CHANGES IN FISH POPULATION REGARDING FISH LARVAL ABUNDANCE IN RESPONSE TO ACIDIFICATION AT THE BAKKHALI RIVER, COX'S BAZAR COAST, BANGLADESH

Humayun Kabir

Roll No. 0120/07

Registration No. 859

Session: 2020-2021

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Fisheries Resource Management

Department of Fisheries Resource Management

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

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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

---	---
Prof. Dr. Gous Miah	**Dr. Sk. Ahmad Al Nahid**
Supervisor	**Co-supervisor**

Dr. Sk. Ahmad Al Nahid

Chairman of the Examination Committee

Department of Fisheries Resource Management
Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

JUNE 2022

Dedicated

To

My Beloved

Parents

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The Author

Humayun Kabir

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LIST OF ABBREVIATIONS

A_T	Total alkalinity
BoB	Bay of Bengal
$CaCO_3$	Calcium carbonate
CO_2	Carbon dioxide
CO_3^-	Carbonate
ºC	Degree Celsius
DIC	Dissolved Inorganic Carbon
DO	Dissolved oxygen
GABAA	Gamma-aminobutyric acid type A
GPS	Global Positioning System
H^+	Hydrogen ion
H_2CO_3	Carbonic acid
HCO_3^-	Bicarbonate
HNO_3	Nitric acid
H_2SO_4	Sulfuric Acid
i.e.	That is
kg	Kilogram
l	Liter
mm	Millimeter
m^3	Cubic meter
mg	Milligram
min.	Minute
mg/ l	Milligram per liter
µatm	Micro atmospheres
mol/kg	Mole per kilogram
Ω	Omega
OA	Ocean Acidification
OM	Organic matter
$p$$CO_2$	Partial pressure of carbon dioxide
pH	potential of hydrogen
ppt	Parts per thousand

PSU	Practical Salinity Unit
ppm	Parts per million
SPSS	Statistical Package for Social Science
UV	Ultra-violate
>	is greater than
<	is less than

ABSTRACT

The uptake of atmospheric CO_2 by the oceans since the beginning of the industrial revolution is considered to be a significant threat to marine ecosystems due to the resulting carbonate chemistry changes. This study tried to evaluate the level of acidity and the larval abundance of the Bakkhali River in Cox's Bazar. Fish larval abundance fluctuates directly in response to the rising acidity of river water. The potential impact of rising atmospheric CO_2 levels on fish larvae caused by ocean acidification is receiving a lot of attention. This research was conducted in the Bakkhali River estuary with monthly sampling from January to December 2021. The key ocean acidification factor, pCO_2, ranged from 19.3642 to 360.6499 μatm. The pH of the water was always found to be alkaline and varied between 8.0 and 8.8 (8.267±0.2146). The range of the DIC (mol/kg) concentration was 0.0005 to 0.001 with a mean of 0.00094686±0.00038532. The aragonite ranges from 0.86174819 to 3.4318 with mean of 2.2771±0.7552 and the calcite ranges from 1.3987 to 5.2121 with a mean of 3.5397±1.1109. Throughout the study, 555 larvae were recorded from the study region. The maximum and minimum number of fish larvae was 109 in August and 11 in February, respectively. The levels of pCO_2 and fish larvae were inversely related. A decline in the quantity of fish larvae had been associated with rising pCO_2 levels and decreasing seawater pH. Fish larvae had significant positive relationships with pH (R^2 =0.5290, $p<0.05$), and fish larvae had significant negative relationships with pCO_2 (R^2 =0.2056, $p<0.05$). The growth and survival of marine organisms during their larval stages could be hampered by ocean acidification. The early life stage was substantially more sensitive to elevated pCO_2 induced mortality than the post larval stage. The results showed that a higher pCO_2 may negative impact on larval fish survival. It is still entirely unknown whether wild populations of marine species are capable of adapting to elevated pCO_2. This finding will stimulate interest in fish biology research in regions where fish larvae are particularly vulnerable to acidification.

***Key words*:** Ocean acidification, Larval abundance, CO_2, pCO_2, pH, Bakkhali river

INTRODUCTION

This chapter describes the introductory information about the research project. These include a brief discussion about the background of the study and objectives of the study. This part also demonstrates and briefly explains the structure of this research project.

1.1 Background of the study

Fish larvae are ecologically and morphologically distinct in contrast with their older counterparts throughout their development phases. They exist in diverse settings, taking various food items and having distinct predators and varying behavior. During larval development, the fish grows from an embryo to a larva, which includes all the completely functional organs. Several species have very distinct larval forms, like spikes on their heads and other features that change or go away when they become adults (Leis and Carson-Ewart, 2000). The larvae stage of the fish development is one of the most crucial stages in the life cycle of the fish. The success of introducing new stocks into the fish population dynamic is typically evaluated at the adult fish stage, but early life history is equally important, thus evaluating early life history is also extremely important. The survival of fish through the larval phase determines the guaranteed stock of several fishing items. One of the important variables in the conservation of fish resources is fish larvae stocks. Disturbance of the early stages of fish life will exert a negative influence on adult fish stocks (Purnomo et al., 2020).

The larval stage is possibly more susceptible to environmental alterations; any alteration in the nature or abundance of ecological factors is detrimental to larval survival and may indicate future recruitment opportunities (Leis and Rennis, 1983). According to Arshad et al. (2012), a great understanding of larvae is often the best technique to provide information of major value to fisheries management, fisheries science and marine biologists. Data from additional oceanic voyages revealed a global view the larval variety and distribution patterns, while early larval studies focused on the timing and location of spawning and the survival of the young of important and abundant species (Moser, 1993). Understanding the number and dispersion of larval fish about environmental circumstances could fill a gap in the research of fish life cycle and provide knowledge for fisheries management.

The present study on ocean acidification (OA), an imminent environmental threat that impacts all marine life, has evolved significantly over the years. As carbon dioxide

(CO_2) levels in the atmosphere rise, so do the acidity levels increasing in the world's oceans. Significant ecological and economic repercussions on fish populations could result from a lack of knowledge about how acidification affects young species. The term "ocean acidification" describes the gradual decrease in pH that the ocean experiences as a result of absorbing CO_2 from the environment. There is the considerable worry that a wide variety of marine creatures may be adversely affected by the rise in dissolved CO_2 concentrations in the oceans and the resulting change in water chemistry (Kroeker et al., 2010, 2013; Wittmann and Pörtner, 2013).

About 25% of anthropogenic CO_2 emissions are taken up by the ocean (Le Quéré et al., 2009), making the ocean a significant sink for CO_2. Since the beginning of the industrial revolution, the average pH of the ocean has decreased by roughly 0.1 due to the increasing atmospheric CO_2 concentration (Orr, 2011). If current trends continue, the pH might drop by almost 0.7 by the year 2300 (Zeebe et al., 2008). Ocean acidification (OA) is a process in which the ocean becomes more acidic as a result of the uptake of CO_2 in the atmosphere into the seas (Feely et al., 2004). This has serious consequences for marine ecology.

Many people are concerned about the effects of ocean acidification on fish larvae as a result of high atmospheric CO_2 concentrations. Rising atmospheric CO_2 levels alter the carbonate balance of the ocean, causing an increase in the concentration of hydrogen ions (H^+), and a consequent decrease in the acidity of the water. Increasing atmospheric CO_2 due to human activities has both specific chemical impacts on ocean waters and indirect bio-physicochemical effects (on things like predator-prey abundance, nutrient recycling, and behavioral responses) on organisms (Hossain, 2015). This process has only lately been considered a human source of stress, with potentially substantial effects on the conservation and maintenance of many economically valuable ocean resources (Miller et al., 2009). It is expected that early stages will be particularly vulnerable to these new circumstances and changes in weather patterns, and if they are unable to endure and adapt, it might be a barrier to the continued existence of species in a changing ocean (Rosa et al., 2012). However, investigations on the consequence of ocean acidification on the performance of fish larvae are mostly rare or have only found minor effects (Franke and Clemmesen, 2011).

The Bakkhali River is a major waterway in Cox's Bazar and is located on the Bay of Bengal's south-eastern coast. It originates in Naikhongchhari and Ramu in Cox's Bazar and falls into the Moheshkhali channel in the Bay of Bengal. This estuary's bottom is significantly impacted by human and industrial operations such as fish harbors, seafood processing plants, and a vast array of shrimp and fish farms. Massive amounts of both physical and chemical pollution in the water body have a severe effect on biodiversity by altering the chemical characteristics of the water body through the release of hazardous substances (Rashed-Un-Nabi et al., 2011). The coastal regions of Bangladesh are home to a diverse fishery typical of the tropics. About 490 fish species (Hossain, 1971) and 19 shrimp/prawn species (Chowdhury and Sanaullah, 1991) can be found in this region.

Fishes are important to estuaries because they are both permanent and transient community members, attracting marine species that use the watery ecosystem for a variety of reasons, including food, shelter, breeding, and growth (Raz-Guzman and Huidobro, 2002). However, coastal and estuarine biomes are extremely ecologically productive and provide extensive, measurable ecosystem services (e.g., commercial and recreational fisheries, nursery grounds for fish and invertebrates, water purification, protection from flood and storm surge, and human recreation). Because of their shallowness, lower salinity, and lower alkalinity, estuaries, coastal, and marine ecosystems are more susceptible to pH fluctuations than the open ocean (Wong, 1979). Natural and manmade sources of carbon dioxide (CO_2) respiration can pose threats to estuaries. As a result, these ecosystems are anticipated to be more severely impacted by rising CO_2 levels in the future decades (Cai and Wang, 1998).

Enriching the atmosphere with CO_2 will simply contribute to raising the baseline further. While this is happening, ocean acidification is causing a steady decline in the number of fish as well as other seafood animals. Reduced availability of seafood threatens not only human populations but also marine life as a whole. Human activity has only recently been recognized as a threat to ecosystems, one that could have far-reaching effects on the long-term viability and maintenance of many economically significant ocean resources (Miller et al., 2009). It is expected that the earliest stages will be particularly vulnerable to the effects of climate change and may be unable to adjust to the changing environment (Cooley et al., 2009). Few studies have been conducted on marine fishes to investigate the consequences of acidification.

According to Arshad et al. (2012), a great understanding of larvae is often the best technique to provide information of major value to fisheries management, fisheries science and marine biologists. It is important to investigate how ocean acidification impacts larval abundance, especially in the context of Bangladesh. This study looks at the changes in fish species regarding fish larval abundance in response to acidification at the Bakkhali River estuary over a year. There hasn't yet been any research done in Bangladesh on how ocean acidification affects the distribution and abundance of fish larvae. The goal of the study was to find out more about the effects of acidification and changes in fish larval abundance at the Bakkhali River, Cox's Bazar coast. Ample research and procedures for careful monitoring must be established in order to estimate the costs of ocean acidification in Bangladesh both now and in the future.

1.2 Significance of the study

This new research offers a significant opportunity to further understanding of species' vulnerability and resilience to ocean acidification. Ocean acidification, recognized as a major threat to marine ecosystems, has developed into one of the fastest growing fields of research in marine sciences. This study will be a great contribution to advance research work on acidification. For the future management of fisheries, it is necessary to know how ocean acidification changes fish larvae. This study may be a secondary source of information for future investigation of larval abundance and changes in response to acidification. The study will also help to manage the abundance of fish larvae and build a management procedure for fisheries sector at the Bakkhali River estuary.

1.3 Objectives of the research

This research is focused on two main goals. The objectives of this study are as follows:

1. To provide an actual scenario of the ocean acidification of the Bakkhali River;
2. To investigate and assess changes in fish larval abundance in response to acidification in the study area

REVIEW OF LITERATURE

Fish larvae is one of the most important elements and the larval abundance fluctuates directly in response to the rising acidity of river water. For completing research objective and there added literature review have been presented and character-wise discussed and possible interpretations have been given. Literatures cited below under the following sub-headings reveal some information about this study.

2.1 General Overview of Ocean Acidification

The process of raising seawater acidity or lowering seawater pH as a result of elevated atmospheric carbon dioxide dissolving is known as ocean acidification (Orr et al., 2005). Ocean acidification has many of the aspects of global changes in the environment that directly threaten marine ecosystems (Royal Society, 2005).

According to Feely et al. (2004), the high levels of carbon dioxide (CO_2) produced by human activities and released into the seas are the primary cause of ocean acidification. Since the beginning of the industrial period, this shift in carbonate biochemistry has caused the mean pH of the ocean surface to fall from 8.21 to 8.10. If the current pace of 22 million tons per day is maintained, the ocean will absorb around one-third of the carbon dioxide (CO_2) emitted into the atmosphere (Cooley et al., 2009). The world's oceans serve as a significant CO_2 sink, therefore their ongoing uptake of CO_2 will alter the chemistry of the saltwater and, as a result, cause an estimated 0.4-0.5-unit decrease in the pH of the seas (Caldeira and Wickett, 2005). Ocean acidification is frequently understood or communicated in terms of this change in pH, but the actuality involves a suite of alterations to seawater chemistry that may not be reflected by a given change in pH. Numerous creatures may not be sensitive to the degree of pH fluctuation associated with contemporary OA, but these changes are thought to have harmful consequences on a wide range of species at different stages of development (Waldbusser et al., 2015).

According to a study by Raven et al. (2005), elevated atmospheric CO_2 levels disrupt the carbonate equilibrium in the ocean, leading to a lower pH. The partial pressure of carbon dioxide (pCO_2) is predicted to approach 900 ppm in an open ocean scenario by 2100, depending on emission trajectory, due to the ongoing intake of additional atmospheric CO_2 (IPCC, 2013). Already observed global trends of a shallower under saturating horizon of $CaCO_3$ (Feely et al., 2004) are expected to become more stable and widespread by 2050 and beyond due to further increases in atmospheric pCO_2 (Gruber et al., 2012).

Le Quéré et al. (2009) stated that burning fossil fuels like coal, oil, and fossil fuels, as well as better land-use practices and deforestation, are all human-caused activities that contribute to the rise in atmospheric CO_2. Enhanced respiration of organic compounds in the soil under global climate change circumstances, as well as other natural processes of decomposition of plant litter (King et al., 2012), could contribute significantly (Knorr et al., 2005). Ocean acidification may influence food webs and carbon cycling. This might alter species composition and primary production rates.

Both calcite and aragonite crystals can create calcium carbonate ($CaCO_3$), which is found in calcareous formations (Kobayashi and Samata, 2006). Already-formed structures are more susceptible to degradation, and calcification may become more difficult for calcareous invertebrates as a result of such ocean acidification. The experiment suggests that coccolithophore, which are planktonic animals having calcified shells, plates, or scales, may be badly affected. Contrarily, there is scant information about the range in which ocean acidification will impact other marine organisms (Fabry et al., 2008; Munday et al., 2008). Numerous studies on fish larval stages show that exposure to elevated CO_2 levels causes abnormal behavior, deformities, and higher mortality rates. Ocean acidification may have a wide variety of negative effects on many coastal ecosystems, with far-reaching consequences across the entire ocean.

2.2 Chemistry of Acidification

Gattuso et al. (2013) stated that acidification is often considered only as a change in acidity (pH), but the reality involves a series of alterations to seawater chemistry that are not always easy to understand or measure. The effects of acidification are demonstrated through a variety of interacting chemical mechanisms that are difficult to predict. A better understanding of the dynamic nature of ocean acidification's global impacts will almost certainly require an investment in understanding the chemistry behind it. It is almost probable that investing in understanding OA will be necessary for a deeper understanding of its effects on the globe. This chemistry (Figure 01) is known as the "marine carbonate system," and it involves a family of molecules that are important for the health, reproduction, and survival of all fish.

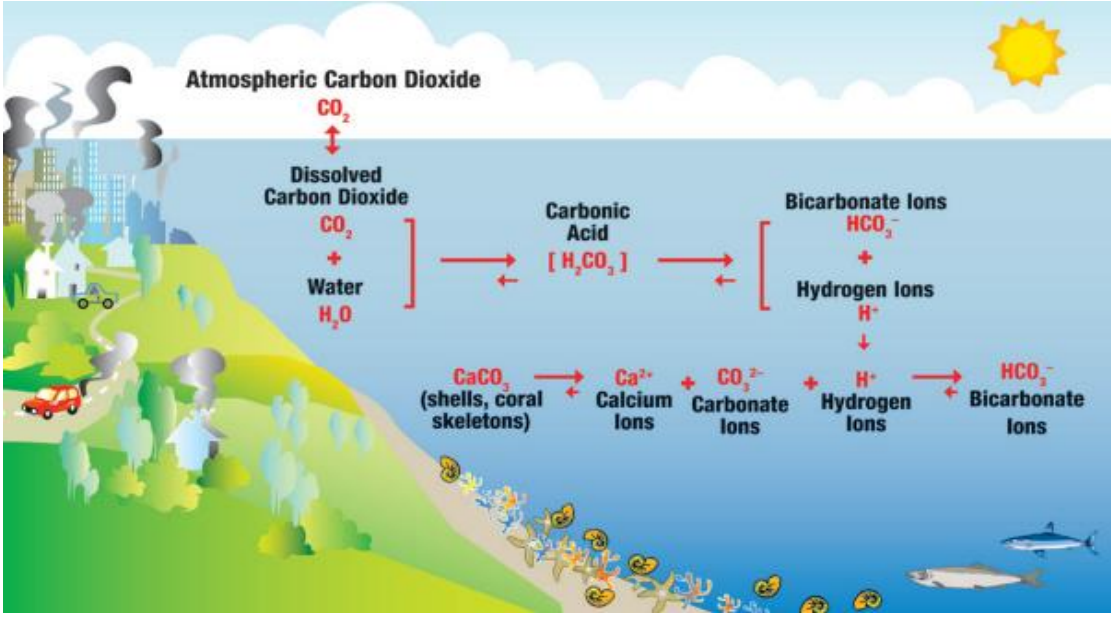

Figure 1. A brief summary of the major changes in ocean chemistry (National Research Council, 2013)

Doney et al. (2009) documented that chemical reactions between atmospheric CO_2 and seas at the air/ocean border are responsible for the observed decrease in surface pH. In the presence of water, CO_2 forms carbonic acid (H_2CO_3), which, upon dissociation, splits into bicarbonate (HCO_3^-) and carbonate (CO_3^-) ions. This process produces hydrogen ions (H^+), which lowers the pH of the seawater. The following reaction describes how atmospheric CO_2 equilibrates with ocean water, leading to a decrease in pH:

$CO_2 \leftrightarrow CO_{2\,(aq)} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{\,2-}$ (Doney et al. 2009)

To put it another way, processes in seawater are reversible and tend toward equilibria (Millero et al., 2002). According to Beaufort et al. (2011), ocean acidification is responsible for changes in the oceanic carbonate system, which affect CO_2, DIC, pH, alkalinity, and calcium carbonate saturation state. A net loss of CO_3^{2-} ions in the surrounding water is observed as CO_2 is sequestered in the ocean and H^+ concentrations rise (Bozlee et al., 2008). This occurs because HCO_3^- is formed when an excess of H^+ combines with CO_3^{2-} (Orr et al., 2005; Bozlee et al., 2008; Doney et al., 2009). As a result, when CO_2 is stored in the ocean, seawater pCO_2 rises, ocean pH falls, carbonate ions rise, and bicarbonate ions fall. Phytoplankton, marine invertebrates and fish may be affected by acidification because of the net reduction of carbonate ions (Fabry et al., 2008).

2.3 Ocean Acidification and Estuarine Ecosystem

Every part of the ocean, from the ocean to the estuary, could be affected by ocean acidification. This could have major implications for marine life (Orr et al., 2005) which might have substantial effects on marine biodiversity (Feely et al., 2010). Since estuaries get such a high volume of water, sediment, organic material, nutrients, and pollutants from both natural and manmade sources, they are particularly vulnerable to ocean acidification. There is more organic matter in estuaries now than there was before because of the rise in atmospheric CO_2 levels (The Royal Society, 2005; Waldbusser et al., 2013).

The baseline of pCO_2 in the ocean changes as a result of anthropogenic carbon inputs. Carbon dioxide (CO_2) released during breathing by species (animals, and microorganisms) dramatically affects the carbonate chemistry of shoreline and estuary waters. Net heterotrophy, the dominant process in many estuaries, causes elevated DIC and decreased pH (Gattuso et al., 1998). Due to these external factors, estuary water chemistry and pH are highly dynamic over space and time. The pH and saturation state (Ω) of calcium carbonate are both significantly lowered when episodic upwelling reaches estuaries (Feeley et al., 2010). The majority of changes in nearshore DIC happen during upwelling as a result of $CaCO_3$ dissolution, organic matter generation, and respiration (Fassbender et al., 2011).

Waldbusser et al. (2013) reported that the application of limestone and nitrogen fertilizer in agricultural operations, as well as the subsequent surface riverine flux, considerably alters the total alkalinity (A_T) and DIC in estuarine ecosystems. Increasing atmospheric CO_2 levels will have a multiplier effect on these alterations in carbonate chemistry. Acidification in estuaries may be made worse by the presence of other environmental characteristics (such as temperatures, salinity, and manmade pollution) that might influence the impacts of rising CO_2 levels in the atmosphere (Lannig et al., 2010; Nikinmaa, 2013). The saturation state (Ω) of calcium carbonate is decreased because river runoff dilutes total alkalinity (A_T) and calcium concentrations (Salisbury et al., 2008).

Kroeker et al. (2013) stated that long-term ocean acidification will have serious consequences for marine ecological systems. Therefore, it is crucial to learn how high CO_2 and low pH levels affect the fitness of marine creatures. Numerous physical and biological processes influence pH changes over a range of periods, particularly in the comparatively shallow and productive near shore region (Booth et al., 2012).

2.4 Ocean Acidification and Shellfish

According to (Talmage and Gobler, 2009), the early life phases of shellfish larva are particularly sensitive when rising partial carbon dioxide, with significant decreases in survivability and slowdowns in metamorphic under rates anticipated to emerge later within a century (Kurihara et al., 2007; Watson et al., 2009). Reduced pH can disrupt calcification by changing the thermodynamics of carbonate minerals and affecting other physiological processes in marine calcifiers (Pörtner, 2008). Many shellfish develop calcareous shells, and rising CO_2 levels have been shown to damage juvenile and adult clams, mussels, and oysters (Green et al., 2004; Gazeau et al., 2007; Kurihara et al., 2007).

Doney et al. (2009) state that research on the impacts of anticipated elevated CO_2 on larval shellfish are consistent with current ocean acidification studies, which suggest that calcifying animals would endure decreased survival and growth, as well as deformed $CaCO_3$ shells and hard portions. Although it is evident that calcifying ocean invertebrates like shellfish are vulnerable to global CO_2 rises, the extent to which the CO_2 increase that has arisen since the onset of the industrial revolution has impacted these populations is unclear. New studies suggest that bivalves in estuarine areas may also be at risk from ocean acidification (Gazeau et al., 2007; Kurihara, 2008; Talmage and Gobler, 2009). Seasonal releases of excessive acidic riverine water would increase acidification in estuaries, according to previous research by Salisbury et al. (2008), potentially poses a significant threat to shellfisheries.

Salisbury et al. (2008) found that seasonal flows of acidic riverine water will enhance acidification in estuaries, which could represent a danger to shellfish industries. Weiss et al. (2002) hypothesized that some bivalve larvae (such as *Crassostrea gigas* and *Mercenaria mercenaria*) produce an even more soluble amorphous $CaCO_3$ as a transient precursor to crystalline aragonite during biomineralization and are consequently less resilient to a range of stresses than adults.

Kurihara et al. (2007) found in their research that the embryonic growth and shell development in *Crassostrea gigas* were found to be inhibited by very high pCO_2 during the first 48 hours following conception (2268 μatm). Environmental chemical and physical cues may delay settling and metamorphosis in invertebrate larvae. Possible stresses that could induce a delay in metamorphosis include extremes in temperature, salinity, nutrition, low dissolved oxygen, pollution, and UV radiation. Furthermore, similar stressors have a greater impact on larvae than on adults (Pechenik, 2006).

2.5 Ocean Acidification and Fish Larvae

Marine calcifying invertebrates, such as mollusks, bivalves and cnidarian, have been the primary focus of studies on the impacts of ocean acidification (Heuer and Grosell, 2014). Although fish were previously neglected, a great collection of research on the impacts of acidification of the oceans on a diverse variety of organisms has been published. In particular, ocean acidification will have a dramatic impact on the health and survivability of fish larvae (Figure 02).

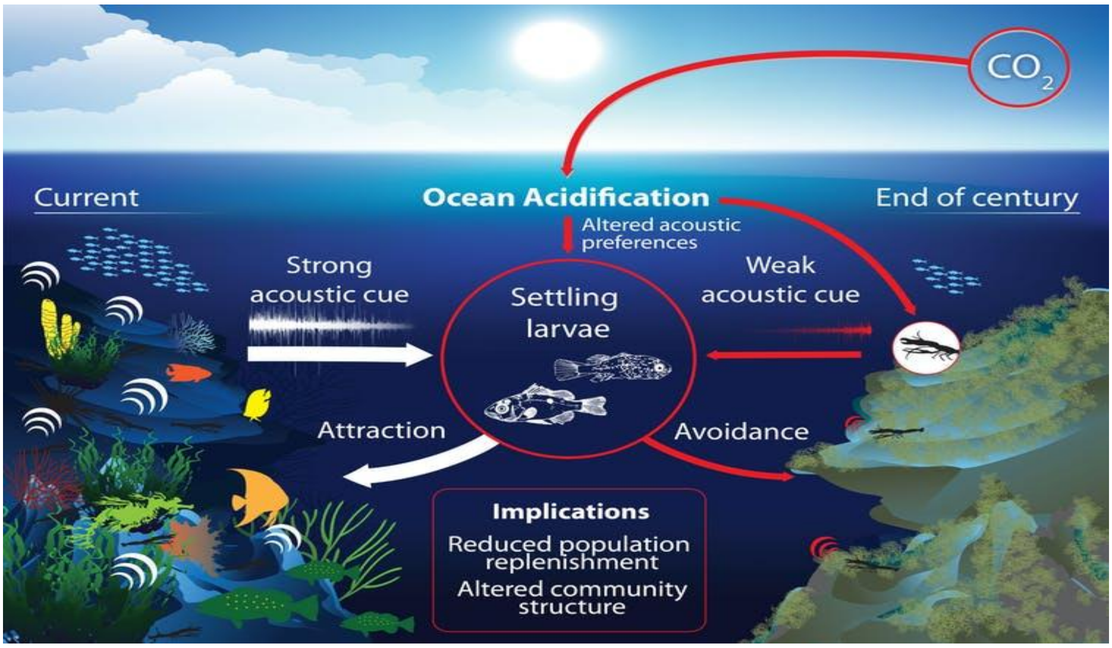

Figure 2. A diagrammatic representation of the direct and indirect effects of acidification on fish larvae (Rossi, 2016)

It is common knowledge that the early development of marine species can be negatively impacted by ocean acidification. So far, most studies on CO_2 induced acidification have focused on open ocean environments, paying little attention to estuaries and other low-salinity, shallow coastal ecosystems in temperate zones. Because fish eggs and larvae are more sensitive to pH changes as juveniles, hence these early life stages are the most likely to face substantial consequences of ocean acidification. Ocean acidification is most likely to have a severe impact on these stages of fish development (Brown, 1989).

Harvey et al. (2013) predict that in the next 50-100 years, the responses of marine creatures to ocean acidification will become increasingly apparent, and most research implies that these responses are predicted to vary since marine organisms have varying responses and degrees of resistance. Higher concentrations of CO_2 dissolved in saltwater tend to decrease physiological pH values in animals that come into contact with it. Cells primarily buffer intracellular fluids, transport important ions, transport carbon dioxide (CO_2) via respiration pigments, and restrict metabolism in response to internal acidification (Seibel and Walsh, 2003; Clairborne et al., 2002).

Larvae face severe cumulative mortality during this early life stage, some of which are driven by gradual shifts in growth, predators, and selected pressure (Houde, 1989). For both pelagic (foraminiferans, pteropods, coccolithophores, marine fish, and mammals) and benthic (corals, echinoderms, mollusks, and crustaceans) taxa, ocean acidification has been shown to have deleterious effects on survival, metabolism, calcification, growth, reproduction, and immune responses (Kroeker et al., 2013).

Fish larvae are thought to be more vulnerable to environmental perturbation because many of the intricate physiological traits that enable juvenile fishes to acclimate to changes in the environment are absent in fish larvae. For this reason, due to the absence of these kinds of physiological characteristics, they are more susceptible to environmental perturbation (McKim, 1977). Impacts on fish embryonic survival, growth, and development have been noted in recent research (Monday et al., 2009; Baumann et al., 2012; Pimentel et al., 2015) as have effects on the biochemical and physiological responses of fish. The early stages of life have been extensively researched because of the likely influence of elevated pCO_2 on these phases (Brauner, 2020). Species susceptibility to ocean acidification has been hypothesized to be associated with the degree to which their preferred environment experiences seasonal changes in CO_2 levels (Munday et al., 2011).

Physiological homeostasis in marine organisms is significantly affected by environmental factors such as salinity, temperature, dissolved oxygen, and pCO_2 (Stapp et al., 2015). Early life cycle stages are thought to be the most dangerous because physiologic homeostasis may not have properly formed and their small size makes them more susceptible to environmental change (Ishimatsu et al., 2008). The latter means that young larvae cannot escape exposure to poor environmental conditions. Importantly, high and variable mortality during the early life history stages, often related to environmental conditions, may be decisive in generating recruitment variability (Houde, 2009). Elevated pCO_2, ranging from 800 to 5000 µatm can result in developmental abnormalities of larvae, such as tissue and organ damage, and changes to fatty acid composition (Chambers et al., 2013).

Fish larvae have been studied extensively, and low pH has been shown to drastically reduce their chances of survival (Kikkawa et al., 2003). Various studies demonstrate the excellent results of fish larval activities along with low pH. Embryonic and larval stages may be especially susceptible to changes in environmental pH because of their high specific surface area ratio and lack of specialized systems for acid-base regulation (Ishimatsu et al., 2008). Disturbances in the olfactory system (Dixson et al., 2010), vision (Chung et al., 2014), lateralization (Domenici et al., 2012), hearing (Simpson et al., 2011) and activity levels have all been suggested by the findings (Munday et al., 2010). These changes are detrimental to predator-prey interactions (Domenici et al., 2012) and compass skills (Siebeck et al., 2015) which could lead to an increase in mortality (Munday et al., 2010). The ability to adapt for cellular acidosis caused by high pCO_2 may explain why marine fish appear to be quite tolerant of modest increases in ambient carbon dioxide (Ishimatsu et al., 2008).

Acidification has two effects on fish larval dispersal. It can induce developmental abnormalities that result in morphological malformations and organ damage, including damage to sensory organs, which can impede their function. Acidification also causes neurotransmitter dysfunction (Nilsson et al., 2012), which has been shown several studies to impair fish larvae's ability to use the sensory systems that are critical for ocean orientation. Swimming larvae will have a much smaller net effect on dispersal if they lack orienting abilities. The ability of larvae to determine depth will most certainly be harmed, resulting in less organized vertical distributions. This has a significant impact on dispersal consequences. It's also feasible that acidification will have an impact on other behaviors, such as swimming abilities that impact dispersal outcomes (Nagelkerken and Munday, 2016).

2.6 Ocean Acidification and Fish Behavior

Ocean acidification has been observed to disrupt the integration of sensory signals in fish, which frequently results in dramatic behavioral changes (Domenici et al., 2012). According to Briffa et al. (2012), ocean acidification is widely recognized as a severe threat to marine species due to its potential to impair critical behavior-mediated life-history processes. Behavior is the result of the interaction of the brain, muscles, and sensory organs, which are all linked by nerves.

The senses of fish, including sight, are affected by low pH (Ferrari et al., 2012). Available studies have shown that the high amounts of carbon dioxide (CO_2) forecast for the century's end can affect a variety of senses, including smell, hearing, and sight (Simpson et al., 2011; Briffa et al., 2012; Chung et al., 2014). Only the olfactory capacity of clownfish (*Amphiprion ocellaris*) larvae has been studied in depth across all stages of development, and only a small number of research papers have looked at the impact of acidification of the oceans on behavior in which was before larvae (Munday et al., 2010; Pimentel et al., 2014).

Cattano et al. (2018) found that fish can adjust to ocean acidification on the most fundamental physiologic levels, but this comes at the cost of changes to their senses and behaviors. The consequences of ocean acidification on a behavior known as lateralization are an interesting example. Individual fish tend to be "handed," or more comfortable turning in one direction compared to the other. Initial experiments with damselfish larvae showed that ocean acidification decreased lateralization, or "handedness" (Domenici et al., 2012). This is an illustration of how ocean acidification can affect cognitive processes at the most fundamental level.

Hamilton et al. (2017) observed that ocean acidification altered behavior in a fish from the same location that was investigated using a similar pH range. Therefore, it is not safe to presume that fish native to areas with naturally low or variable pH levels are less vulnerable to the effects of ocean acidification. It has also been shown that ocean acidification reduces predator avoidance by hampering vision (Ferrari et al., 2012; Chung et al., 2014). The distribution of fish and the dynamics between predators and prey could be affected by sensory factors that bring about such behavioral shifts.

Briffa et al. (2012) found that reef fish and sticklebacks (*Gasterosteus aculeatus*) exhibited a decrease in aggressiveness, lateralization, and learning capabilities when exposed to high CO_2. In addition, increased CO_2 levels were found to have a systemic influence on the brains and cognition of marine fish, as seen by their poorer responses to both auditory and visual threats.

Chivers et al. (2014) demonstrated that larval clownfish *(Amphiprion ocellaris)* and damselfish (*Pomacentrus wardi*) are particularly vulnerable to the effects of elevated pCO_2 levels on sensory experiences and neuronal performance. The Gamma-aminobutyric acid type A (GABAA) receptor is a key inhibitory neurotransmitter receptor in the brains of vertebrates.

2.7 Ocean Acidification and Fish Physiology

Ocean acidification can present a physiological challenge for marine fish because the reduced outward diffusion gradient of CO_2 from the body to the seawater can result in higher CO_2 and lower pH in the blood plasma and intracellular fluids (Perry and Gilmour, 2006). The effects of climatic changes on marine fish are often linked to increased disease incidence and mortality due to direct physical stress brought on by factors including lower pH, temperature increases, and decreasing oxygen levels (Crozier and Hutchings, 2014). Existing research on the consequences of high CO_2 on fish reveals considerable alterations in several critical physiological systems, including respiration (CruzNeto and Stevenson, 1997), blood circulation (Lee et al., 2003), and metabolism (Perry et al., 1988).

The respiratory and circulatory systems are the most directly affected by pH in fish physiology. Specifically, fish physiology is most sensitive to changes in pH in the respiratory and cardiovascular systems. Like human lungs, fish gills filter out dissolved oxygen from the water and flow it directly into the bloodstream. These gases and other elements necessary for life are subsequently distributed throughout the body via blood circulation (Brauner and Baker, 2009). Dissolved carbon dioxide in seawater can diffuses through epithelial surfaces, potentially upsetting the acid-base balance of an organism. It is believed that changes in the organism's acid-base status greatly affect the physiological effects of elevated partial carbon dioxide.

Pörtner (2008) and Heisler (1986) showed that environmental hypercapnia causes a transient but considerable reduction in arterial pH while the pCO_2 remains high. Within 3-24 hours, this fall in blood pH is accounted for by the exchange of acid-base-related ions with the surrounding water, primarily through the gills, thanks to biochemical balancing by proteins and other low-molecular-weight buffering molecules. Stronger acid-base control shouldn't endanger a fish's life, but there is a biological cost associated with acid-base regulation just as there is with every other physiological response (Heuer and Grosell, 2014).

Osmoregulation is thought to cost marine fish between 6 and 15 percent of their resting oxygen intake. Energy expenditure for acid-base control on top of this baseline cost for osmoregulation would be required if seawater pCO_2 were to increase. Fish emit extra H^+ ions into the surrounding water through many epithelia (gills, kidneys, gut) when body fluid pH drops below a certain critical value (Heisler, 1986). Extracellular acid-base regulation is typically more efficient in fish than in invertebrates (Widdicombe and Spicer, 2008).

Evans et al. (2005) observed that fishes may have to expend more energy on physiological adjustments in response to increases in ambient pCO_2, particularly in acid-base control and cardio-respiratory control. In the face of increasing atmospheric CO_2, adaptive physiological tolerance of larvae may not be adequate to support populations of calcifying benthic species, including such widely distributed commercially significant fisheries (Miller et al., 2009).

MATERIALS AND METHODS

This chapter mainly provides a detailed description of the research project area (study area) and discusses the various systematic techniques that are used to conduct this research project.

3.1 Study area

The Bakkhali River is one of the most significant rivers in the Cox's Bazar district of Bangladesh, which is located on the southeastern coast of the Bay of Bengal. This waterway is recognized as a significant intermediate route for the Maheskhali channel. This research was conducted in the Bakkhali river estuary with monthly sampling from January 2021 to December 2021 (Figure 03).

Figure 03. Bakkhali River Estuary

3.2 Site Selection

Geographical coordinates of the selected area were recorded by using "GPS coordinates" software, and a map was constructed by using QGIS (version 3.4.5) (Figure 04) and sampling was conducted in the morning in the middle of each month. The approximate geographical location of this study area was between 21.487971° N and 91.965496° E.

Figure 04. Map of Cox's Bazar region and study site

3.3 Sampling procedure

Fish larvae were collected from the selected spot by Bongo Net (0.50 m mouth diameter, 1.3m long, and 500µm mesh at the body). During the sampling in the river, a flow meter (Model: KC Denmark A/S 23.090-23.091) was affixed to the net's mouth to measure the amount of seawater passed during each tow. The sample time was approximately 10 minutes of surface tow-in daylight. The collected specimen was preserved in 90% ethanol and transported to the Aquatic Ecology laboratory at Chattogram Veterinary and Animal Sciences University for sorting out based on morphology and other attributes.

3.4 Fish larvae sorting

Usually, for taxonomic identification, larvae were sorted from the whole sample. The first step of sorting was to discard ethanol from the sample. To do this, samples were sieved through meshes of 0.1 mm and thoroughly washed with distilled water so that sand particles, plastics, leaves, and other unwanted matters could easily be removed. Washed larvae were placed in a jar with fresh 90% ethanol, and each specimen was put one by one in a petri dish to be analyzed under a stereo microscope (OPTIKA ITALY C-B3), at low magnification (10x) and several pictures were taken. Each image was assigned a unique code so that it could be easily found later.

3.5 Morphological identification of fish larvae

Using the stereo microscope (OPTIKA ITALY C-B3), fish larvae were identified to the family level considering characteristics of related taxa supplied by Leis and Rennis (1983) and Leis and Carson-Edwart (2000). The body structure, coloring pattern, and meristic and morphometric traits are the most significant characteristics to identify larval fish.

3.6 Estimation of fish larval quantity

To measure the amount of water flowing through the Bongo net, a flow meter was attached to it. Later, each fish larva was separated from the zooplankton. The samples were standardized based on the number of fish larvae found per 1,000 m^3 of filtered seawater. The quantity of fish larvae per 1000 m^3 is calculated using the equation (Lirdwitayaprasit et al. 2008) below:

The volume of water passed in each sampling=

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

Where,

Diameter of the bongo net, d= 0.50m

So, net radius, r = 0.25m

Net opening area= πr^2

$=3.1416\times0.25^2$

$=0.19635\times2$; as each net has two openings

$=0.3927$

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

3.7 Determination of water quality parameter

Gas-tight bottles were used to collect seawater, preventing air bubbles from getting trapped when the bottles were sealed to measure pH and alkalinity. Water samples were taken using a long-weighted tube for integrated samples. Electronic Prove was used to estimate dissolved oxygen (DO) and temperature on the spot.

3.7.1 Temperature

Water temperature was determined by using a Celsius thermometer.

3.7.2 pH

Water pH value was determined by using pH meter (YSI pH100A).

3.7.3 Salinity

Salinity was measured by using refractometer meter (PC Stester 35). The practical salinity scale was used to measure salinity, which is expressed in practical salinity units (PSU).

3.7.4 Alkalinity

Total alkalinity was measured using the titrimetric method with diluted H_2SO_4. To measure the alkalinity 50 ml of sample water was taken into a conical flask. Then 2-4 drops of phenolphthalein indicator were added in the sample. As the color of the sample didn't change, it indicated that phenolphthalein alkalinity was absent. After that fresh 50 ml water sample was taken into another flask and 2-4 drops of Methyl Orange indicator were added in the sample. The color turned into yellow. Then the sample was titrated against standard H_2SO_4 (0.02N). Titration was continued until the yellow color turned into pink. The required amount of acid (H_2SO_4) was recorded and the result was calculated by the following formula:

$$\text{Alkalinity} = \frac{\begin{array}{c}\text{Acid used(ml)} * 0.02\text{N(Normality of acid)} \\ * 50\text{(Gram Equvalent weight of CaCO3)} * 1000\end{array}}{\text{Sample volume}}$$

3.8 Determination of ocean acidification factors

The other parameters of ocean acidification were deduced using R programming language and CO2SYS software. The program was parameterized specifically with the two dissociation constants K_1 and K_2 for carbonic acid in coastal waters as of Waters et al. (2014). The concentrations of acid-base compounds in seawater may be estimated using salinity, temperature, and two of the four CO_2 related factors: total dissolved inorganic carbon (DIC), total alkalinity (A_T), H^+ concentration, and pCO_2 are also factors to consider.

3.9 Data analysis and interpretation

The collected data were double-checked, coded, summarized, classified, and entered into a database with Microsoft Excel 2019. Those data were analyzed using SPSS (Version 22.0), and Programming Language R (Version 3.6.3), which were used to perform all statistical analyses.

PHOTO GALLERY

Plate 1. Bongo net

Plate 2. Bongo net operation

Plate 3. Sample of Fish larvae

Plate 4. Sorting of fish larvae

Plate 5. Counting of fish larvae

Plate 6. Identification of fish larvae

Plate 7. Observation of fish

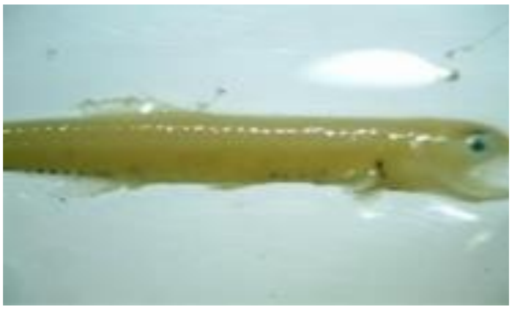

Plate 8. Engraulidae

Plate 9. Sillaginadae

Plate 10. Clupeidae

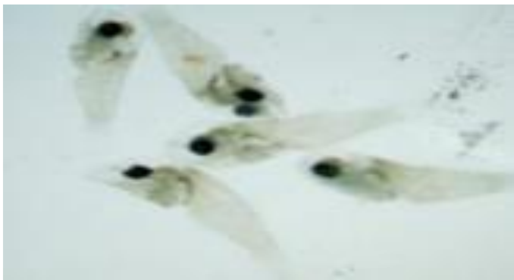

Plate 11. Ambassidae

Plate 12. Gobiidae

Plate 13. Labeling and documentation of fish larvae

Plate 14. Determination of Alkalinity

RESULTS

The results of the experiments under the field condition have been presented sequentially in consistence with the objectives set forth to reach a conclusion regarding the effects of acidification and changes in fish larvae at the Bakkhali River, Cox's Bazar coast. The study has been presented and compared in this chapter through different tables, figures, and appendices. These results have been presented and character-wise discussed and possible interpretations have been given under the following headings.

Table 1. Acidification state of the Bakkhali River (Jan'21 to Dec'21)

Seawater measurements					Calculated factors for acidification			
	Temperature (°C)	**pH**	**Salinity (psu)**	**Alkalinity (mg/l)**	***p*CO_2 (µatm)**	**DIC (mol/kg)**	**ΩAragonite**	**ΩCalcite**
Mean	28.85	8.267	24.117	117.75	143.9857	0.00094686	2.2771	3.5397
Std.	2.1898	0.2146	6.604	40.53	102.274	0.00038532	0.7552	1.1109
Min.	25.1	8	9	59	19.3642	0.00048363	0.86174819	1.3987
Max.	31.5	8.8	32.5	185	360.6499	0.00158147	3.4319	5.2122

4.1 Temporal Variation of Water Quality Parameters

4.1.1 Temperature

The temperature varied from 25.1-31.5 ºC from January to December 2021 (Figure 05). The maximum temperature was recorded 31.5 ºC in April followed by 31.1, 30.8, 30.7, 30.2, 29.5, 29.1, 28.8, 27, 26.5, and 26 ºC in June, September, November, May, July, March, February, October, August and January 2021. The minimum temperature was recorded 25.1 ºC in December 2021. The mean temperature was 28.85 ± 2.1898 °C.

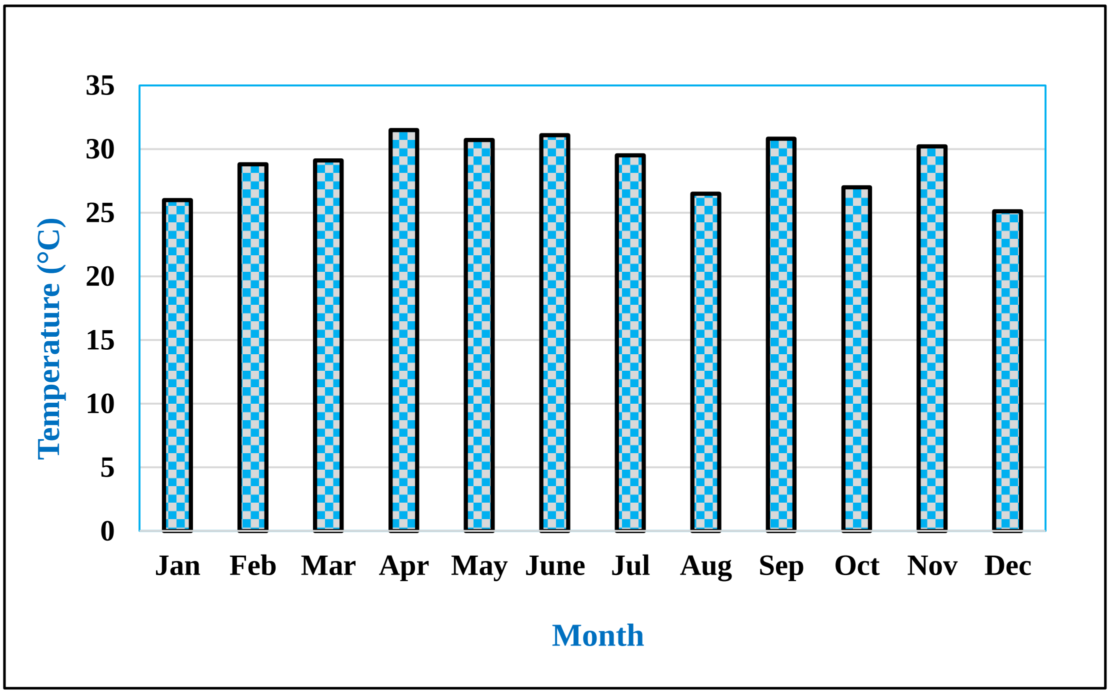

Figure 05. Monthly variation of temperature

4.1.2 Salinity

The salinity varied from 9-32.5 PSU from January to December 2021 (Figure 06). The maximum salinity was recorded 32.5 PSU observed in November followed by 32, 29.6, 28.5, and 26.7 PSU noted in April, March, February, January, 24 PSU May, June, October, and 21, 20, 18.1 PSU recorded in December, July, August 2021. The minimum salinity was recorded 9 PSU in September 2021. The mean salinity value was 24.117 ±6.604 PSU.

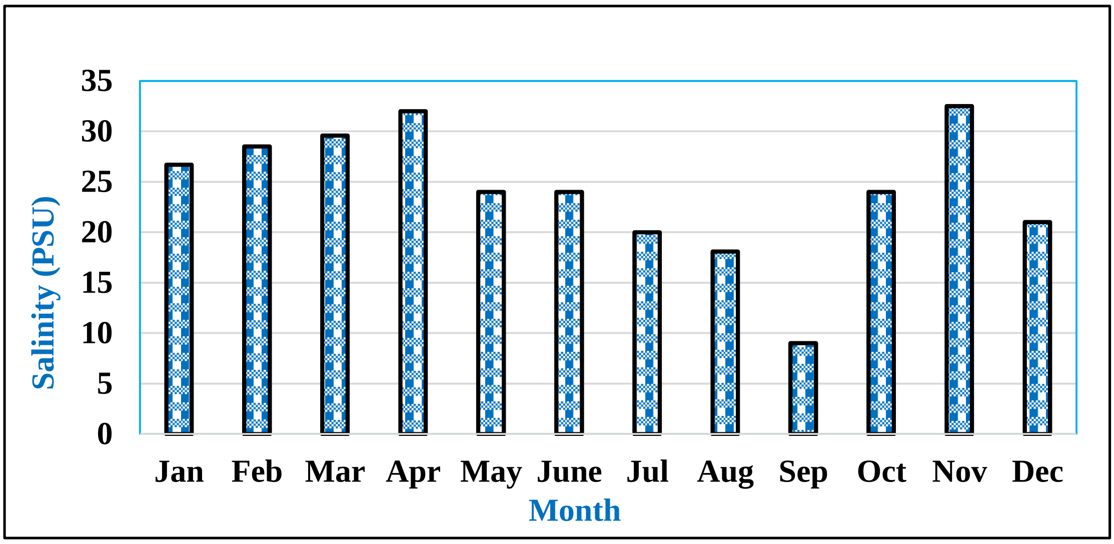

Figure 06. Monthly variation of salinity

4.1.3 Alkalinity

The alkalinity varied from 59-185 mg/l from January to December 2021 (Figure 07). The maximum alkalinity was recorded 185 mg/l in February followed by 170 mg/l recorded in January, and March, 134, 128, 111, 104, 98, 96, 88, and 70 mg/l alkalinities in December, April, May, November, June, October, July, September, respectively in 2021. The lower alkalinity values was recorded at 59 mg/l in August 2021. The mean alkalinity was 117.75 ± 40.53 mg/l.

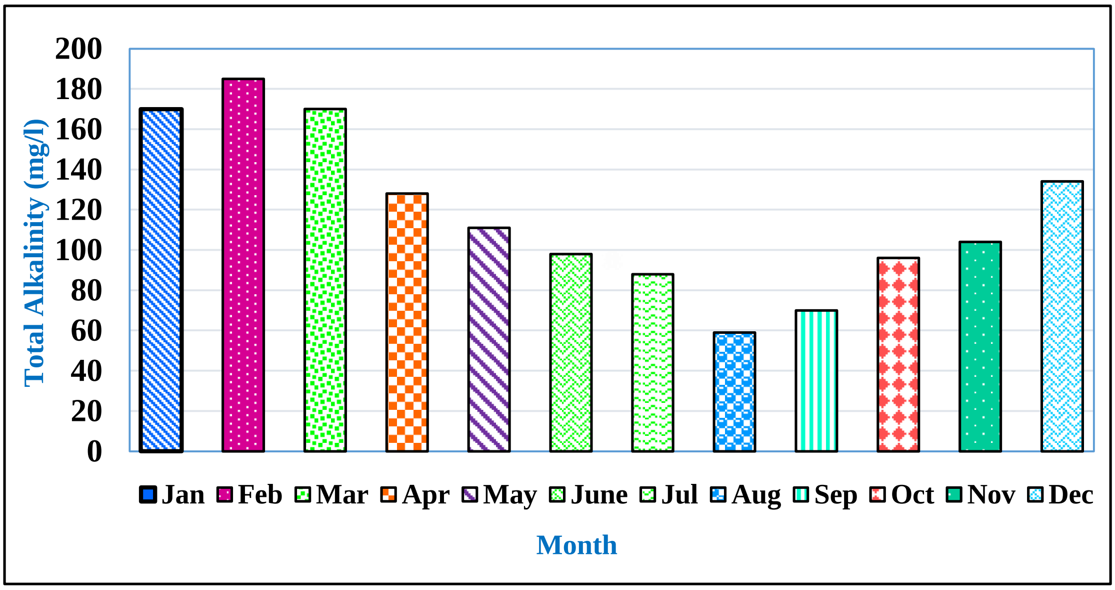

Figure 07. Monthly variation of alkalinity

4.1.4 pH

The pH varied from 8.0-8.8 from January to December 2021 (Figure 08). The maximum pH values was 8.8 in September followed by 8.5, 8.4, and 8.3 pH observed in November, June, and April, and 8.2 pH noted in March, July, August, October, December, and 8.1 pH recorded in February 2021. The minimum pH values was 8 in January. The mean pH value was 8.267 ± 0.2146.

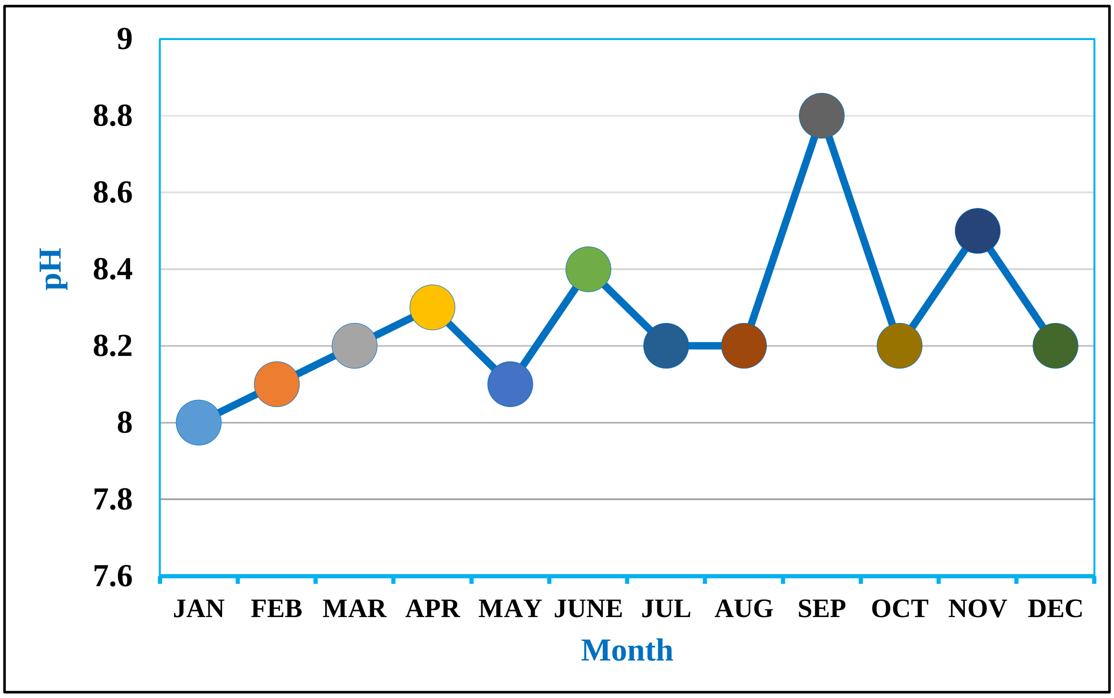

Figure 08. Monthly variation of pH

4.1.5 Monthly comparison of hydrological parameters of Bakkhali River

The mean temporal variation in the concentration of physico-chemical parameters are presented in Figure 09. The seasonal temperature variation were small, the maximum temperature was recorded 31.5 °C in April and the minimum temperature was recorded 25.1°C in December. Unlike temperatures, salinities in the estuary vary greatly seasonally. The salinity varied from 9-32.5 PSU from January to December 2021. The variation in mean pH is approximately similar throughout the study period. The highest pH measurement was recorded 8.8 in September, while the lowest was 8 in January. Within the lower alkalinity portion of the estuary, salinity and temperature levels display distinct variations. The lower alkalinity values was recorded at 59 mg/l in

August. These variations may be largely attributed to changes in river flows and, consequently, to variations in the ratios of the source waters.

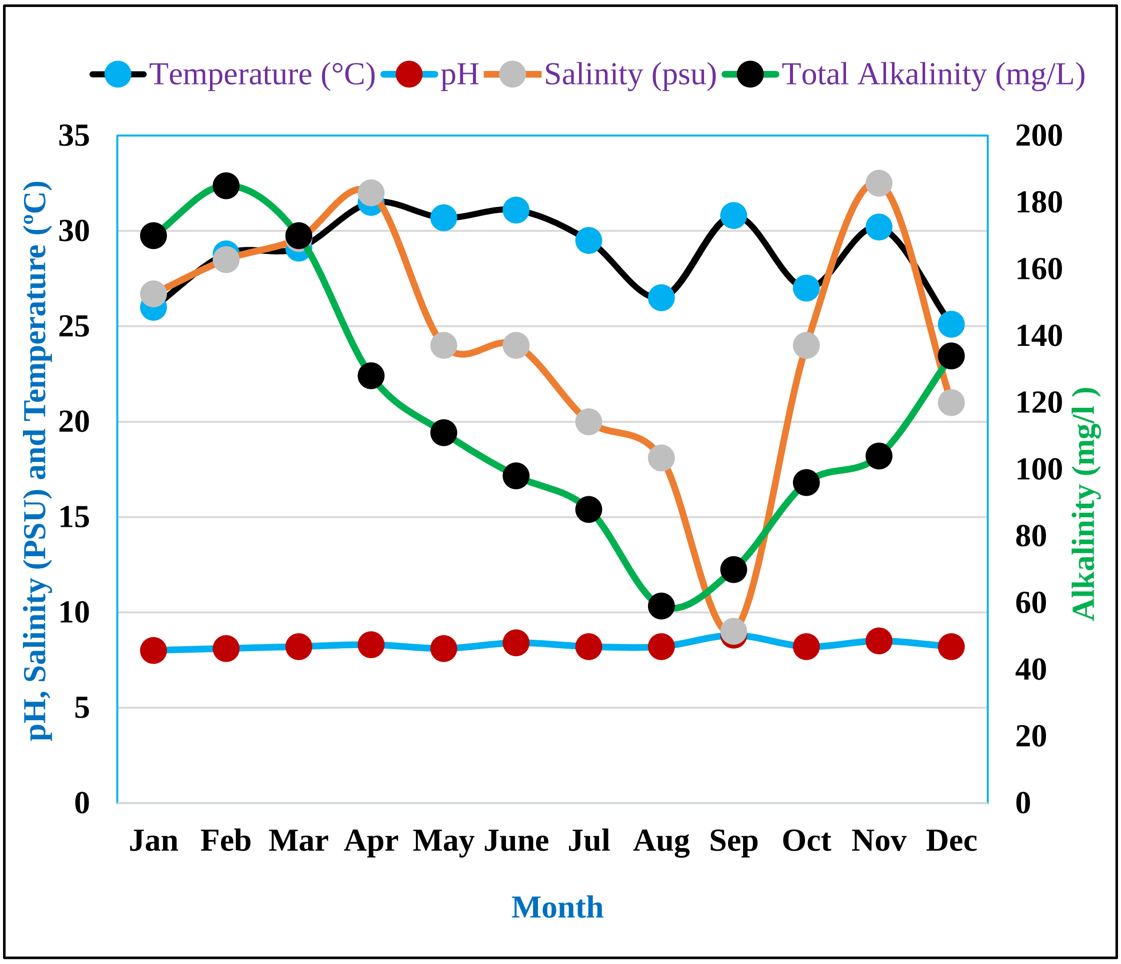

Figure 09. Monthly comparison of hydrological parameters

4.2 Temporal Variation of Ocean Acidification Factors

4.2.1 DIC (Dissolved Inorganic Carbon)

The DIC ranged from 0.00048363-0.00158147 mol/kg from January to December 2021 (Figure 10). The highest value of DIC was recorded at 0.0016 mol/kg in February and followed by 0.0015, 0.0014, 0.0012, DIC value was stated in January, March, December 2021, 0.0009 mol/kg DIC value was stated in April, May, October, 0.0007 mol/kg DIC value was noted in June, July, 0.0006 mol/kg DIC value was noted in November 2021. The lowest value of DIC was recorded at 0.0005 mol/kg in August. The mean DIC value was 0.00094686±0.00038532 mol/kg.

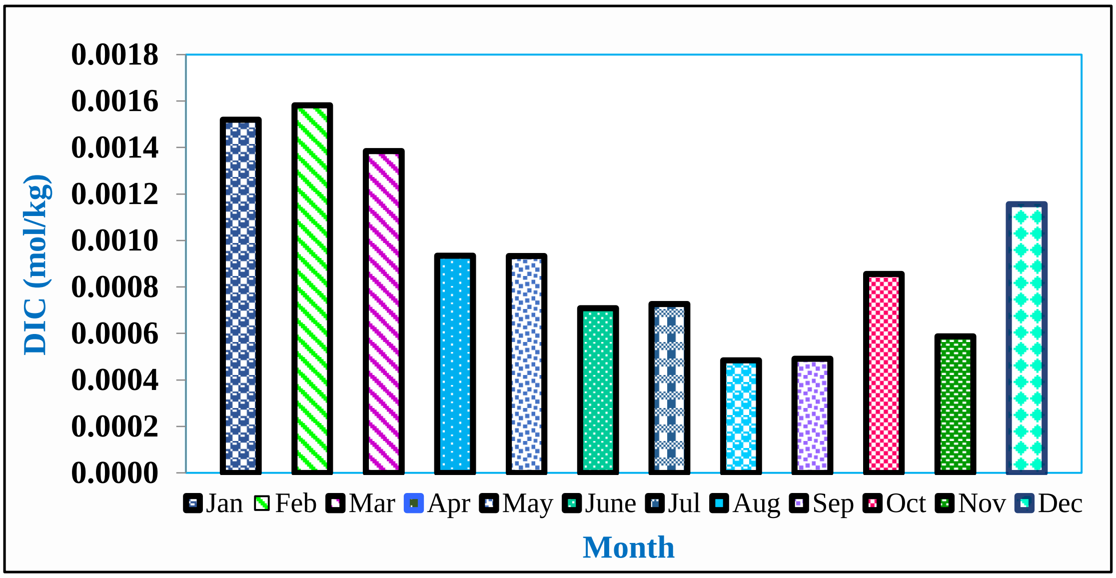

Figure 10. Monthly variation of DIC

4.2.2 ΩAragonite

The ΩAragonite ranged from 0.8617-3.4319 from January to December 2021 (Figure 11). The highest value of ΩAragonite was recorded at 3.4319 in March and followed by 3.1711, 3.1074, 2.7241, 2.4976, 2.3368, 2.0945, 2.0482, 1.7539, 1.8023 and 1.4956 value of ΩAragonite was observed in April, February, November, June, September, January, December, October, May, and July, respectively in 2021. The lowest value of ΩAragonite was noted at 0.8617 in August 2021. The mean value of ΩAragonite was 2.2771±0.7552.

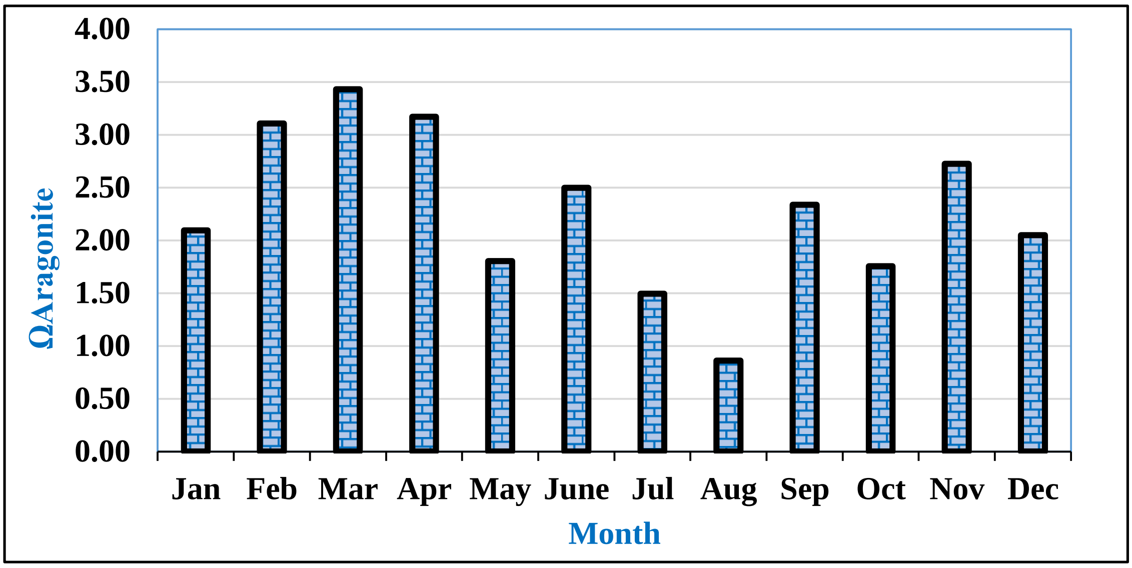

Figure 11. Monthly variation of ΩAragonite

4.2.3 ΩCalcite

The ΩCalcite ranged from 1.3988-5.2122 from January to December 2021 (Figure 12). The highest value of ΩCalcite was recorded at 5.2122 in March followed by 4.7428, 4.7419, 4.0862, 3.9917, 3.8623, 3.2779, 3.2478, 2.7906, and 2.3781 ΩCalcite was noted in February, April, November, September, June, December, May, January, October, and July. The lowest value of ΩCalcite was recorded at 1.3987 in August. The mean value of ΩCalcite was 3.5397±1.1109.

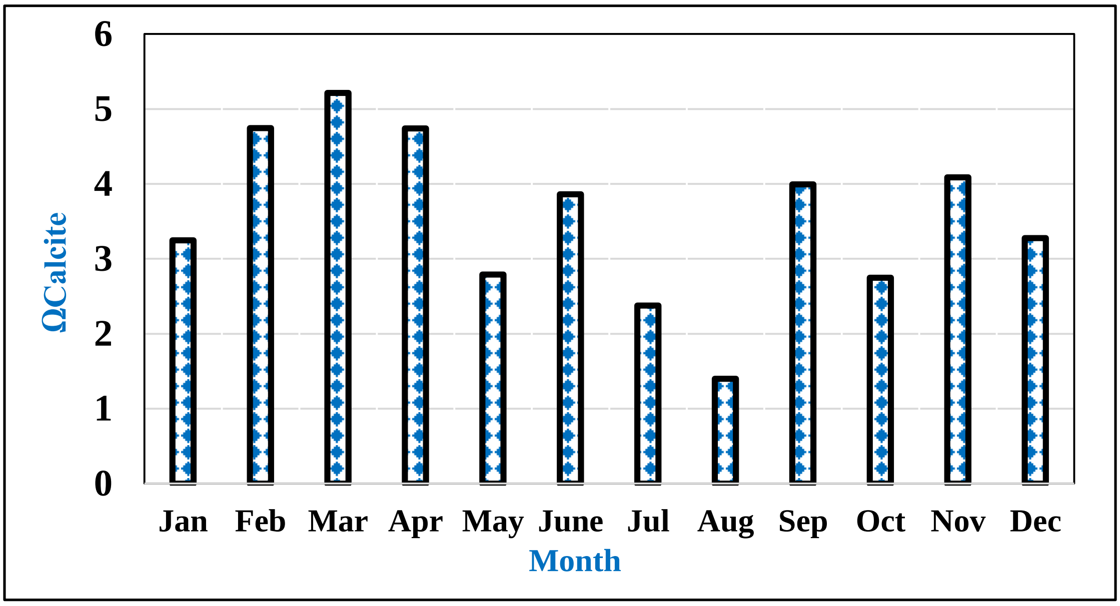

Figure 12. Monthly variation of ΩCalcite

4.2.4 *p*CO_2

The *p*CO_2 ranged from 19.3642-360.6499 µatm from January to December 2021 (Figure 13). The maximum *p*CO_2 was recorded at 360.6499 µatm in January followed by 288.8298, 193.1420, 179.5786, 176.8834, 126.5883, 113.7661, 97.1385, 75.8629, 61.5759, and 34.4489 µatm *p*CO_2 recorded in February, March, May, December, October, July, April, August, June, and November. The minimum value was recorded at 19.3642 µatm in September 2021. The mean value of *p*CO_2 was 143.9857 ±102.2742 µatm.

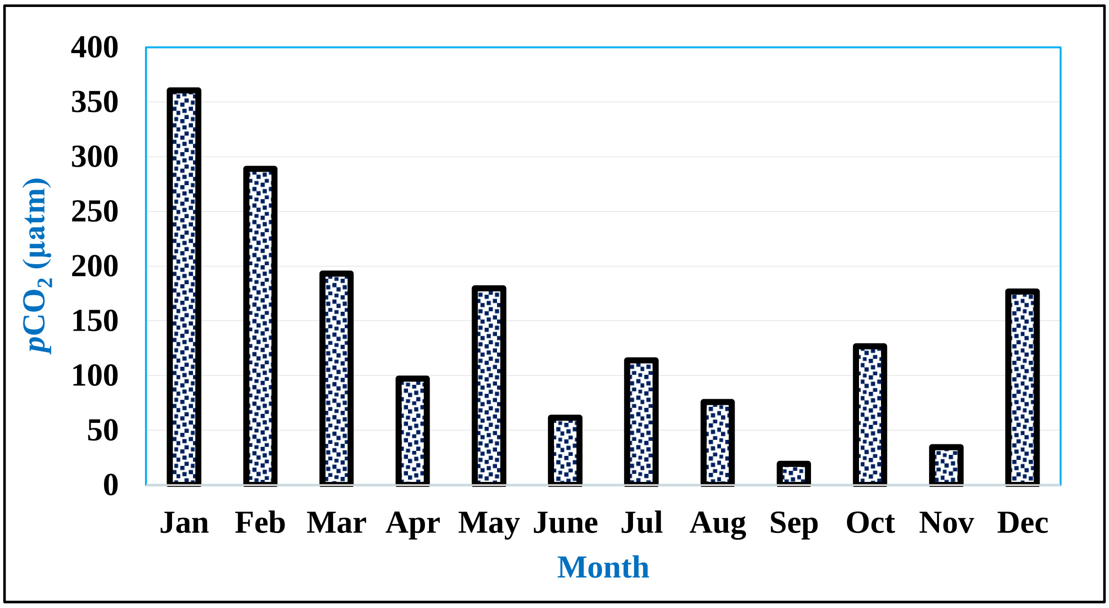

Figure 13. Monthly variation of *p*CO_2

4.3 Fish larval abundance

The highest number of fish larvae was recorded at 109 in August and followed by 99, 74, 58, 45, 37, 29, 28, 25, 21, and 19 fish larvae noted in July, September, June, March, May, January, April, October, November, and December 2021. The lowest number was recorded at 11 in February. The mean fish larvae was 46±32 (Figure 14).

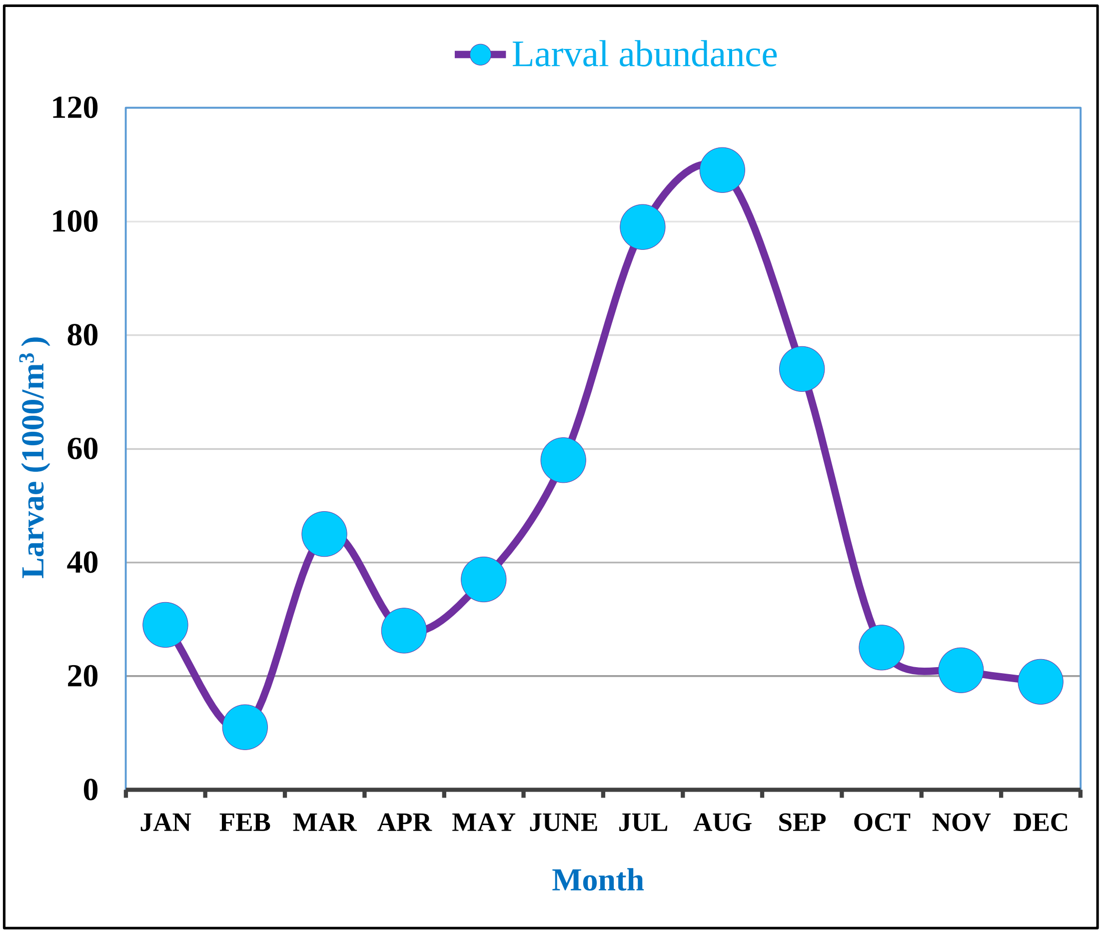

Figure 14. Monthly variation of fish larval abundance

4.4 Comparison between pH & *p*CO_2

An inverse relation between *p*CO_2 and pH was observed during the sampling period. The *p*CO_2 ranged from 19.3642 -360.6499 µatm from January to December 2021.The maximum *p*CO_2 was recorded at 360.6499 µatm in January. The minimum was recorded at 19.3642 µatm in September 2021 whereas the pH varied from 8.0-8.8 from January to December 2021. The maximum pH values were 8.8 in September, 8.1 pH was recorded in February 2021. The minimum pH value was 8 in January (Figure 15).

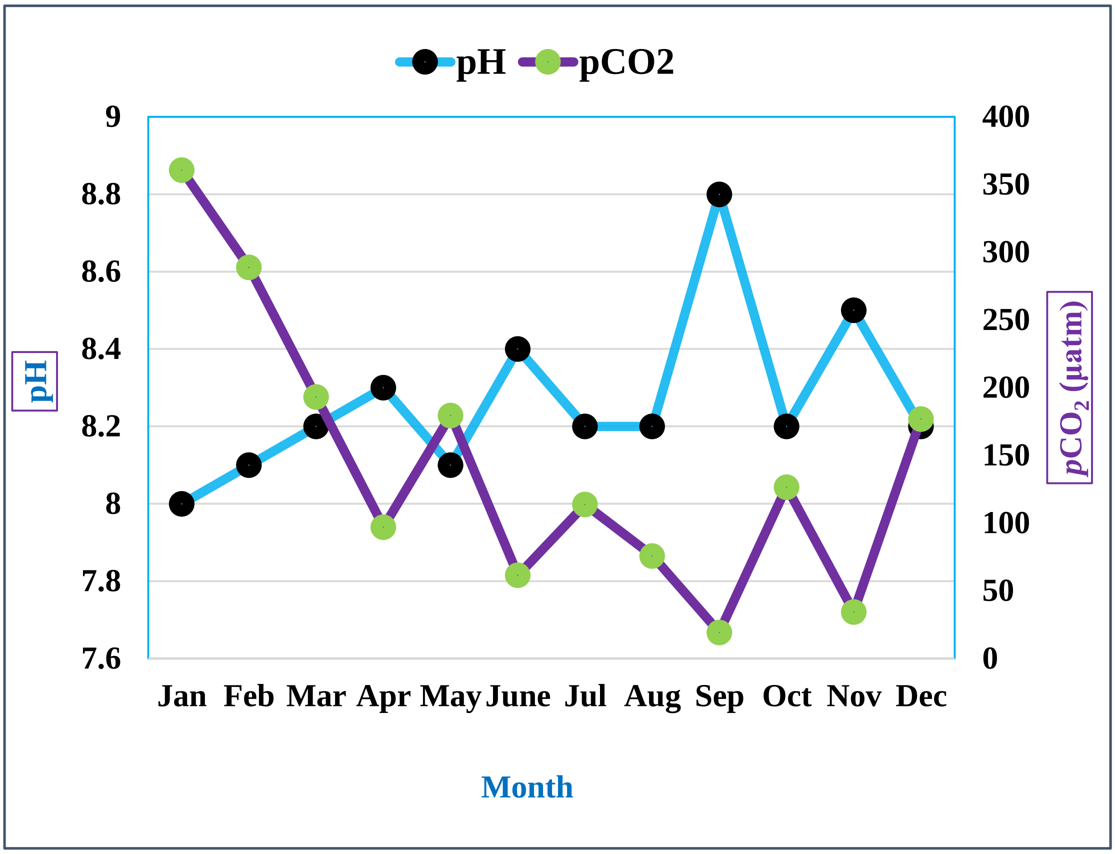

Figure 15. Comparison between pH and pCO_2

4.5 Relationship among different ocean acidification factors with fish larvae

The relationship of fish larval abundance with the different ocean acidification factors such as pCO_2, DIC, omega aragonite, and omega calcite were shown in scatterplot below (Figure 16). This Scatterplot matrix showed the negative relationship among different ocean acidification factors with fish larval abundance. The scatter plot matrix below demonstrates a positive correlation between DIC, omega aragonite, and omega calcite and pCO_2, but a negative correlation between these parameters and fish larvae. The availability of fish larvae was significantly changed by these variables as well.

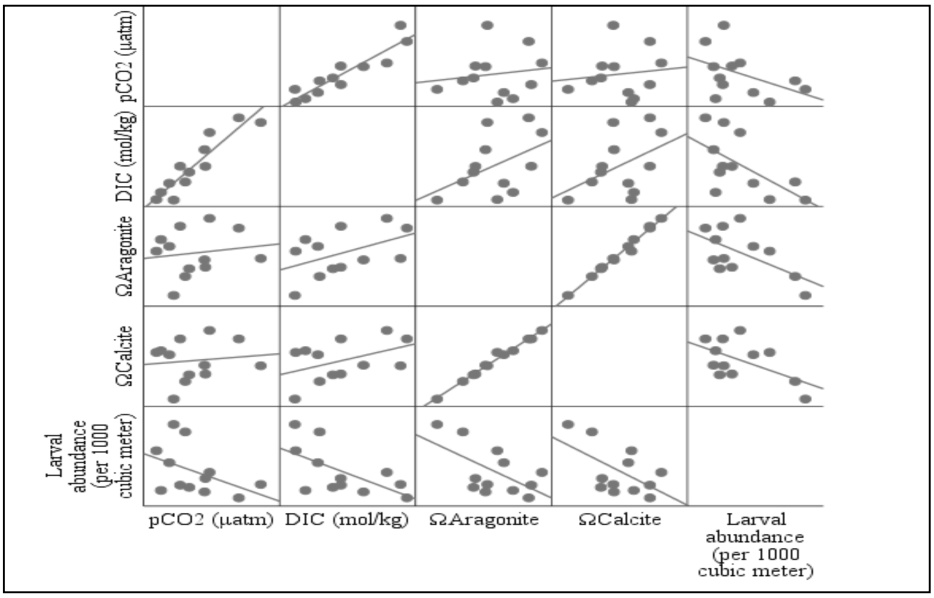

Figure 16. Scatterplot matrix of ocean acidification factors with larval abundance

4.6 Changes in larval abundance with pCO_2

This graph demonstrated a negative relationship between pCO_2 and fish larvae. The number of larvae increases when pCO_2 is low, and it decreases when pCO_2 is high (Figure 17).

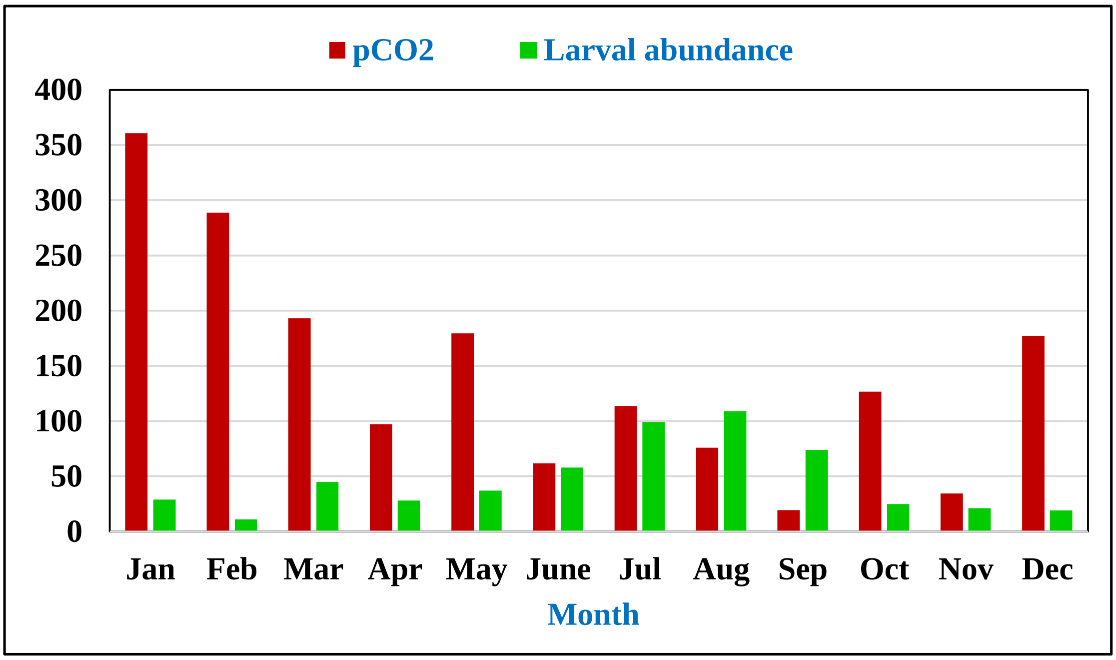

Figure 17. Changes in larval abundance with pCO_2

4.7 Relationships between pH, pCO_2, and fish larval abundance

Table 2. The correlations between pH, pCO_2, and fish larval abundance

Items	Regression equation	% Role of individual factor	F value	Significance	R^2 value
pH	y =0.0015x+8.1958	52.90	0.2300	$p<0.05$	0.5290
***p*CO2**	y=-1.4391x+210.55	20.56	0.4533	$p<0.05$	0.2056

Y, pH, and pCO_2; X, fish larvae.

A correlation study was done to establish the relationship between fish larvae and pH and pCO_2 in research study. From Table 2, it was revealed that a significant positive correlation was observed between pH and fish larvae ($p<0.05$), and a negative relationship was found in pCO_2 ($p<0.05$) (Figure 18 and Table 2).

It was evident that the equation y = 0.0015x+8.1958; y= -1.4391x+210.55 gave a good fit to the data and the co-efficient of determination $R^2 = 0.5290$, 0.2056 fitted regression line had a significant regression coefficient. In the case of pH, the relationship can be explained by the 52.90% increase of fish larvae contributed to enhancing the pH by 52.90%. On the other hand, for pCO_2, the negative relationship can be explained by the 20.56% increase in fish larvae contributing to enhancing pCO_2 by 20.56% accordingly.

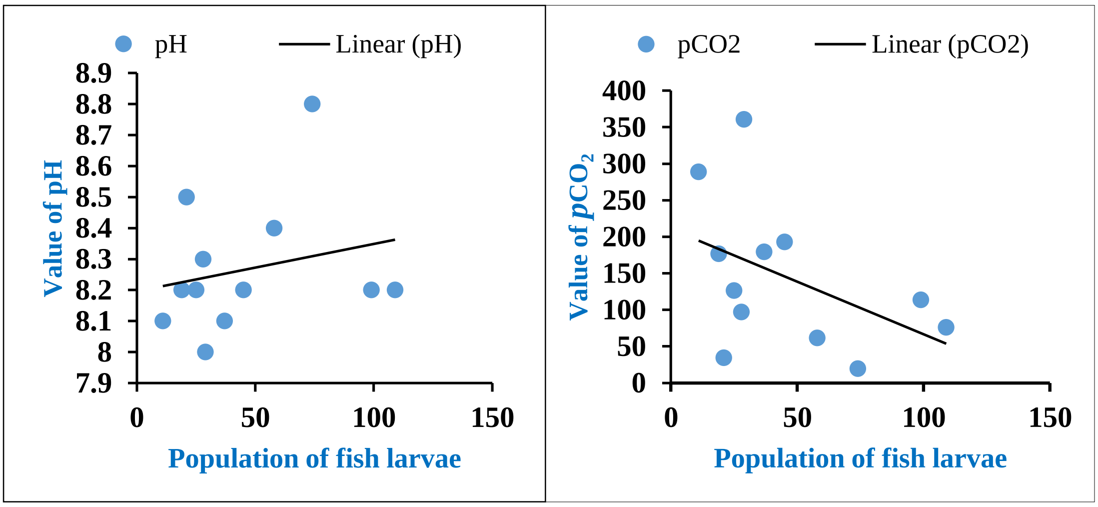

Figure 18. The relationships between pH, pCO_2, and fish larval abundance

DISCUSSION

5.1 Water Quality Parameters

5.1.1 Temperature

Water temperature is an essential physical element that influences chemical and biological processes in water. In this present study, the maximum temperature was recorded 31.5°C in April and the minimum temperature 25.1°C in December 2021. This range indicates the moderate fluctuations that occur in the present situation. According to Boyd (1990), aquatic species in the tropical and subtropical regions do not grow far below 26-28°C. This range indicates the moderate fluctuations that occur in the present situation. During a study of the water quality at Kalatoly in Cox's Bazar, the lowest temperature of 24.5°C and the highest temperature of 28°C were recorded in January and May, respectively (Aftabuddin et al., 2009).

Munday et al. (2008) demonstrated that temperature has been shown to have an impact on the development, growth, and survival of reef fishes, especially during the early larval stages. In general, higher temperatures typically shorten embryonic and larval lifespans while accelerating larval development (Sponaugle et al., 2006; Munday et al., 2009). Another research on coastal pollution revealed that the water temperature of the Bakkhali River on the Cox's Bazar coast ranged between 21.3°C and 27.5°C (Uddin, 2011). As a result, the water temperature in the study area proved optimal for aquatic life. This indicates that the complex interactions of temperature and acidification will eventually decide the effects of climate change on the larval stage of marine fishes.

5.1.2 Salinity

The maximum salinity was recorded 32 PSU in November and the minimum salinity 9 PSU in September 2021. Salinity is influenced by different factors in this study area. Salinity levels in the Bakkhali River water were observed to vary seasonally, with summer readings of 12 ppt and winter readings of 27 ppt (Raknuzzaman et al., 2018). Rashed-Un-Nabi et al. (2011) found in their research that the salinity levels of 2.33 and 5.33 ppt during the monsoon, 29.66 and 30.66 ppt during the winters, and 25.66 and 26.33 ppt during the pre-monsoon at two estuary sites varied widely. Salinity increase due to evaporation but decreases with the addition of freshwater through runoff or rainfall (Science Learning Hub, 2010). According to Rozengurt and Hedgepeth (1989), natural recruitment and species abundance in the Caspian Sea have changed owing to salinity increases. McAllister et al. (2001) also found similar results that change in species abundance as a result of increased salinity. Brinda et al. (2010) recorded the greatest abundance during the monsoon season from the Vellar estuary, and they concluded that more species were found during the monsoon season, which may favor low salinity and low temperature. The current findings are consistent with the previously mentioned publications.

5.1.3 pH

It is one of the most significant water chemistry parameters and is measured as the degree of acidity or alkalinity on a scale from 0 to 14. The value of pH is another key element of the aquatic ecosystem that impacts the distribution and abundance of aquatic organisms. This study found that the maximum density of fish larvae was observed with the highest pH value. The maximum pH values recorded 8.8 in September and the minimum pH values observed 8 in January 2021. The optimal pH range for all living things, especially aquatic life, is 6.0 to 9.0 (Kabir et al., 2020). According to (Swingle, 1967) acidic pH reduces the metabolic rate, growth rate, and other physiological activity of fish. This study showed the pH range was in optimal condition. According to Rashed-Un-Nabi et al. (2011), pH is a key factor in regulating the distribution and abundance of fish in the Bakkhali River estuary. Extreme pH levels have a deleterious impact on fish development and reproduction (Zweig et al., 1999), and can potentially result in mass death. Sensitivity to severe pH levels varies by fish species and age, with fish exhibiting less resilience during the embryonic and larval phases (Lloyd and Jordan, 1964). Some fish species that live in high pH environments must migrate to regions where the pH is near to neutral (Parra and Baldisserotto, 2007). Finally, marine oceanographers should investigate how short-term pH fluctuations influence marine life.

5.1.4 Alkalinity

Alkalinity refers to the buffering capacity of the water body which neutralizes acids and bases and maintains a stable pH level. The highest alkalinity values 185 mg/l in February and the lowest alkalinity values 59 mg/l in August 2021. Alkalinity shows a positive relationship between pH and salinity. With increasing salinity, the pH values also rise (Wong, 1979). This study showed alkalinity was more or less similar. The alkalinity of Bakkhali River was 146.85 mg/l, according to a study on the physicochemical evaluation of surface and groundwater quality in the larger Chittagong region of Bangladesh (Ahmed et al., 2010).

5.2 Ocean Acidification Factors

5.2.1 Dissolved Inorganic Carbon (DIC)

Dissolved inorganic carbon (DIC) comprised of CO_3^{2-} and HCO_3^- is a major constituent of sea water and an important indicator of ocean acidification. Nearly 90% of the dissolved inorganic carbon in surface seawater is found as bicarbonate ions, while just 1% is found as dissolved CO_2 (DIC). The highest value of DIC 0.00158147 was recorded in February and the lowest value of DIC 0.00048363 was recorded in August 2021. Sarma et al. (2015) stated in their study that DIC was distributed in a manner consistent with salinity ranging from 1840 to 1930 μmol kg $^{-1}$ in 1991 and 1936 to 2086 μmol kg $^{-1}$ in 2011. In 1991, DIC between the northern and southern coastal regions of the Bay of Bengal was lower (2 μmol kg $^{-1}$) than it was in 2011 (131 μmol kg $^{-1}$). This shows that between 1991 and 2011, the amount of DIC in surface waters at a rate of 1.6 μmol kg $^{-1}$ y^{-1} in the north and 8.2 μmol kg $^{-1}$ y^{-1} in the southwest Bay of Bengal. Spyres et al. (2000) noted that the ideal value of DIC for organisms is less than 0.002 mol/kg. It is said that this research illustrates the optimal DIC which is congenial fish larvae survival and this statement is agreed with Spyres et al. (2000).

5.2.2 ΩAragonite

The aragonite saturation state is commonly used to determine ocean acidification because it is a measure of carbonate ion concentration. Shells and other aragonite structures disintegrate when the saturation level of aragonite drops below 1, whereas species suffer when it drops below 3 (Jiang et al., 2015). The highest value of ΩAragonite was recorded 3.4319 in March and the lowest value of ΩAragonite was 0.86174819 noted in August 2021. This value represents an aragonite state that exceeds the optimum level in the Bakkhali River, but shortly this rate may also be altered due to atmospheric CO_2. The effects of under saturating aragonite on marine mollusks have often proven detrimental. Kurihara et al. (2007) found that the growth of *Crassostrea gigas* larvae was hampered throughout the stage of calcification and shell formation when seawater remained under saturated about aragonite (ΩAragonite =0.68).

5.2.3 ΩCalcite

Calcifying organisms are corrosive to under-saturated saltwater with a ΩCalcite of less than 1 (Corliss and Honjo, 1981). The highest value of ΩCalcite was recorded 5.2122 in March and the lowest value of ΩCalcite was recorded 1.3987 in August 2021. This value indicates a moderate level of ΩCalcite performed on this study site. The complex interrelated alterations to carbonate chemistry that occur during ocean acidification make determining which factors are most adversely harming biota challenging. The decrease may be the cause of many calcifying organisms' declines in development, calcification, population, and survival (Kleypas et al., 1999; Bednarsek and Ohman, 2015). Increases in surface temperatures of up to 4ºC over the next decade may mitigate the effect of carbon sequestration on global ΩCalcite and ΩCalcite saturation, making the impact of climate change on these quantities difficult to estimate (Andersson et al., 2008). Saturation levels of CO_3^{2-} and Ca^{2+} ($\Omega > 1.0$) suggest that enough of the ions are present to construct calcareous structures, while saturation values (1.0) are likely to promote dissolution or prevent the creation of calcareous structures (Fabry et al., 2008; Doney et al., 2009). The derived value of ΩCalcite was greater than 1 and so it was not dangerous for the calcifying organisms.

5.2.4 Partial Pressure of Carbon dioxide (pCO_2)

The larvae of fish are especially sensitive to increased pCO_2 due to cutaneous gaseous exchange and a deficiency of functioning gills. Similarly, as *p*CO2 rises, the external diffusion gradient of CO_2 from the fish muscle to the saltwater decreases, resulting in acidosis and disturbance of internal pH homeostasis (Rombough, 1988). Finally, the larval stages have the highest mortality rates, and the patterns of these stages are considered to have a large effect on recruitment into fisheries (Houde, 1987).

According to Kumar et al. (1996), low pCO_2 levels 275-400 µatm were seen in the coastal Bay of Bengal during the pre-monsoon seasons in both the southwest and northeast. Since there was a 50-100 µatm variation in the pCO_2 levels in the Bay of Bengal, it was less than the ambient value 355 µatm. As per reports, the Bay of Bengal was a significant atmospheric CO_2 sink in 1991. The pCO_2 levels were much higher in 2011 (342-504 µatm) than they were in 1991, with increases of 1.5 µatm per year in the south and 6.7 µatm per year in the northern coastal Bay of Bengal. The rates of change in the inorganic carbon components in the southwestern coastal Bay of Bengal were similar to those described elsewhere in the world (Astor et al., 2013; Church et al., 2013). As a result of recent increases in sulphate and nitrogen aerosol loadings in this area throughout winter and spring, the pH of surface waters decreased. In contrast, a substantially larger increase in pCO_2 (by an order of magnitude) was seen in the northwest coastal Bay of Bengal in 2011.

The present study reveals that the pCO_2 that are found in the sampling period in Bakkhali River waters is not in alarming condition for larval abundance for the whole year of 2021. But in January the pCO_2 was higher resulting lower larval abundance. Similar statement was given by Scanes et al. (2014).

5.3 The correlations between pH, pCO_2, and fish larval abundance

The acidification of the oceans driven by anthropogenic CO_2 emissions has already lowered marine pH. The effects of ocean acidification may be particularly harmful to fish larvae and habitat loss could exacerbate the detrimental consequences of ocean acidification upon coastal biodiversity. Threats posed by decreased ocean pH levels are urgent and alarming, yet acidification of the oceans is still a contemporary phenomenon and study is still in its infancy. Scanes et al. (2014) observed that larval abundance is negatively correlated with pCO_2 and level more than 300 µatm has negative impact for larval abundance. The impairment of several sensory organs and overall cognitive function in the larvae and juveniles of various fish species suggests that pCO_2 influences on central brain processing. The attraction of juvenile (mainly tropical) fish to predator scents and noises is reversed by exposure to increased pCO_2 (Simpson et al., 2011). Settlement-stage damselfish (*Pomacentrus wardi*) larvae grown at high pCO_2 (700-850 µatm) with a damaged olfactory system and poor anti-predator response behavior had 5-9 times greater fatality than control larvae (Ferrari et al., 2011). Furthermore, whereas decreasing the $CaCO_3$ saturation point may be the most concerning aspect for calcifying species in a highly acidic ocean (Doney et al., 2009), pCO_2 may be the most dangerous for fish (Rombough, 1988; Kikkawa et al., 2004; Heuer and Grosell, 2014).

Increased pCO_2 has a variety of complicated impacts on marine fish eggs and larvae (Heuer and Grosell 2014). Elevated pCO_2 levels ranging from 800 to 5000 µatm have been linked to developmental defects in larvae, including tissue and organ damage and alterations in fatty acid content (D'iaz-Gil et al., 2015; Frommel et al., 2016). The sensitivity of marine fishes to changes in seawater chemistry differs considerably. In a series of lab investigations, Hurst et al. (2012) found that the growth of walleye pollock (*Gadus chalcogrammus*) eggs and larvae showed relatively moderate responses to high ambient CO_2 levels.

The pH of the Bakkhali River waters was relatively basic (8.267 ± 0.2146) which was supported by Sarma et al. (2012), who found low pCO_2 and a high pH during peak discharge in the northern coastal Bay of Bengal. In this study, the effect of acidification on fish larval abundance at Bakkhali River was examined. Acidification variables played a significant role in controlling the diversity and distribution of aquatic species. These variables have a significant impact on the survival of aquatic species. The maximum and minimum pCO_2 value in the Bakkhali river estuary was recorded 360.6499 μatm at January and 19.3642 μatm in September, respectively. A total of 555 larval individuals were recorded throughout the year. The number of larval individuals was recorded as higher in August (109 individuals/1000m^3) and lower in January (11 individuals/1000m^3).

According to Diaz et al. (2019), pCO_2 is the main factor of ocean acidification for biological organisms and elevated level of pCO_2 (550 μatm) have severe effects on larval growth. As the concentration of pCO_2 ranged between 360.64 μatm to 19.36 μatm, the intensity of ocean acidification is not so severe resulting the high survival rate of larvae and high larval abundance. There might be some larvae family which can sustain in high acidification condition though it is subjected to long term observation.

From Table 2, it was revealed that a significant positive relationship with fish Larvae whereas pCO_2 has a negative relation with larvae. In the case of pH, the relationship can be explained by the 52.90% increase of fish larvae contributed to enhancing the pH by 52.90%. On the other hand, for pCO_2, the negative relationship can be explained by the 20.56% increase in fish larvae contributing to enhancing pCO_2 by 20.56% accordingly. It is evident the availability of fish larvae is lower in January as the pH was minimum and pCO_2 was higher whereas fish larvae higher in September due to higher pH level and lower pCO_2. These alterations would have a major influence on ecosystem functioning in this vulnerable location, demanding more careful consideration in future research.

CONCLUSION

The declining pH levels of the ocean waters appear to be an indication of acidification as the concentration of atmospheric CO_2 rises. This study predicted that acidification brought on by CO_2 loading may have an immediate effect on fish physiology, behavior, and biochemical performance as well as on the survival, growth, excavation, and behavior of fish. For the future management of fisheries, it is necessary to know how ocean acidification changes fish larvae. A few researches on marine fishes has been undertaken to test the effects of ocean acidification. This result shows that increased CO_2 levels in the water impact on recruiting efficiency, which has far-reaching implications for the sustainability of fish larval stocks. While CO_2 dissolves in the water, the acidity rises to the surface and the acidity rises to the surface, inhibiting most marine animal shell development and causing reproductive problems in a variety of fish. Because their larvae cannot develop normally in a more acidic environment, several coastal fishes are also in a bind as a result of ocean acidification. More heat is generated by the ocean when its acidity increases. Together, these variables create a hostile habitat for marine life. These studies will assist in the protection and management of estuaries to promote species distributions and sustainable production.

RECOMMENDATIONS AND FUTURE PERSPECTIVES

According to this research work, the following recommendations should be done:

7.1 General recommendations

To mitigate the consequences of acidification in coastal regions, the following tasks might be implemented:

- ✓ Limiting primary productivity by controlling eutrophication, including nutrient release from agricultural operations.
- ✓ Limiting the release of pollutants into the atmosphere, such as nitrogen and sulphur oxides, which are precursors of the HNO_3 and H_2SO_4 that are involved in acid rains.
- ✓ Reducing the amount of organic matter (OM) that enters the ocean by soil runoff, means regulating and limiting land use, soil erosion, and wastewater discharges.
- ✓ To minimize anthropogenic carbon emissions and mitigate the serious effects of ocean acidification, management measures must be ambitious and implemented immediately.

7.2 Policy recommendations

- ✓ A deeper understanding of the impacts of ocean acidification is needed to develop effective management plans, which requires that further extensive research be conducted in this field.
- ✓ It is important to develop connections between economists and scientists to examine the socioeconomic effects and costs of ocean acidification.
- ✓ Communication between scientists and policymakers must be improved so that policies are founded on scientific discoveries.
- ✓ Reducing human-caused CO_2 emissions into the atmosphere, should be done as part of a larger attempt to fight global warming.
- ✓ The implementation of measures aimed at CO_2 captures, such as a worldwide increase in green plantations.
- ✓ From an international standpoint, the most effective way to mitigate the impacts of ocean acidification is to set carbon emission limits and develop worldwide agreements to lower carbon emissions.

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APPENDICES

Appendix 1: Hydrological parameters of Bakkhali River

Month	Temperature (°C)	pH	Total Alkalinity (mg/l)	Salinity (psu)
Jan	26	8.0	170	26.7
Feb	28.8	8.1	185	28.5
Mar	29.1	8.2	170	29.6
Apr	31.5	8.3	128	32
May	30.7	8.1	111	24
June	31.1	8.4	98	24
Jul	29.5	8.2	88	20
Aug	26.5	8.2	59	18.1
Sep	30.8	8.8	70	9
Oct	27	8.2	96	24
Nov	30.2	8.5	104	32.5
Dec	25.1	8.2	134	21

Appendix 2: Derived ocean acidification factors of Bakkhali River

Month	*p*CO_2 (µatm)	HCO_3^- (mol/kg)	CO_3^{2-} (mol/kg)	DIC (mol/kg)	ΩAragonite	ΩCalcite
Jan	360.6499	0.0014	0.0001	0.0015	2.0945	3.2478
Feb	288.8298	0.0014	0.0002	0.0016	3.1074	4.7428
Mar	193.1420	0.0012	0.0002	0.0014	3.4319	5.2122
Apr	97.1385	0.0007	0.0002	0.0009	3.1711	4.7419
May	179.5786	0.0008	0.0001	0.0009	1.8023	2.7906
June	61.5759	0.0006	0.0001	0.0007	2.4976	3.8623
Jul	113.7661	0.0006	0.0001	0.0007	1.4956	2.3781
Aug	75.8629	0.0004	0.0001	0.0005	0.8617	1.3988
Sep	19.3642	0.0004	0.0001	0.0005	2.3368	3.9917
Oct	126.5883	0.0007	0.0001	0.0009	1.7539	2.7463
Nov	34.4489	0.0004	0.0002	0.0006	2.7241	4.0862
Dec	176.8834	0.0010	0.0001	0.0012	2.0482	3.2779

Appendix 3: Abundance of fish larvae (larvae/1000 m^3) in Bakkhali River

Month	**Larval Abundance**
Jan	29
Feb	11
Mar	45
Apr	28
May	37
June	58
July	99
Aug	109
Sep	74
Oct	25
Nov	21
Dec	19

Appendix 4: Descriptive statistics of ocean acidification factors

Descriptive statistics								
		Sampling Month	***p*CO_2 (µatm)**	**HCO_3^- (mol/kg)**	**CO_3^{2-} (mol/kg)**	**DIC (mol/kg)**	**ΩCalcite**	**ΩAragonite**
N	**Valid**	12	12	12	12	12	12	12
	Missing	0	0	0	0	0	0	0
Mean		6.50	143.9857143865	0.0008090576	0.0001338423	0.0009468666	3.5397096661	2.2770826662
Std. Deviation		3.606	102.27419381351	0.00036151727	0.00004576675	0.00038532518	1.11086918908	0.75516846650
Min.		1	19.36422834	0.00036363	0.00005047	0.00048363	1.39877237	0.86174819
Max.		12	360.64990900	0.00138873	0.00020503	0.00158147	5.21217696	3.43189902

Appendix 5: The correlations between pH, pCO_2, and fish larval abundance

Correlations					
		Sampling Month	**pH**	**Larval abundance (1000/m³)**	**pCO_2 (µatm)**
Sampling Month	**Pearson Correlation**	1	.493	.128	-.669*
	Sig. (2-tailed)		.103	.692	.017
	N	12	12	12	12
pH	**Pearson Correlation**	.493	1	.230	-.788**
	Sig. (2-tailed)	.103		.472	.002
	N	12	12	12	12
Larval abundance (1000/m³)	**Pearson Correlation**	.128	.230	1	-.453
	Sig. (2-tailed)	.692	.472		.139
	N	12	12	12	12
pCO_2 (µatm)	**Pearson Correlation**	-.669*	-.788**	-.453	1
	Sig. (2-tailed)	.017	.002	.139	
	N	12	12	12	12
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

BRIEF BIOGRAPHY OF THE AUTHOR

This is Humayun Kabir, son of Md. Ruhul Amin and Mst. Nurunnahar who came from Ishwarganj under Mymensingh district. He passed Secondary School Certificate examination in 2012 from Uchakhila Higher Secondary School and Higher Secondary Certificate examination in 2014 from Agricultural University College, Mymensingh. He achieved his B. Sc. Fisheries (Hons.) Degree in 2019 from Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU). Now, he is a candidate for the degree of MS in Fisheries Resource Management under the Department of Fisheries Resource Management, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.