary since it has the potential to have adverse affects on fish populations and the economies of people who rely on them. This research will help decision makers to take appropriate decisions on reducing the degree of acidification in Cox’s Bazar coasts of the Bay of Bengal.

1.3 Objectives of the research

1. To provide an actual scenario of acidification in Rezu Khal estuary of Cox’s bazar coast along with the deviations of water quality parameters.

2. To identify the impact of acidification on fish larvae abundance at premature stage in Rezu khal estuary.

Chapter 2

Review of literature

Sarma et al., (2015) observed on ocean acidity and carbon dioxide exchange patterns in the coastal Bay of Bengal and found that in 1991 compared to 2011, the Bay of Bengal was categorized being more basic, clearer and having lower pCO_2. In addition, rates in the northwestern (NW) coast were observed to be 3-5 times higher. The rates of pH decreasing, DIC increase, and pCO_2 per year were all similar with global trends in the southwestern (SW) coast.

Sridevi and Sarma (2021) studied on role of river discharge and warming on acidification of the oceans and pCO_2 levels in the Bay of Bengal, found that increasing freshwater input and atmospheric pollutant accumulation were the dominant controlling factors on oceans pH and pCO_2 in the BoB between 1998 and 2015 and that the area is actually acting as a stronger sink for atmospheric CO_2 than it did in the previous two decades.

Rajalakshmi & Achyuthan (2021) observed that climate change in the Bay of Bengal, cyclone strength has increased over the past 20 years. Several fish species, a major constituent of the coastal food chain, are now susceptible to its productivity due to sea acidity and an elevation in sea surface temperature.

Sarma et al., (2021) studied on increased percentages of acidification due to atmospheric pollution accumulation in the coastal Bay of Bengal, this has been recorded that a decrease in pH over the past 10 years is accompanied by a rise in aerosol optical depth (AOD), total dissolved particles (TSP) in the environment, and concentrations of sulfate and nitrate in TSP.

Melzner et al., (2020) studied on how ocean acidification influences mechanism of marine invertebrates and try to highlights links behind these disturbances of organisms' functions. Coastal environment are subjected to acidification that causes many disfunctions in marine species that reduces their survivability, growth and recruitment.

Baumann et al., (2012) studied on reduced early life growth and survival in a fish which has direct response to increased carbon dioxide and find that the early life stages of a common estuarine fish (*Menidia beryllina*) were severely affected by CO_2 concentrations projected in the world's bodies of water later this century. Compared to the post-hatch larval stage, the egg stage was significantly more vulnerable to increased CO_2-induced mortality.

Munday et al., (2011) conduct experiment on the effect of ocean acidification on the growth of otoliths in tropical marine fish larvae. Results reveal that sensitivity varies greatly among species and support the notion that pH regulation in the otolith endolymph may cause an increase in $CaCO_3$ precipitation in the otoliths of larval fish exposed to elevated CO_2.

Hurst et al., (2013) studied on impacts of ocean acidification on walleye pollock (*Theragra chalcogramma*) hatching sizes , larval growth and reveals that there were only minor effects of CO_2 level on size and growth rate, but fish in the ambient treatments tended to be slightly smaller than fish reared at elevated CO_2 levels. These finding suggest that the early life stages of walleye pollock are resilient to the direct physiological effects of ocean acidification in terms of its growth potential.

Kroeker et al., (2013) studied on impacts of ocean acidification on marine organisms: quantification of sensitivity and interaction with warming. The findings show an enhanced sensitivity of mollusk larvae but show that an enhanced sensitivity of early life cycle stages is not widespread across all taxonomic groupings.

Mostofa et al., (2016) reviews and syntheses the effects of ocean acidification on marine ecosystems together with their potential negative effects on marine organisms, ecosystem services and processes and suggest that marine environment are highly affected by increasing acidity that increase the mortality rate of fish larvae, specially invertebrates.

Munday et al., (2009) studied on increasing CO_2 and the impacts of ocean acidification on a tropical marine fish's early developmental cycle which show that reduced pH had no influence on the maximum swimming rate of larvae in the settlement stage. From these studies, the growth and productivity of larvae from fish that spawn in water bottom are significantly impaired by rates of ocean acidification that are likely to be experienced in the near future.

Rossi et al., (2015) studied on the rise in larval fish development caused by ocean acidification also reveals that there is still a gap between ontogenetic growth and the time of orientation behaviors, which may creates difficulties for larvae to find habitat or causes settlement in unfavorable habitat.

Silva et al., (2016) studied on impacts of ocean acidification on sand melt larvae's abilities to swim, develop, and act biochemically and found that swimming speed was unaffected by treatment, but exposure to higher pCO_2 levels generates big larvae in high pCO_2 treatment and small larvae in medium pCO_2 treatment, which lead to higher energetic costs.

Munday et al., (2010) conducted experiment on ocean acidification that poses a threat on replenishing fish populations. At 700 ppm CO_2, there was indication of altered larvae behavior, with many of these attracted to the predators' odour.The capacity to detect predators was entirely compromised at 850 ppm CO_2.In their natural habitat on coral reefs, larvae exposed to higher levels of CO_2 were much more active & showed risky behavior's per the findings, adding additional CO_2 to the ocean will reduce reproduction efficiency and have profound impacts on the sustainability of fish stocks.

Ishimatsu et al., (2005) studied on effects of a high CO_2 atmosphere on the physiological functions of fish and reveals that hypercapnia has a direct effect on vital physiological functions like respiration, circulation, and metabolism. Adjustments to these mechanisms are likely to slow growth and population size through failure to reproduce and alter geographical distribution since fish avoid high-CO_2 waters or swim less actively.

Chapter 3

Materials and Methods

In any research, methodology is an indispensable and integral part. The acceptability of a scientific research results depends to a great intent on the appropriate methodology. This part deals with the methods that were followed and materials that were used to achieve the objectives of the study. In this study a scientific and logical methodology has been followed by the researcher, the following steps shown in Fig 3.1 were persuaded in order to achieve the goals of this research.

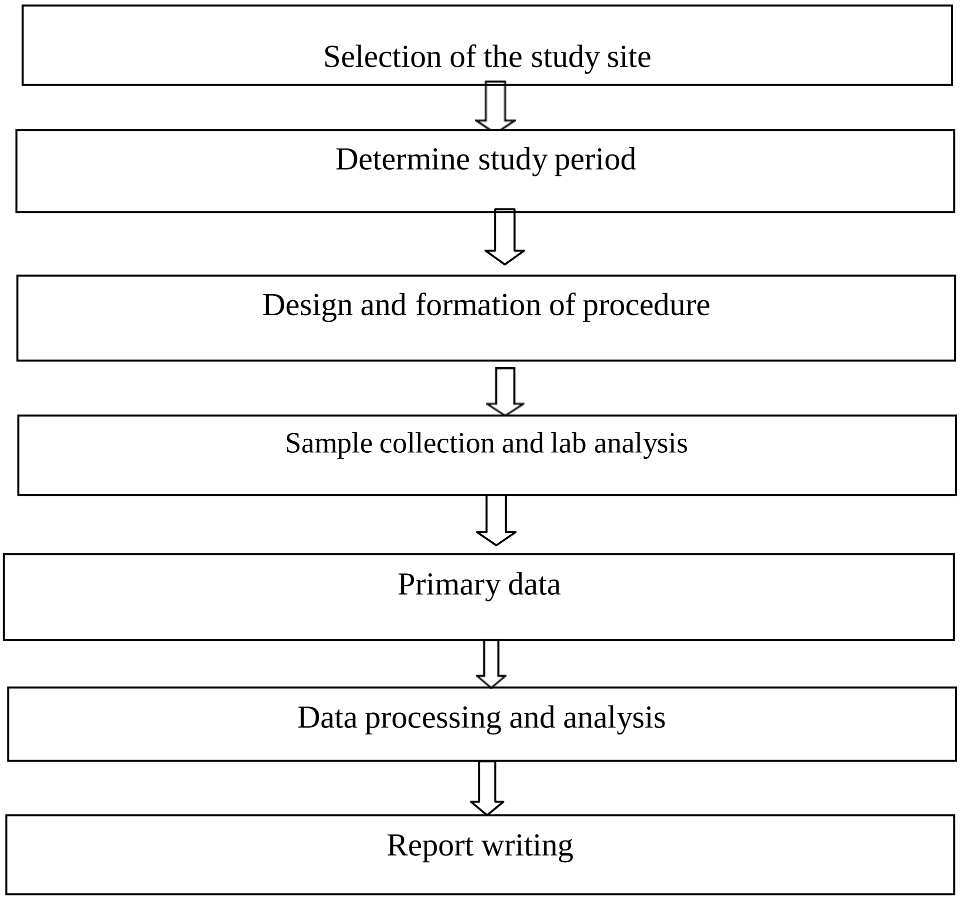

Fig 3.1: Methodology followed for the study

3.1) Study site

To estimate the relationship between larval abundance and ocean acidification, sampling site Rezu khal-Cox's Bazar coast of Bay of Bengal, was selected considering river discharge and other parameters by using GPS, Arc GIS geographical mapping software.

3.2) Locations of Sampling Station

In Cox's Bazar, Bangladesh, water samples and larvae were collected from Rezu khal Estuary (21° 22′ to 21° 15′ N and 92° 4′ to 92° 12′ E).

3.3) Study periods

This research was conducted in Cox's Bazar coasts, Bangladesh with continuous monthly sampling from January to December 2021 at the Rezu Khal estuary. This research station covered estuarine environments shown in Fig 3.2.

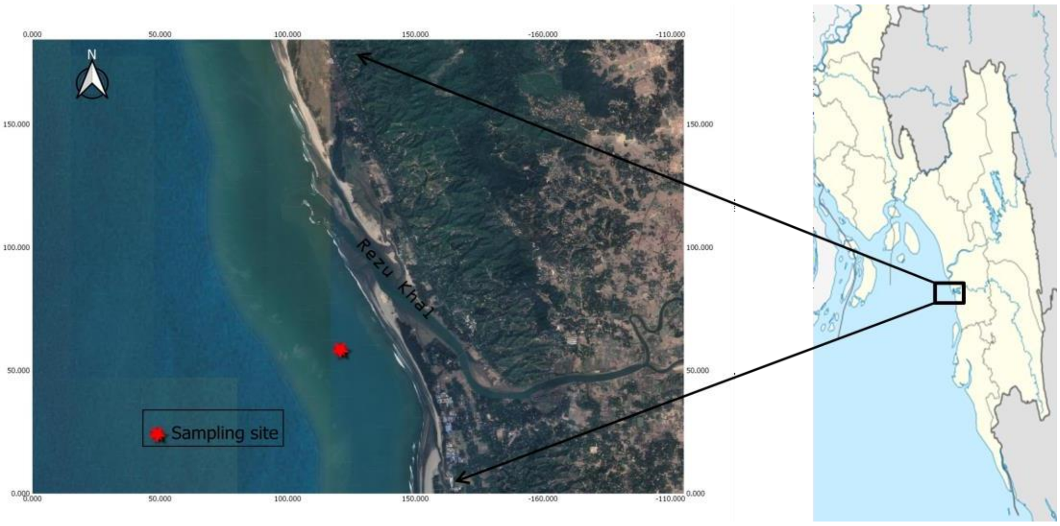

Figure 3.2: Locations of sampling stations, Cox's Bazar coasts, Bangladesh.

3.4) Sampling Procedure

Samples were collected in each month of research time frame. Water hydrological characteristics (temperature, salinity, pH and alkalinity) and larvae abundance have been measured and recorded for assessing potential impacts on larval abundance due to changes of acidification at the research station.

3.5) Determination of water quality parameter

Necessary equipment such as pH meter, thermometer, refractometer etc.were carried during sampling time. Temperature, pH, salinity were recorded on the spot. Alkalinity was measured in aquatic lab, Faculty of Fisheries, CVASU. For collecting sea water and preventing air bubble, gas tight bottle was used.

3.5.1) Water temperature

A Celsius thermometer had been used to assess the water's temperature.

3.5.2) pH

Using a pH meter (Model YSI pH 1000A), pH was measured.

3.5.3) Salinity

Salinity was measured by using refractometer meter (Model PC Stester 35).

3.5.4) Determination of Alkalinity (Titrimetric Method)

- ✓ At first, sample was collected from the surface of the water body.
- ✓ In the conical flask, 50 ml volume of water was taken.
- ✓ Then, the conical flask received 2 drops of phenolphthalein.
- ✓ After that burette was filled by sulphuric acid (0.02N H_2SO_4).
- ✓ After that the content was titrated with Sulfuric acid until the color became colorless.
- ✓ Afterwards, the solution got 2 droplets of mixed indicator.
- ✓ After that the content was again titrated with sulphuric acid &continued until the color turned red.
- ✓ At last, total alkalinity was measured and recorded.

Final result was calculated by the following formula:

Alkalinity= {Acid used (ml) ×.02 N (Normality of acid) ×50(Gram equivalent weight of $CaCO_3$) × 1000} ÷ sample volume

3.6) Study on fish larvae

3.6.1) Sample collection

Bongo Net (mouth diameter: 0.50 m, length: 1.3 m, mesh size: 500µm at body and cod end) was used to collect fish larvae. The volume of seawater filtered during each tow was recorded using a flow meter (Model: KC Denmark A/S 23.090-23.091) attached to the mouth of the net. The sampling period extends around 10 minute's surface tow-in daylight. The collected specimen was preserved in 90% ethanol and transported to the Aquatic Ecology Lab, CVASU for sorting out based on morphology and other attributes.

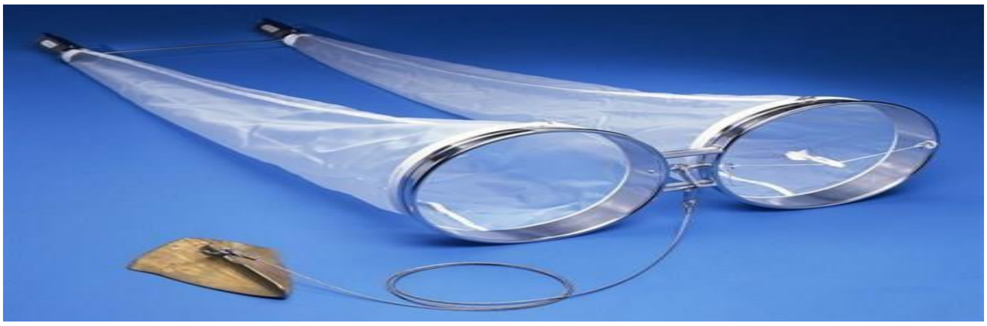

Figure 3.3: Bongo Net

3.6.2) Fish larvae sorting

Larvae were typically sorted from the entire sample for taxonomic classification. Removing alcohol from the mixture was the first step in sorting. To do this, samples were thoroughly rinsed with tap water and sieved through meshes of 0.1 mm to easily remove sand particles, plastic, twigs, as well as other unwanted material. Cleaned larvae then put in a bottle with new alcohol.

3.6.3) Sample identification

Each specimen was placed one at a period in a petri dish for observation below a stereo microscope (OPTICA Microscope ITALY C-B3) at different magnifications (10x) and picture of identified fish larvae were taken using a desktop (Win pad 10, 1 HD-WPU 10). To make it simple to locate images afterwards, each was given a different code.

3.6.4) Sample counting

Depending on the proportion of fish larvae detected per 1,000 m^3 of filtering sea water, the samples were calibrated. The following formula (Lirdwitayaprasit et al., 2008) was used to measure the number of fish larvae**:**

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

Water volume passed in each sampling=Indicated number of revolutions× Pitch of the impeller (0.3) ×Opening area of net (m^2) ×1000

The number of revolutions associated with a tow was 1988 (noted from the Digital Flow Meter attached to the net). The water volume passed through the plankton net was:

Volume= 1988×0.3×0.3927×1000

= 126615.72L

$=126.62m^3$

Number of larvae per 1000 m^3 = (Number of larvae in sample×1000) ÷Water volume passed

Larvae were counted from a sample of Rezu khal estuary in November. The volume of water passed in that sampling was 126.62 m^3.

So, the number of larvae per 1000 m^3 = (35×1000) ÷126.62m^3

= 27.6417 ≈ 28

3.7) Determination of ocean acidification factors

Salinity, temperature, and two of the four CO_2 related parameters, comprising whole dissolved inorganic carbon (DIC^-), total hardness (A_T), H^+ concentrations or pCO_2 could be used to determine the concentrations of acid-base species in seawater. Ocean acidification parameters were deduced using R programming language and validated from CO_2SYS software which was designed by Lewis and Wallace for CO_2 system calculation (1998). The input parameters for "seacarb" package of R programming were: pH, A_T, salinity, temperature.

3.8) Data collection and analysis

The data on water quality parameter, acidification factor and total larvae counting was recorded. Collected data have been classified, summarized & analyzed by programming language R (Version 3.6.3), SPSS (Version 22.0) and Microsoft Excel (Version 2007).

3.9) Report writing

Final steps for conducting this research were to make an overall report where all data was included sequentially and statically.

Photo gallery

Plate 3.1: Sample of fish larvae

Plate 3.2: Sorting of fish larvae

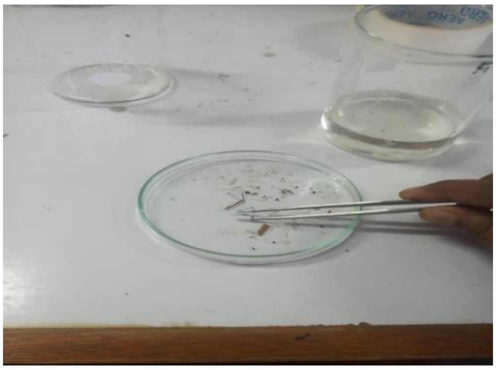

Plate 3.3: Counting of fish larvae

Plate 3.4: Labeling of fish larvae

Plate 3.5: Micoscopic observation of fish larvae

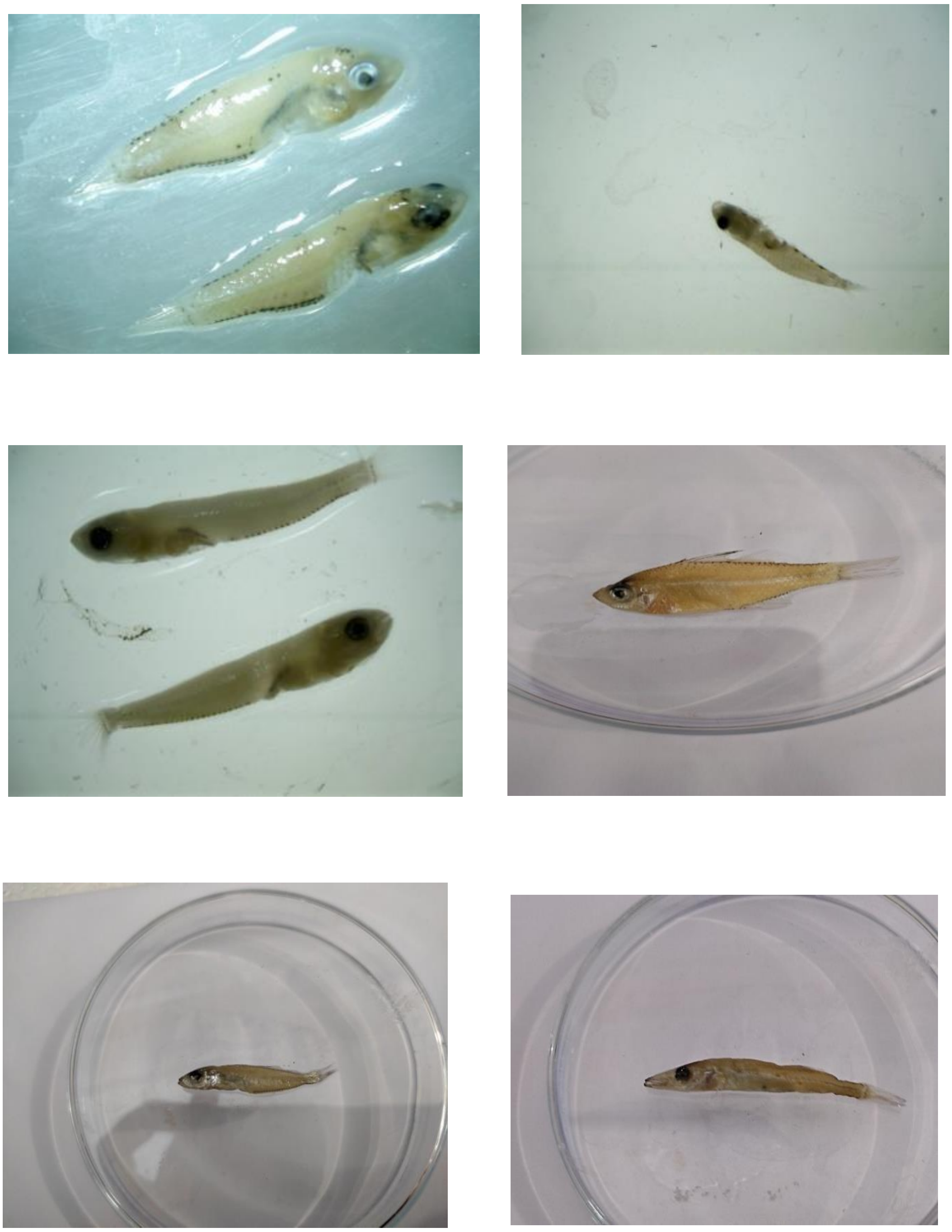

Plate 3.6: Some identified fish larvae

Chapter 4

Results

The results of hydrological parameter of water, acidification factor and larvae abundance of sampling station are shown in graph.

4.1) Water Parameters

4.1.1) Temperature

The temperature (^{0}c) was recorded 22.7, 26.6, 26.2, 28.3, 30, 26.3, 29.1, 28.4, 30.4, 28.9, 30 and 24.9 from January to December respectively. Highest temperature (30.4^{0}C) was recorded in September. The lowest temperature was (22.7^{0}C) recorded in January. The mean temperature was 27.65±2.3423^{0}c. Monthly variation of temperature is shown in Fig 4.1.

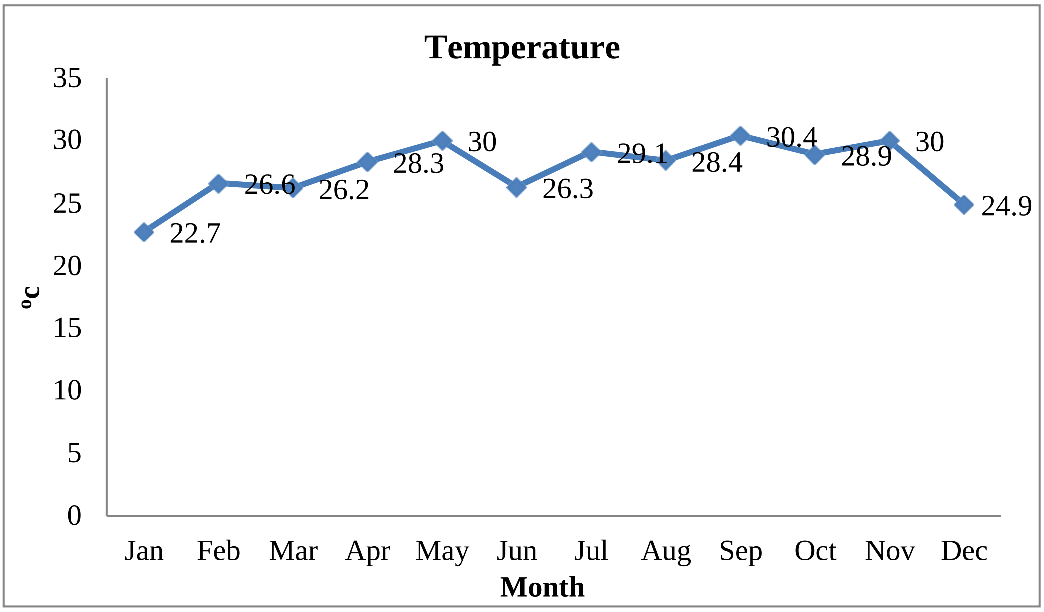

Fig 4.1: Monthly variation of temperature in Rezu Khal

4.1.2) pH

The value of pH was recorded 8.4, 8.5, 8.3, 8.2, 8.2, 8.3, 8.1, 8.8, 8.6, 8.3, 8.3 and 8.5 from January to December respectively. Highest pH was (8.8) recorded in August. Lowest pH was (8.1) recorded in July. The mean value was 8.3± .19598. Monthly variation of pH is shown in Fig 4.2.

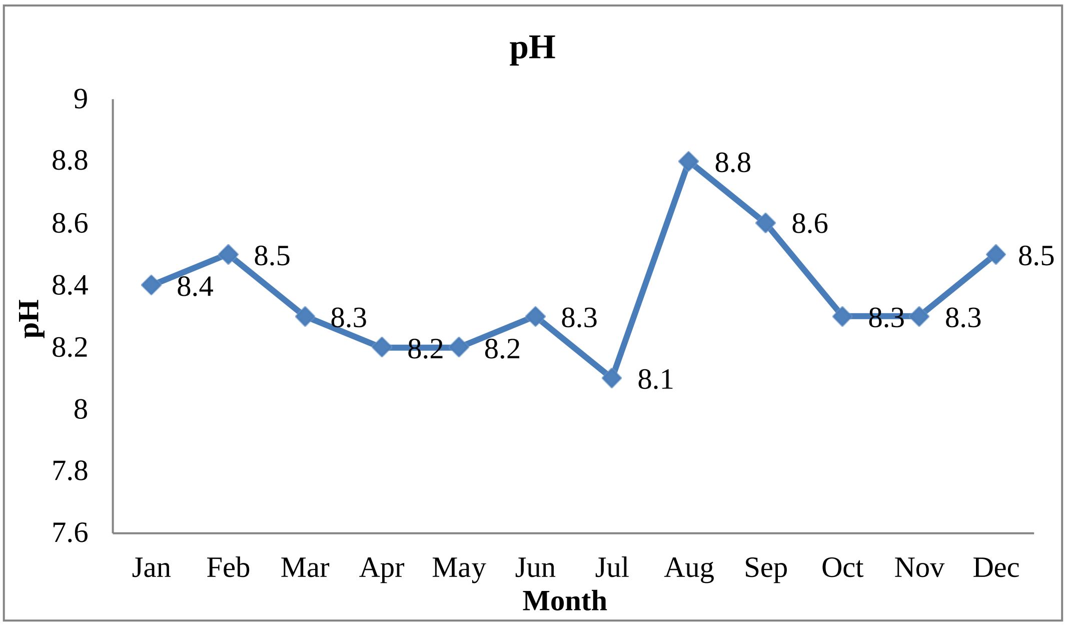

Fig 4.2: Monthly variation of pH in Rezu Khal

4.1.3) Alkalinity

The value of alkalinity (mg/l) was recorded 138 , 120 , 110 , 110 , 109 , 100 , 82 , 65 , 78 , 92 , 108 and 138 from January to December respectively. Highest alkalinity was (138 mg/l) recorded in January and December. Lowest alkalinity was (65mg/l) recorded in August. The mean value was 104.17 ±22.405 mg/l. Monthly variation of alkalinity is shown in Fig 4.3.

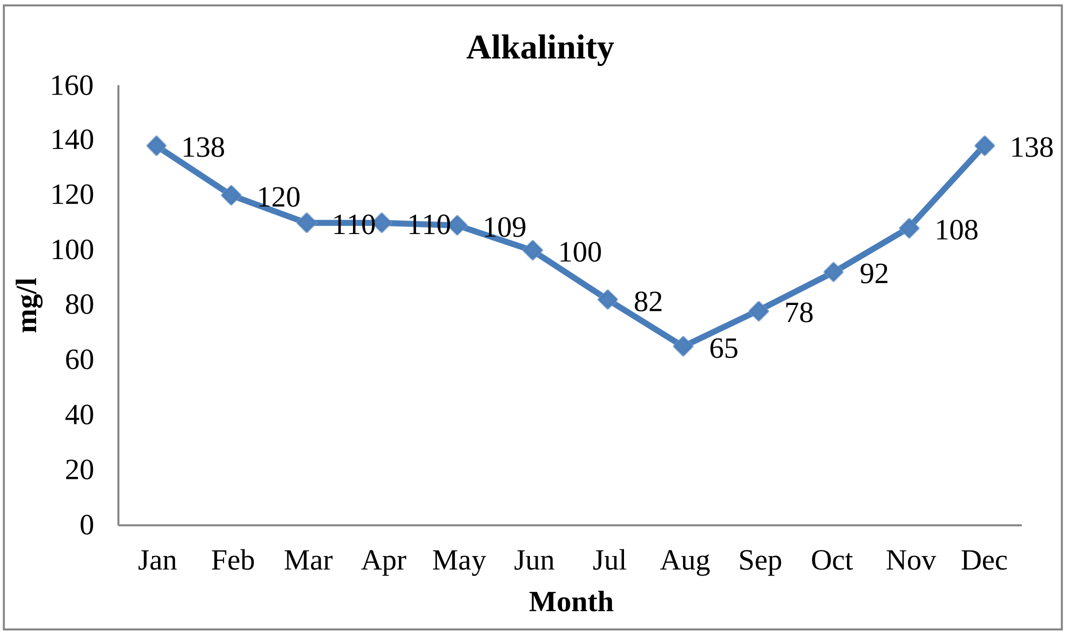

Fig 4.3: Monthly variation of alkalinity in Rezu Khal

4.1.4) Salinity

The value of salinity (psu) was recorded 28.1 , 25 , 24.7 , 27 , 28 , 21 , 22 , 14 , 13 , 25 , 30 and 30 from January to December respectively. Highest salinity was (30 psu) recorded in November and December. Lowest salinity was (13 psu) recorded in September. The mean value was 23.98 ±5.6432 psu. Monthly variation of salinity is shown in Fig 4.4.

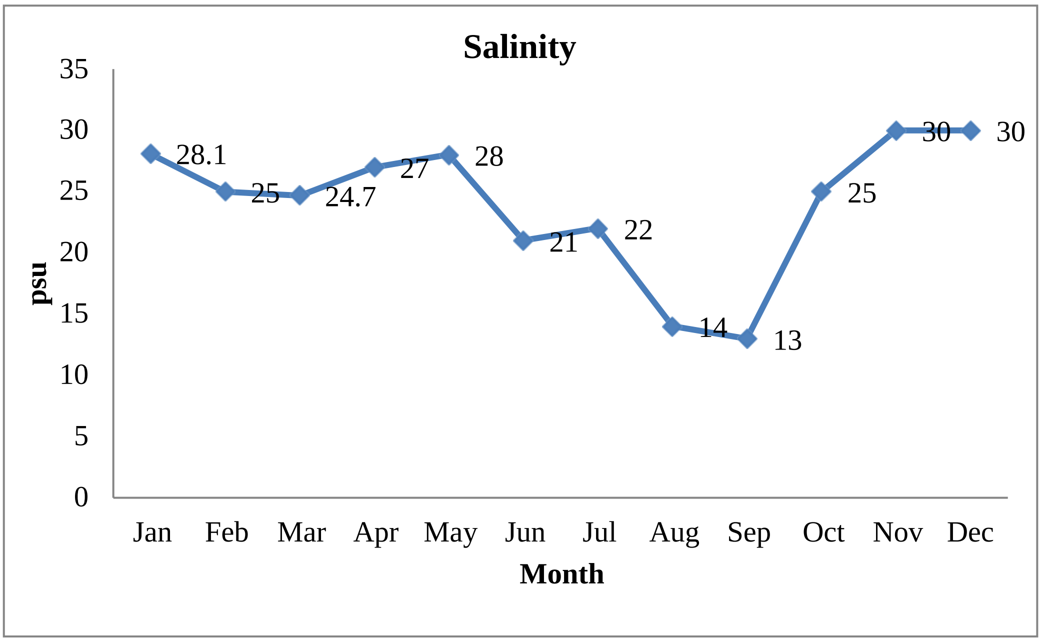

Fig 4.4: Monthly variation of salinity in Rezu Khal

4.2) Ocean acidification factor of Rezu khal

4.2.1) pCO_2

The ocean acidification factor pCO_2 (μatm) was recorded 88.7602, 56.8227, 98.6071, 126.3713, 121.5864, 96.2138, 135.2675, 13.5113, 34.4957, 78.8180, 84.3012 and 60.5543 from January to December respectively. Highest value was (135.27 μatm) in July. Lowest value was (13.51 μatm) in August. Mean value was 82.94 ± 36.85695 μatm. Monthly variation of pCO_2 is shown in Fig 4.5.

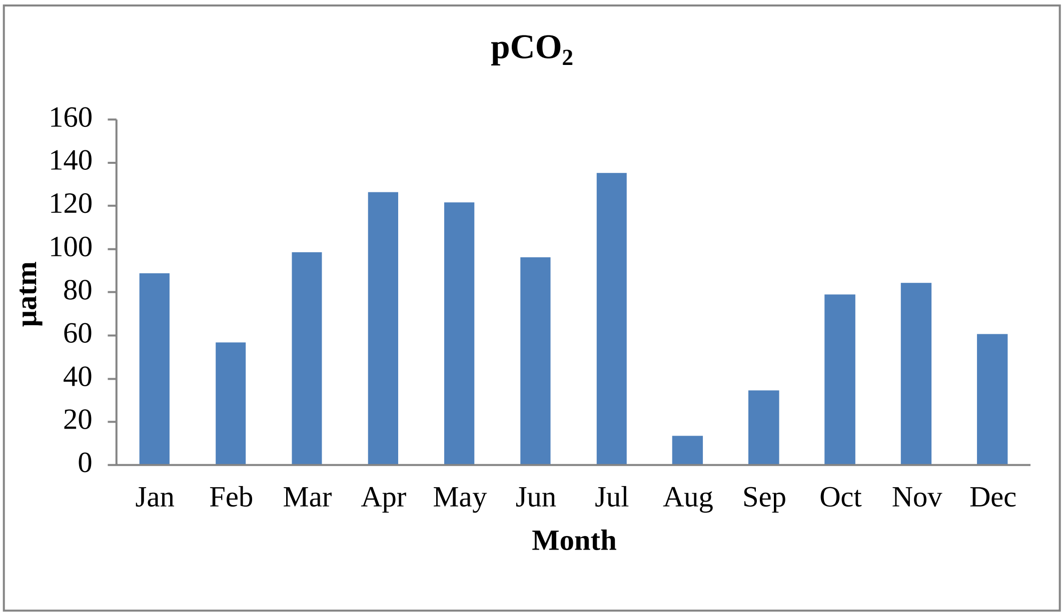

Fig 4.5: Monthly variation of pCO_2 in Rezu Khal

4.2.2) HCO_3^-

The acidification factor HCO_3^- (mol/kg) was recorded 0.0009, 0.0007, 0.0007, 0.0008, 0.0007, 0.0007, 0.0006, 0.0003, 0.0004, 0.0006, 0.0006, 0.0008 from January to December respectively**.** Highest value was (0.0009 mol/kg) in January. Lowest value was (0.0003mol/kg) in August. Mean value was 0.00065 ±0.0001679 mol/kg . Monthly variation of HCO_3^- is shown in Fig 4.6.

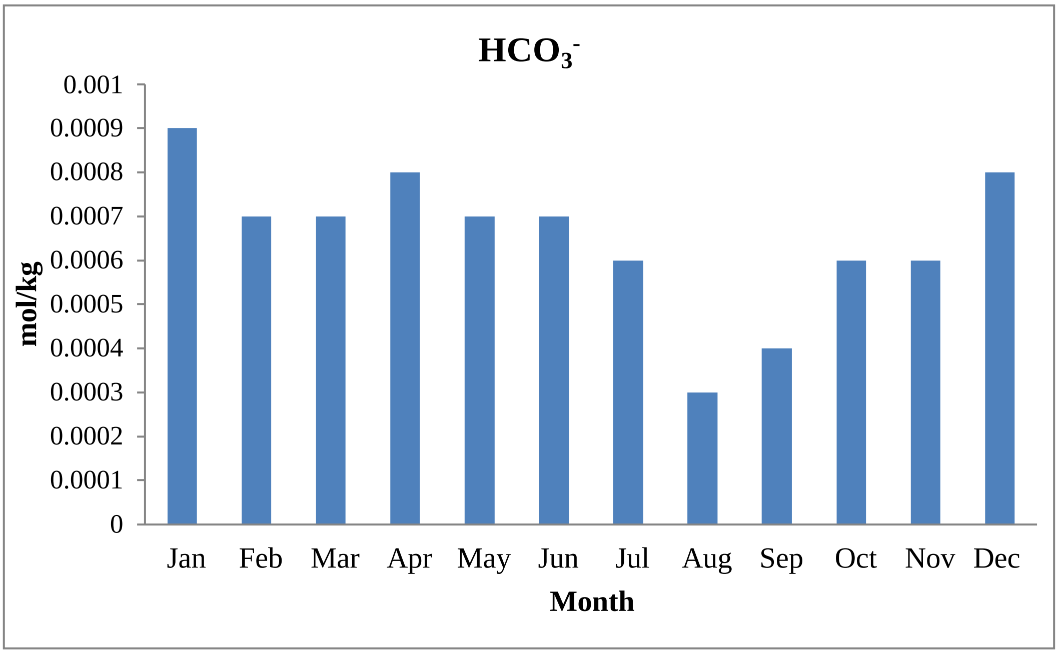

Fig 4.6: Monthly variation of HCO_3- in Rezu Khal

4.2.3) CO_3^{2-}

The acidification factor CO_3^{2-}(mol/kg) was recorded 0.0002, 0.0002, 0.0001, 0.0001, 0.0001, 0.0001, 0.0001, 0.0001, 0.0001, 0.0001, 0.0001, 0.0002 from January to December respectively**.** Highest value was (0.0002 mol/kg) in January, February and December. Lowest value was (0.0001 mol/kg) from March to November. Mean value was 0.000125±0.0000452 mol/kg. Monthly variation of CO_3^{2-} is shown in Fig 4.7.

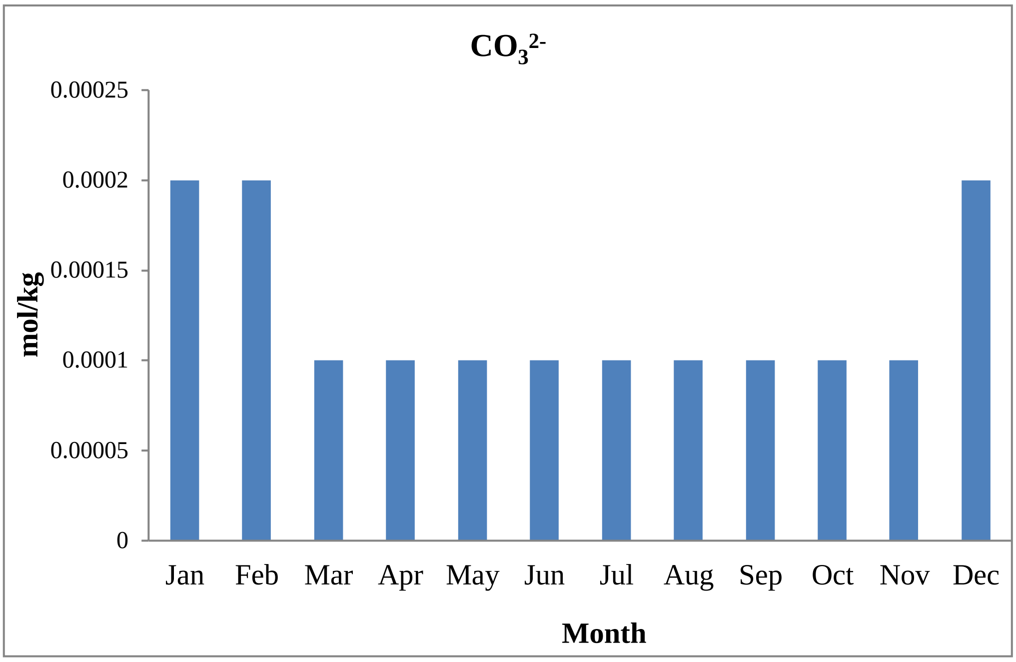

Fig 4.7: Monthly variation of CO_3^{2-} in Rezu Khal

4.2.4) DIC^-

The acidification factor DIC^- (mol/kg) was recorded 0.0011, 0.0009, 0.0009, 0.0009, 0.0009, 0.0008, 0.0007, 0.0004, 0.0006, 0.0007, 0.0008, and 0.0010 from January to December respectively**.** Highest value was (0.0011 mol/kg) in January. Lowest value was (0.0004 mol/kg) in August. Mean value was 0.000808± 0.0001881 mol/kg. Monthly variation of DIC^- is shown in Fig 4.8.

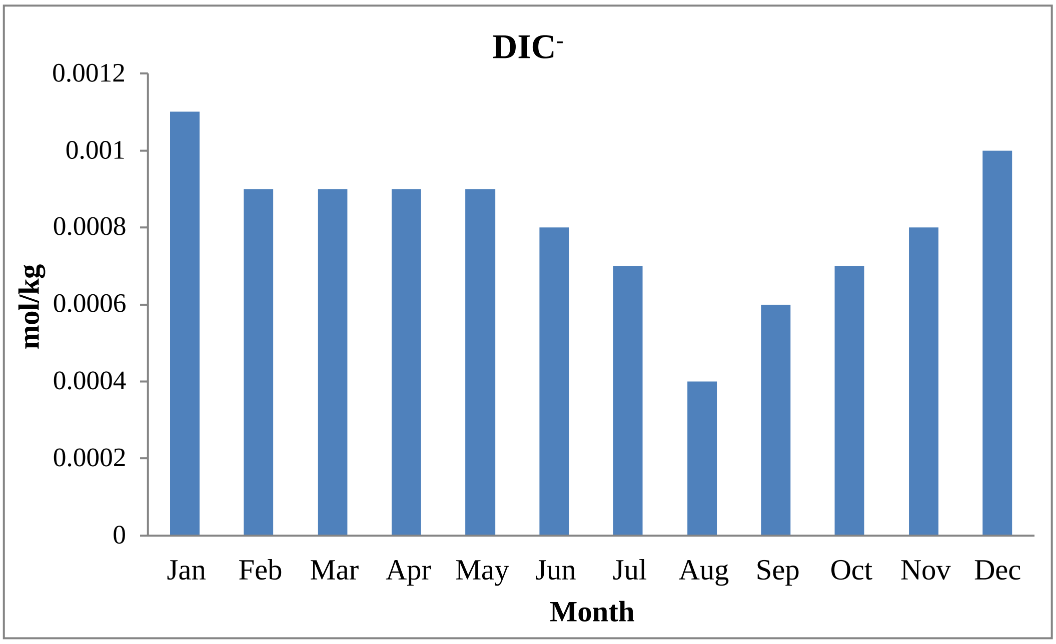

Fig 4.8: Monthly variation of DIC^- in Rezu Khal

4.2.5) $\Omega_{Aragonite}$

The acidification factor $\Omega_{Aragonite}$ was recorded 3.0109, 3.1943, 2.1528, 2.0245, 2.1363, 1.8464, 1.1914, 2.1504, 2.2129, 1.9207, 2.4798 and 3.7165 from January to December respectively. Highest value was (3.7165) in December. Lowest value was (1.1914) in July. Mean value was 2.3364±.6782995. Monthly variation of $\Omega_{Aragonite}$ is shown in Fig 4.9.

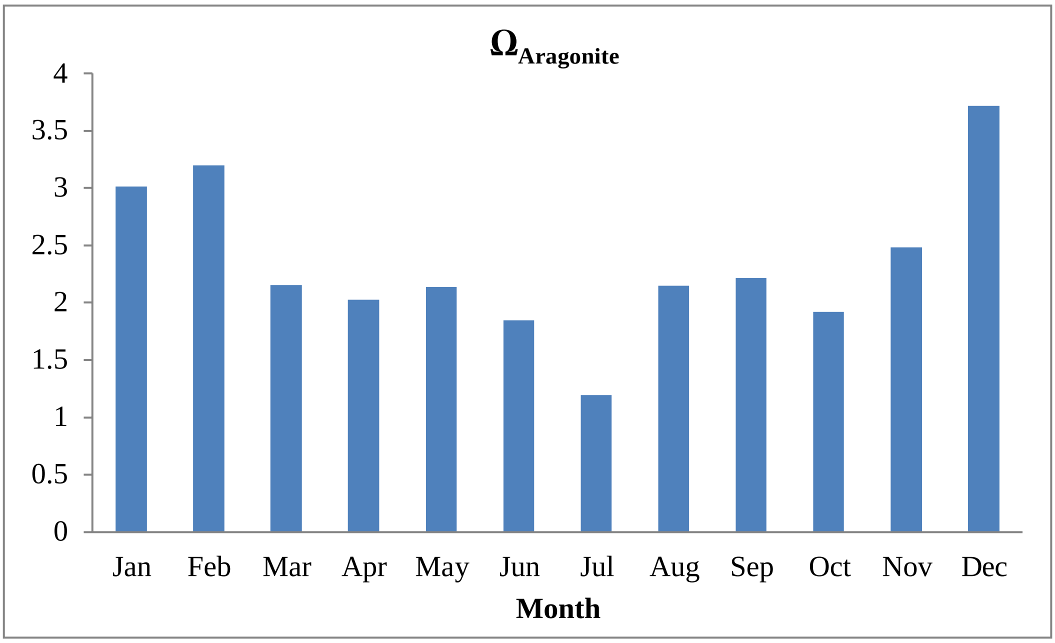

Fig 4.9: Monthly variation of $\Omega_{Aragonite}$ in Rezu Khal

4.2.6) $\Omega_{Calcite}$

The acidification factor $\Omega_{Calcite}$ was recorded 4.6857, 4.9829, 3.3671, 3.1136, 3.2545, 2.9452, 1.8742, 3.5714, 3.6815, 2.9756, 3.7501, and 5.7099 from January to December respectively. Highest value was (5.7099) in December. Lowest value was (1.8742) in July. Mean value was 3.6593±1.0329938. Monthly variation of $\Omega_{Calcite}$ is shown in figure 4.10.

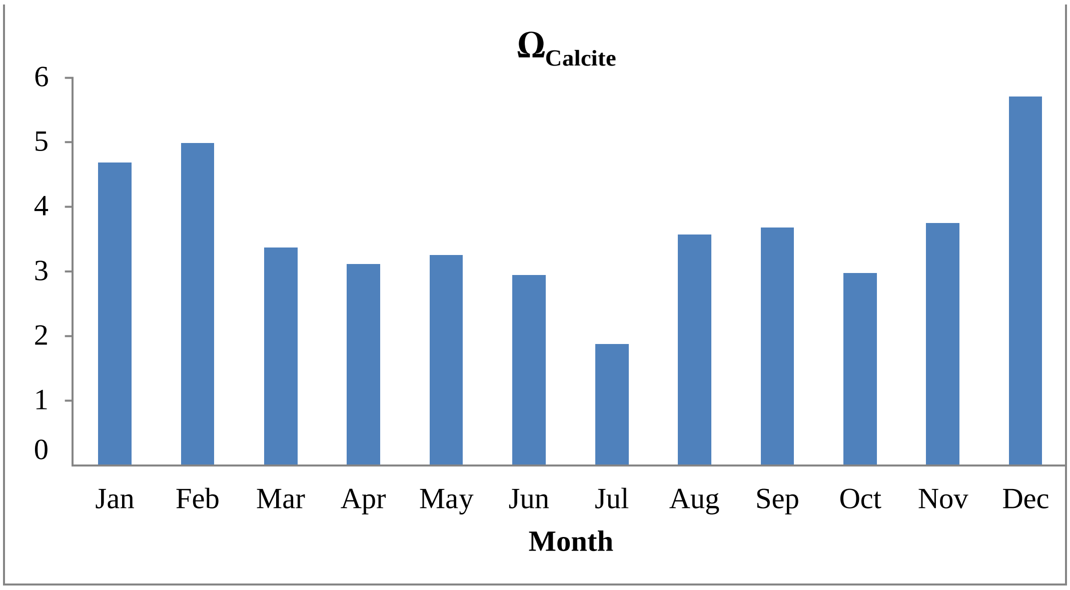

Fig 4.10: Monthly variation of $\Omega_{Calcite}$ in Rezu Khal

4.3) Determination of larvae abundance

The number of larvae varied 14, 47, 5, 131, 58, 72, 367, 390, 154, 216, 28 and 29 from January to December respectively. Highest larvae number 390 was found in August. Lowest larvae number 5 was found in March. Mean value was 125.92±133.71 . Monthly variation of larvae number is shown in Fig 4.11.

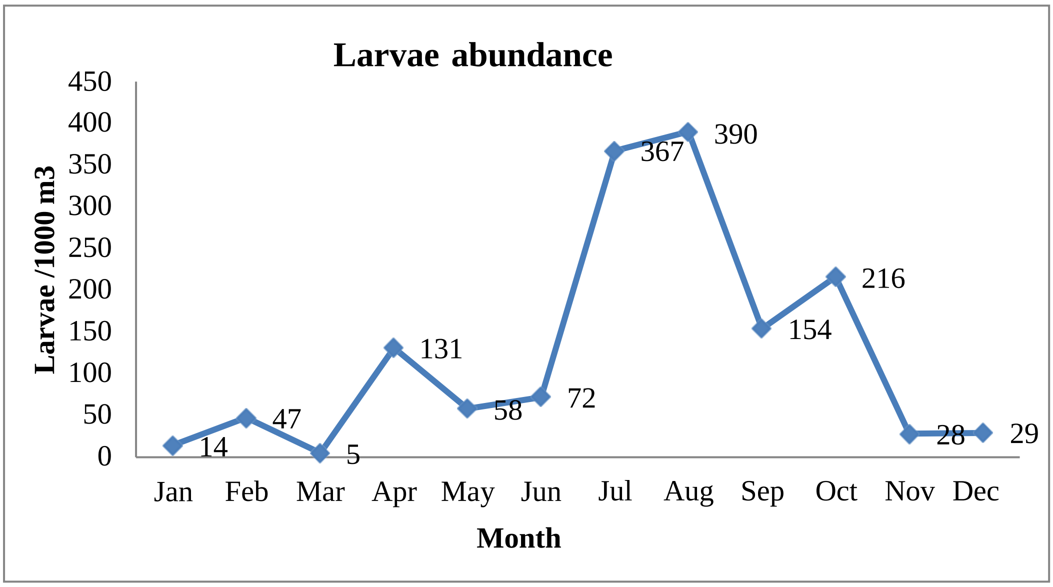

Fig 4.11**:** Monthly variation of fish larvae in Rezu Khal

4.4) Larvae abundance in relates to pCO_2 (µatm)

The larvae number was found (5-390) and pCO_2 found (13.5113-135.2675) µatm from January to December respectively. Larvae number was found highest (390) at lowest pCO_2 (13.51) µatm in August. Larvae number was found lowest (5) at higher pCO_2 (98.6071)µatm in March. Monthly variation between larvae number & pCO_2 is shown in Fig 4.12.

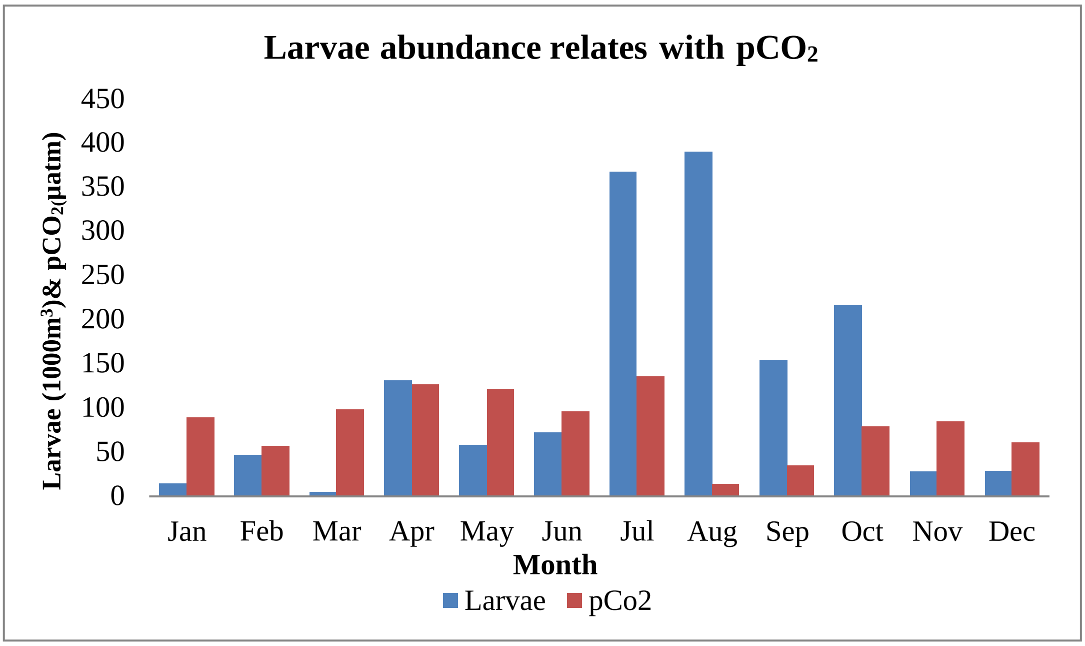

Fig 4.12: Monthly variation of fish larvae and pCO_2 in Rezu Khal

4.5) Larvae abundance relates with pH

The larvae number was found (5-390) and pH found (8.1-8.8) from January to December respectively. Larvae number was highest (390) at highest pH (8.8) in August. Larvae number was lowest (5) at lower pH (8.3) in March. Monthly variation of larvae number & pH is shown in Fig 4.13.

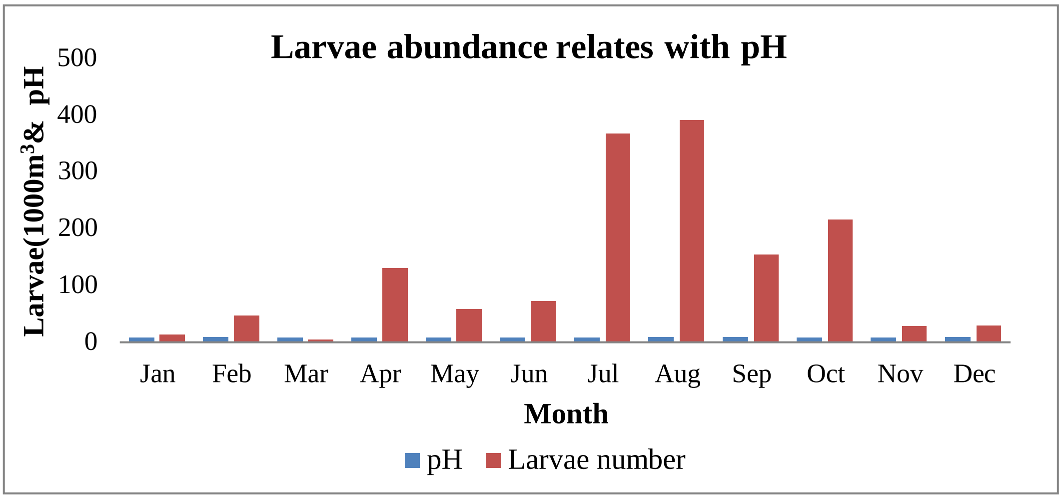

Fig 4.13: Monthly variation of fish larvae and pH in Rezu Khal

4.6) Statistical analysis

Larvae number, water parameter & ocean acidification factor were analysed through SPSS which derived value were shown in Table 4.1.

Tab 4.1: Statistical analysis of hydrological parameters & acidification factor

Parameters	Minimum	Maximum	Mean	Std. Deviation
Larvae (1000m^3)	5	390	125.92	133.711
pH	8.10	8.80	8.3750	.19598
Temperature(^{0}c)	22.7	30.4	27.650	2.3423
Alkalinity(mg/l)	65	138	104.17	22.405
Salinity(psu)	13.0	30.0	23.983	5.6432
pCO_2	13.51	135.27	82.9425	36.85695
HCO_3^-(mol/kg)	.0003	.0009	.000650	.0001679
CO_3^{2-}(mol/kg)	.0001	.0002	.000125	.0000452
DIC^-(mol/kg)	.0004	.0011	.000808	.0001881
$\Omega_{Aragonite}$	1.1914	3.7165	2.336408E0	.6782995
$\Omega_{Calcite}$	1.8742	5.7099	3.659308E0	1.0329938

4.7: Correlation matrix

A negative correlation found between PCO_2, pH &larvae abundance and positive correlation found between pH and larvae abundance which were shown below table (Tab 4.1, Tab 4.2 and Tab 4.3).

Table 4.2. Correlations between pCO_2 & pH of Rezu Khal

Correlations			
		pCO_2	pH
pCO_2	Pearson Correlation	1	-.970**
	Sig. (2-tailed)		.000
	N	12	12
pH	Pearson Correlation	-.970**	1
	Sig. (2-tailed)	.000	
	N	12	12
**. Correlation is significant at the 0.01 level (2-tailed).			

Table4.3.Correlations between pCO_2 & Larval abundance of Rezu Khal

Correlations			
		Larvae abundance	pCO_2
Larvae abundance	Pearson Correlation	1	-.166
	Sig. (2-tailed)		.605
	N	12	12
pCO_2	Pearson Correlation	-.166	1
	Sig. (2-tailed)	.605	
	N	12	12

Table4.4. Correlations between pH & Larval abundance of Rezu Khal

Correlations			
		Larvae abundance	pH
Larvae abundance	Pearson Correlation	1	.181
	Sig. (2-tailed)		.573
	N	12	12
pH	Pearson Correlation	.181	1
	Sig. (2-tailed)	.573	
	N	12	12

Chapter 5

Discussion

Aquatic environment has a complicated connection of various Physico-Chemical parameter of water with its biota. Physico-chemical parameter of water play role as factors for fish larvae abundance. A comparative study of Physico-Chemical parameter in relation to acidification factor and larvae abundance of Rezu Khal estuary has been conducted and relationship between acidification factor and larvae number has been discussed.

5.1 Water Temperature

The study showed that water temperature was close to average value (Fig 4.1). In the Reju Khal estuary, the range of water temperature found between 22-30 °C & mean value was 27.65◦C, which was observed to be around the acceptable limits of surface water temperature 20-30 ºC (Standard,1997). The recorded temperature was optimum range (22-31°C) for the growth of the fish (Billah et al., 2016). Based on a study, water temperature is an important factor influencing marine life (Korai et al., 2008). Besides, in the estuary surface water temperature is regulated by the tidal cycles or fresh water input from the upstream (WHO, 2004).

5.2 Water pH

The present study found pH value 8.1-8.8 and average was 8.3 (Fig4.2). The observed pH value of the Reju Khal estuary was within the limit (6.5-9.20), recommended by the WHO (Hossain et al., 2012).The dissolved minerals in the water, dumping of garbage, photosynthesis, bacterial activity, water turbulence, chemical components of precipitation moving into the body of water, sewage overflows, and even aerosol and fine particulates all can generate pH fluctuations (Faires, 1993).

5.3 Alkalinity

The alkalinity was recorded 65-138 mg/l (Fig 4.3) and mean value was 104.17 mg/l. The observed value of alkalinity was found within ideal range. The optimal value of alkalinity is more than 100 mg/l (Faires, 1993). Medium salinity in the water is characterized as moderate alkalinity (Biswas et al., 2015). Alkalinity of Bakkhali River was 146.85 mg/l as per a study on the physico-chemical assessment of ground and surface water quality in the greater Chattogram region of Bangladesh (Ahmed et al., 2010).

5.4 Salinity

Salinity was recorded 13-30 psu and average was 23.98 psu (Fig 4.4), which is similar with reffered range. The salinity in an estuary fluctuates between (5-35) ppt (Iqbal et al., 2014; Vernberg, 1974). Mahmood (1990) reported that the salinity in the estuary area of Chakaria varied from (6.14- 32.5) ppt, while Ahmed (1983) found that the salinity in the Karnafully River ranged from (3.30- 22.93) ppt. Seawater's salinity is enhanced by evaporation and it is lowered by the addition of freshwater via runoff or rainfall, which neutralized the seawater (Hasan et al., 2019). Levels of salinity in the Bakkhali River water fluctuated periodically, having summer reading of 12 ppt and winter reading of 27 ppt, based with one investigation (Raknuzzaman et al., 2018).

5.5 Ocean acidification factors

During the study period, partial pressure of carbon dioxide concentration was found between (13.5113-135.2675)µatm (Fig 4.5) and mean value was 82.942µatm. From a partial pressure (pCO_2) of approximately 280 µatm to its present level of nearly 400 µatm, the concentration of atmospheric carbon dioxide (CO_2) has increased by almost 40% (Solomon et al., 2007). Results shows that derived pCO_2 level is below referred range. So, acidification did not occur in Rezu khal estuary.

During the study period, dissolved inorganic carbon dioxide (DIC^-) concentration was found 0.0004-0.0011mol/kg (Fig 4.8) and mean value was 0.000808mol/kg. DIC^- is a vital element of ocean water as well as important indicator of acidification which is generally formed by CO_3^{2-}, HCO_3^-, and CO_2 concentration. The optimum level of DIC^- for living organism is considered less than.002 mol/kg (Spyres et al., 2000). DIC^- value is found below optimum range, which is not harmful for living marine organisms.

Ocean acidification factor $\Omega_{aragonite}$ was found 1.1914-3.7165 (Fig 4.9) and mean value was 2.3364. In marine environment, biological organisms became stressed when aragonite saturation level is below 3 and shell structure began to dissolve below 1 (Jiang et al., 2015). The $\Omega_{aragonite}$ level was found above ideal range and organisms remain stress free at this range.

Ocean acidification factor $\Omega_{calcite}$ was found 1.8742-5.7099 (Fig 4.10) and mean value was 3.6593. Calcifying organism especially marine invertebrates which is lack of defensive mechanism are mostly vulnerable to unsaturated ($\Omega_{calcite}$ <1) sea water (Corlis & Honjo, 1981).The $\Omega_{calcite}$ level was found greater than the ideal range which is not harmful for marine calcifying organisms.

5.6 Relation of fish abundance and pCO_2

Present studies shows that larvae number varies from higher to low in different month (Fig 4.12). Larvae number was quite high in April, July, August, September and October when pCO_2 level fluctuate significantly. Fish population was found quite low in January, March, November and December at moderate pCO_2 level. Larvae number reached its peak in August at lowest pCO_2 level. Larvae number was lowest in March at lower pCO_2 value. Rising levels of pCO_2 (550 μatm) have crucial effects on larvae development, which is considered main ocean acidification factors for living organisms (Diaz et al., 2019). Negative correlation exist between larvae abundance and pCO_2 and pCO_2 level more than 300μatm show negative impact on fish larvae number (Scanes et al,2014). One crucial phase is the development of fish population is the extremely vulnerable larval stage. Because of modest changes in growth; predation, and reproductive pressure, larvae experience high cumulative mortality during this early phase (Houde, 1989). Whenever the larval stage is completed successfully, it influences ecosystem processes in the young or adult habitat, stock replenishment & population's connectivity (Cowen & Sponaugle, 2009). Consequently, fish may be environmentally affected by acidification (Pankhurst & Munday, 2011). Due to the lower CO_2 partial pressures (pCO_2) of their body fluids, aquatic organisms, like fish, are more exposed to an increase in the atmospheric CO_2 concentration than terrestrial animals (Ultsch & Jackson, 1996). Finding reveals that larvae abundance is not affected by changing pCO_2 level.

5.7 Fish abundance relates with pH

During study period, pH value remains almost same although larvae number goes through ups and downs in different month (Fig 4.13). In August, larvae number found highest when pH value remain above average. In March, lowest larvae number was found when pH value remains within average. Fish population found quite low in January, March, November and December when pH remain almost stable. Larvae number was found quite high in April July, August and September, although pH did not fluctuate significantly. The pH varies around 6.5 to 8.5 on average proved safe for marine life (Standard, 1997). Some evidence suggests that increasing CO_2, decreasing pH in ocean water can alter the growth, maturity in various marine organisms (Fabry et al., 2008). Research on larval development has so far shown how low acidity greatly reduces survival (Kikkawa et al., 2003). Compared to adding acid by its own, CO_2-induced acidification has more negative effects (Hayashi et al., 2004). Since juveniles and adults are often less susceptible to pH changes than fish eggs and young larvae are, it is most likely that significant impacts of ocean acidification would be seen in these early life stages (Ultsch &Jackson, 1996). Results shows that pH fluctuation did not affect larvae abundance at Rezu Khal estuary.

5.8 Correlation between pH & pCO_2

According to Pearson correlation, if the value is between 0.70-1.00, it means the correlation between the two variables is very strong and the negative value means negative correlation. In this research, a very strong negative correlation (r= -0.97) was found between pH and pCO_2, which is significant at $p<0.01$ level (Tab 2). So, the correlation between pH and pCO_2 is significant of Rezu Khal.

5.9 Correlation between pCO_2 & larvae abundance

The rise of pCO_2 in ocean water mainly affects the carbonate system of ocean water. The increase in the value of pCO_2 decreases the value of water pH and ultimately reduces the survivability of larvae. According to Pearson correlation, if the value is between 0.10-0.29, it means the correlation between the two variables is weak and the negative value means negative correlation. In this research, a weak negative correlation(r=-0.16) was found between pCO_2 and larval abundance. According to Pearson's correlation, if the p value is > 0.05, it means an insignificant relationship between two variables. In this research, the p value was found 0.605 >0.05 (Table 3). So, the correlation between pCO_2 and larval abundance is not significant for Rezu Khal.

5.10 Correlation between pH & larvae abundance

Carbonic acid is formed as a result of CO_2 dissolving in the ocean. In the water, this carbonic acid diffuses, producing hydrogen ions and bicarbonate. Since the pH scale applies the equation pH =-log [H+] to describe acidity, a rise in hydrogen ion concentration results in an increase in acidity. pH is an important parameter to describe the actual condition of ocean acidification. If the value of pH decreases, the ocean water will become more acidic. According to Pearson correlation, if the value lies between 0.10-0.29, it means the correlation between the two variables is weak and the positive value means positive correlation. In this research, a weak positive correlation(r=0.18) was found between pH and larval abundance. According to Pearson's correlation, if the p value is > 0.05, it means an insignificant relationship between two variables. In this research, the p value was found 0.573 > 0.05 (Table 4). So, the correlation between pH and larval abundance is not significant of Rezu Khal.

Chapter 6

Conclusion

The objective of the study was to measure the effects of acidification on the richness of fish larvae and the level of carbon dioxide (pCO_2) which is the main factor of ocean acidification. Ocean acidification occurs with increasing pCO_2 by reducing pH concentration which has impact on larvae number. Analyzing result and discussion, it can be concluded that larvae abundance remain unaffected with changing pCO_2 and pH level. There can be many reasons which are responsible for variation in larvae number. Fluctuation of pCO_2 was noticible which has influence on other acidification factor (HCO_3^-, CO_3^{2-}, DIC^-, Ωaraganite, Ωcalcite). It is not easy to find out specific reason for acidification that is responsible for decreasing larvae stock. Water parameter (temperature, pH, alkalinity, salinity) was found within referred value. From this, it provides knowledge about the present condition of acidification of Rezu Khal. Research on this location will help to gather information on ocean acidification scenario of Cox's Bazar coasts of the Bay of Bengal. Using these outputs, impact of ocean acidification on fish larval population abundance can be easily identified. This research will help decision makers to take appropriate decisions on reducing the degree of acidification in Cox's Bazar coasts of the Bay of Bengal. This will also be an educative work for a researcher because it will include both field and laboratory work. It also provides updated data that is helpful for further research.

Chapter 7

Recommendation

Pollution and global warming are two of the many factors that accelerate ocean acidification. The following actions will be helped to save the ocean in the long term. According to this research work; the following recommendation can be done:

- ✓ Acting authority should ban that industry who disposes their waste material without proper treatment.
- ✓ Use of fossil fuels should reduce which are responsible for the emission of carbon dioxide
- ✓ Use of renewable energy should increase to return the environment to its original state.
- ✓ Waste material should recycle as much as possible to protect the environment from ocean acidification.
- ✓ Tree plantation should be introduced broadly to produce more oxygen.
- ✓ Growth of aquatic vegetation should ensure to improve marine environment.
- ✓ Effective way to limit acidification is to act on climate change.
- ✓ Water should be protected from being polluted.
- ✓ Awareness should be developed at national level.
- ✓ As it is a pilot study, further studies may be conducted on similar or different field to make a concrete remark on effect of acidification.

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Appendices

Appendix A: Hydrological parameters of Rezu Khal

Month	Temperature (◦C)	pH	Alkalinity (mg/l)	Salinity (psu)
Jan	22.7	8.4	138	28.1
Feb	26.6	8.5	120	25
Mar	26.2	8.3	110	24.7
Apr	28.3	8.2	110	27
May	30	8.2	109	28
Jun	26.3	8.3	100	21
Jul	29.1	8.1	82	22
Aug	28.4	8.8	65	14
Sep	30.4	8.6	78	13
Oct	28.9	8.3	92	25
Nov	30	8.3	108	30
Dec	24.9	8.5	138	30

Appendix-B: Derived ocean acidification factor of Rezu Khal

Month	pCO_2(µatm)	HCO_{3^-} (mol/kg)	CO_3^{2-} (mol/kg)	DIC (mol/kg)	$\Omega_{Aragonite}$	$\Omega_{Calcite}$
Jan	88.7602	.0009	.0002	.0011	3.0109	4.6857
Feb	56.8227	.0007	.0002	.0009	3.1943	4.9829
Mar	98.6071	.0007	.0001	.0009	2.1528	3.3671
Apr	126. 3713	.0008	.0001	.0009	2.0245	3.1136
May	121.5864	.0007	.0001	.0009	2.1363	3.2545
Jun	96.2138	.0007	.0001	.0008	1.8464	2.9452
Jul	135.2675	.0006	.0001	.0007	1.1914	1.8742
Aug	13.5113	.0003	.0001	.0004	2. 1504	3.5714
Sep	34.4957	.0004	.0001	.0006	2.2129	3.6815
Oct	78.8180	.0006	.0001	.0007	1.9207	2.9756
Nov	84. 3012	.0006	.0001	.0008	2.4798	3.7501
Dec	60.5543	.0008	.0002	.0010	3.7165	5.7099

Appendix C: Abundance of fish larvae (1000/m^3) in Rezu Khal

Month	Larvae number
Jan	14
Feb	47
Mar	5
Apr	131
May	58
Jun	72
Jul	367
Aug	390
Sep	154
Oct	216
Nov	28
Dec	29