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Management of Euglenophytes Bloom in Fish Ponds and its Effect on the Growth of Common Carp (Cyprinus Carpio L.) as an Algal Meal

Rahman, Md. Mahabubur

University of Rajshahi

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MANAGEMENT OF EUGLENOPHYTES BLOOM IN FISH PONDS AND ITS EFFECT ON THE GROWTH OF COMMON CARP (*CYPRINUS CARPIO* L.) AS AN ALGAL MEAL

A THESIS

SUBMITTED TO THE
UNIVERSITY OF RAJSHAHI, RAJSHAHI, BANGLADESH
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY (Ph. D.)

By
Md. Mahabubur Rahman
B.Sc. Fisheries (Honours), M.S. in Fisheries Management (BAU)
Roll No. 12605
Registration No. 0663
Season: 2012-2013

DEPARTMENT OF FISHERIES
FACULTY OF AGRICULTURE
UNIVERSITY OF RAJSHAHI
RAHSHAHI, BANGLADESH

NOVEMBER, 2013

Dedicated

To

My Beloved Mother

and

Late Father

DECLARATION

I do hereby declare that the research work submitted as thesis entitled **"MANAGEMENT OF EUGLENOPHYTES BLOOM IN FISH PONDS AND ITS EFFECT ON THE GROWTH OF COMMON CARP (*CYPRINUS CARPIO* L.) AS AN ALGAL MEAL"** in the Department of Fisheries, University of Rajshahi, Bangladesh for the degree of **Doctor of Philosophy** is the result of my own investigation. This thesis or part of it has not been submitted to any other University or institution for any degree or other purposes.

Md. Mahabubur Rahman
(Assistant Professor

Department of Fisheries
University of Rajshahi)

-The Researcher

University of Rajshahi
November, 2013

CERTIFICATE

This is to certify that the Thesis entitled **"MANAGEMENT OF EUGLENOPHYTES BLOOM IN FISH PONDS AND ITS EFFECT ON THE GROWTH OF COMMON CARP (*CYPRINUS CARPIO* L.) AS AN ALGAL MEAL"** has been completed by **Mr. Md. Mahabubur Rahman**. The work is original and independently pursued by the candidate. It embodies some interesting observations contributing to the existing knowledge on the subject.

The candidate is permitted to submit the work for the award of degree of **Doctor of Philosophy** in **Fisheries** of the University of Rajshahi, Rajshahi, Bangladesh. This thesis has not been submitted elsewhere for any degree or other purposes.

Principal Supervisor

(Dr. Md. Delwer Hossain)
Associate Professor

Department of Fisheries
University of Rajshahi
Rajshahi, Bangladesh
Email: delwerhru@yahoo.com

Co-Supervisor

(Dr. Ananda Kumar Saha)
Professor

Department of Zoology
University of Rajshahi
Rajshahi, Bangladesh
Email: anandroma@yahoo.com

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Md. Mahabubur Rahman

ABSTRACT

As harmful effects of euglenophytes bloom on fish production, three experiments were conducted to investigate the management of euglenophytes bloom in fish ponds and its effect on the growth of common carp (*Cyprinus carpio* L.) as an algal meal during 2010-2012 in Rajshahi district, North-west part of Bangladesh.

The first experiment was conducted to investigate the relationships of euglenophytes bloom to environmental factors in nine fish ponds for twelve months from July 2010 to June 2011. Among the ponds, three ponds with bloom were selected at Raighati in Mohanpur Upazila (BP-R), another three ponds with bloom at Yusufpur in Charghat Upazila (BP-Y) and three non-bloom ponds (NBP) at Meherchandi in Motihar Thana. The environmental factors (water temperature, dissolved oxygen, pH, NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu), soil organic matter, and planktonic algal community and density were examined monthly by using standard methods. There was no significant difference in water temperature among the study ponds (BP-R, BP-Y and NBP) but significantly lower dissolved oxygen and pH, higher concentrations of NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn, Cu and soil organic matter, higher density of euglenophytes, and lower density of cyanophytes, chlorophytes and bacillariophytes were recorded in BP-R and BP-Y as compared to NBP ($P<0.05$). The euglenophytes were occurred by three genera, *Euglena, Phacus* and *Trachelomonas* among which *Euglena* was the dominant genus. The density of euglenophytes in the bloom ponds showed an increasing trend from September (early autumn) and peaked in November (late autumn) and December (early winter). The density of these algae showed negative correlation with water temperature, dissolved oxygen and pH while positive correlation with NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu concentrations ($P<0.05$).

In second experiment, an attempt was made to investigate the management of euglenophytes bloom by using duckweed and lime for five months from August to December 2011 in twelve euglenophytes bloom forming ponds at Raighati, Mohanpur Upazila under four treatments such as T1 (the ponds treated with duckweed), T2 (the ponds treated with lime), T3 (the ponds treated with both duckweed and lime) and T4 (the ponds without duckweed and lime) with three replications. The study ponds were stocked with the fish species comprising *Labeo rohita*, *Catla catla*, *Hypophthalmichthys molitrix*, *Puntius gonionotus* and *Cirrhina mrigala* at 60/dec. Water quality parameters, soil organic matter, algal community and density, and growth performances (in terms of mean weight gain, average daily weight gain and specific growth rate), gut contents and electivity index of the fishes were examined by using standard methods. The results

showed that use of duckweed and lime in the euglenophytes bloom forming ponds had positive effects on water quality parameters, soil organic matter, euglenophytes density and growth of fish. Better water quality, lower density of euglenophytes and higher growth of fish were recorded in T3 as compared to other treatments ($P<0.05$). The results of the gut contents and electivity index revealed that grazing of fish had no significant effects in controlling euglenophytes bloom.

The third experiment was conducted to investigate the effects of euglenophytes algae supplemented feed on the growth and carcass compositions of common carp (*Cyprinus carpio* L.) for 12 weeks feeding trail from August to October 2012. Four feeds containing 0 % (Feed-1, Control feed), 20% (Feed-2), 30% (Feed-3) and 40% (Feed-4) euglenophytes algae were used in combination with conventional fish feed ingredients (Rice bran and mustard oil cake). The study was carried out in 12 glass aquariums at the wet laboratory of the Department of Fisheries, University of Rajshahi, Rajshahi under four treatments such as T1(the fish group fed with Feed-1), T2 (the fish group fed with Feed-2), T3 (the fish group fed with Feed-3) and T4 (the fish group fed with Feed-4) with three replications. Nutritive values of euglenophytes algae and experimental feeds, physico-chemical parameters of water, growth performance and feed utilization (in terms of mean weight gain, average daily weight gain, specific growth rate, feed intake and feed conversion ratio) and carcass compositions of fish were examined by using standard methods. The chemical analysis showed that euglenophytes algae contained average 49.64% crude protein, 14.40% crude lipid, 15.96% total carbohydrate, 9.29% moisture and 10.41% ash, and the experimental feeds (four combinations) had different nutritional value. During the study period, physico-chemical parameters of water among the treatments did not show any significant difference ($P>0.05$) and remained within the suitable ranges for fish growth. The results showed that euglenophytes algae supplemented feeds had positive effects on the growth and carcass compositions as compared to the control feed. Significantly higher growth, improved FCR and better carcass nutrients recorded in T3 as compared to other treatments ($P<0.05$).

The findings of the present research indicates that higher concentrations of nutrients and heavy metals under lower water temperature, dissolved oxygen and pH are responsible for euglenophytes bloom; use of both duckweed and lime is better for management of euglenophytes bloom; and euglenophytes algae could be used as a feed ingredient and 30% supplementation of these algae in the conventional feed is better for growth and carcass nutrients of common carp. More comprehensive investigations are required in long-term basis and future design including more ponds/aquariums would increase the statistical power in order to base conclusions on the effect of different treatments.

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CHAPTER ONE

General Introduction

Chapter One

GENERAL INTRODUCTION

Fishes and fisheries have been linked to the development of the human's earliest civilization. Even since, the fishes have been of considerable nutritional importance and human society has been extended to culture aspects of food, behaviour, belief and religion (Kreuzwe, 1974). Fishes have great significance in the live of mankind, being the most important and cheap source of protein and providing certain useful products for human worldwide including Bangladesh.

Bangladesh is a land of rivers. It lies in the north-eastern part of south Asia, between 20º 34′ and 26º 38′ North latitudes and 88º 01′ and 92º 41′ East longitudes. The country is surrounded by India on the west, the north and the north-west, Myanmar on the south-east and the Bay of Bengal on the south. Except a few part of hilly regions in the north-east and the south-east, some areas of high lands in north and north-west parts, the basic characteristics of the land of the country are low, flat and fertile. The total area of the country is 147,570 sq km (BBS, 2008). It is the most densely populated country of the world. But, this country is endowed with an abundance of water bodies filled with diversity of fish species and is blessed with more or less unique aquatic environment for fisheries resources advancement.

1.1. Fisheries resources in Bangladesh

Fisheries in Bangladesh comprise inland capture, inland culture and marine capture fisheries. The inland capture fisheries exploit open water areas of rives and their tributaries, estuaries, the Sundarbans mangrove forest area, permanent wetlands and seasonal flood plains. The inland culture fisheries include production from closed water bodies such as ponds and ditches, oxbow lakes, baors, and coastal and inland shrimp and fish farms. The marine capture

fisheries comprise industrial and trawl fisheries and small scale artisanal fisheries by coastal fisher communities. There are huge water resources scattered all over the country in the form of rivers and estuaries (8.53 lakh ha.), beels (1.77 lakh ha.), baors (8556 ha.), ponds (3.50 lakh ha.) Kaptai lake (68800 ha.), Sundarbans (2.0 lakh ha.) and floodplains (28.30 lakh ha.) (DoF, 2011). Relative share of different water bodies in Bangladesh is shown in Figure 1.1.

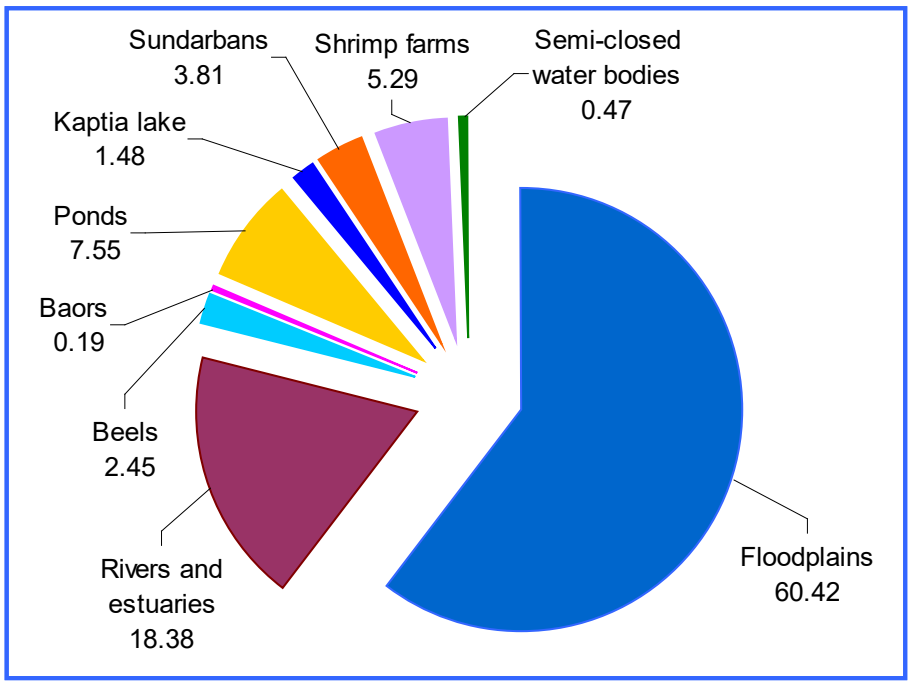


Figure 1.1: Relative share (%) of different water bodies in Bangladesh (Source: DoF, 2011)

Due to suitable environment, the water bodies of this country provide the richest grounds for fish biodiversity (Mazid, 2002). The country abounds in a large variety of fish species which include 260 indigenous freshwater fish species, 24 species of freshwater prawn, 475 species of marine fish, 36 species of marine shrimp and 12 species of exotic fish (DoF, 2011). There are 93 species of exotic fish introduced in the country of which 18 were introduced for culture fisheries and the rest for aquaria.

Inland fisheries are the major source of fish to meet the nation demand. Carps, catfishes, snakeheads, live fishes, *Hilsha* fishes and small indigenous fishes are the most important fisheries in commerce (FAO, 2007). Due to favourable climatic conditions, the water bodies of this country have great potential to produce enough fish for food, increasing income and livelihood of the rural population of the country.

1.2. Fish production potential in Bangladesh

The country enjoys a sub-tropical climate, fertile soil and water more or less ideally suited for fish production. In the overall agro-based economy of the country, the contribution of the fisheries is very promising and important. Fisheries is one of the rich potential sector of agriculture and over the last three decades aquaculture has developed to become the fastest growing food producing sector in the world as well as in Bangladesh.

Fisheries sector has been gradually gaining higher position in the developing economy of Bangladesh since last few years (Shafi, 2003). The inland fisheries of Bangladesh is one of the most productive resources in the world. In terms of overall production, Bangladesh ranks third in inland fish production among the countries of the world (Islam, 1989). In regarding animal protein supply, employment generation, foreign currency earning, poverty alleviation and economic development, fisheries play a unique role.

Fisheries play a vital role in national economy and contribute 3.74% of the GDP, 22.23% to the agriculture products and 2.70% to the export earnings (DoF, 2011). In 2009-2010, the highest ever export earning of Tk. 3408.51 crore was earned through the export of shrimp and fish products (DoF, 2011). The total fish production in 2009-2010 was 2.9 million tons. About 14.5 million people are directly or indirectly involved in this sector which is increasing approximately by 3.5% annually.

The average fish production was calculated as 103 and 3253kg/ha. for capture and culture fisheries, respectively. The growth rate of production from 2000 to 2010 gradually increased. From 1999 to 2010, capture fisheries declining nearly to 93988 mt (8.63%) pre year, whereas culture fisheries increase 8.93% and combined total fish production increase by 8.93%. Percentage contributions of fish production in capture, culture and marine fisheries is shown in Figure 1.2.

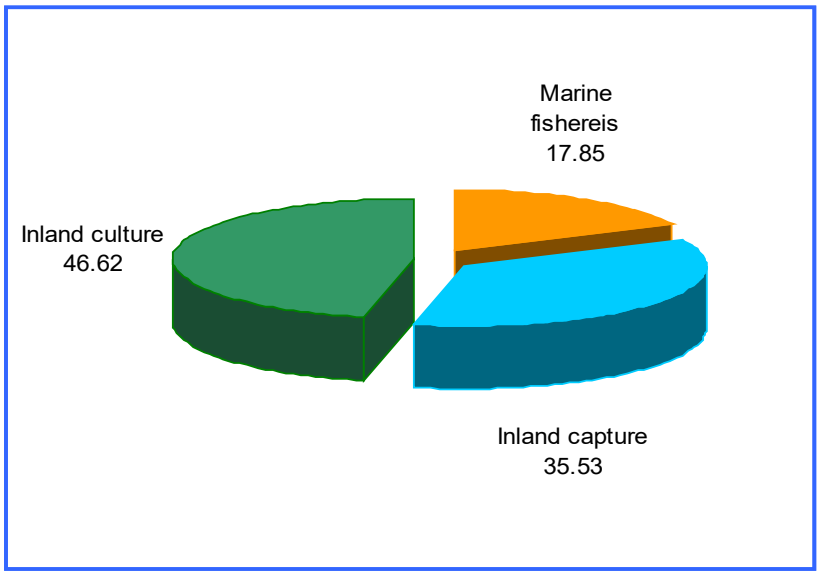


Figure 1.2: Percentage contributions of fish production in capture, culture and marine fisheries (Source: DoF, 2011)

Notwithstanding Bangladesh possesses vast and invaluable inland water bodies, the total production of fish from capture fishery are being reduced rapidly due to indiscriminate harvesting of fish, pollution by agro-chemical and sewage effluents, constructions of unplanned flood control devices (Ali, 1991). Concurrently, aquaculture production increased due to the development and implementation of improved culture techniques and expansion of the pond aquaculture (Alam and Thomson, 2001; Gupta *et al*., 1999). But, the growth rate of fish production in our country is not coping with the ever-increasing growth of population as a result the per capita fish consumption rate has shown a declining trend in recent years. Thus the nation's of total area of waters having fish production potential is of very great importance.

With more than 95% of population being fish eaters and the present level of production inadequate to feed the local population, the production levels have to be expanded both horizontally and vertically to meet the demand. Interestingly, the area still available for aquaculture is larger than the area under aquaculture, providing opportunities for increasing in production through lateral expansion (Munilkumar and Nandeesha, 2007).

1.3. Aquaculture: Production factors and environmental issue

Aquaculture has a low-energy expenditure and high-protein yield in comparison to other agricultural sectors. It has gained a momentum throughout the world during recent decades, which is probably unparalleled in other branches of food production. Pond aquaculture, the common existing fish framing system of Bangladesh, has tremendous potential as evidenced in various studies (Khan, 1985; Ahmed, 1992). But, it has not yet been able to meet up the total requirement of fish for consumption in spite of the increasing tendency of aquaculture practices. Environmental degradation, absence of sound management policies, lack of entrepreneurship in modern aquaculture, inadequate extension support to fish farmers etc. are the main factors responsible for this situation.

Since aquaculture production is affected by multiple factors, many characteristics should be measured and analyzed to explain production. Water quality, seed quality, stocking density, season, culture system, feeding etc. are the important factors affecting the aquaculture production. Moreover, development of scientific aquaculture depends on the limnological information, nutrient availability, knowledge of planktonic algae and their relation to each other. The value of planktonic algae in a water body, forming the basic link in the food chain of fish, has been well recognized. The quality and quantity of planktonic algae are both equally important for fish production. Water quality is also dependent on the abundance of planktonic algae. However, proper management of all these factors is essential for successful aquaculture.

Inspite of great potential, unfortunately, environmental degradation due to natural events or human activities create various problems for aquaculture in Bangladesh. Among different environmental problems, eutrophication is a growing inclusive problem in large number of inland waters like ponds and lakes. It is considered as one of the most pressing environmental problems in both the developed and the developing countries (Harper, 1992; Ryding and Rast 1989). Decomposition of organic wastes and unutilized feeds plus direct application of fertilizers are the major sources of eutrophication in aquaculture pond. A common symptom of pond or lake eutrophication is the appearance of large number of algae. Algae represent the important nutritive base and have a significant effect on the biological productivity of a water body. But, they are considered to be disastrous in the pond or lake when in bloom.

1.4. Algal bloom: Causes and types

Algae in the form of microscopic plants are called "phytoplankton" and develop in a wide variety of shapes and forms. Various types of algal populations quickly develop in fertile waters under suitable environmental conditions. Development of high concentration of algal populations turn water a green or blue green or red colour referred to as a bloom. Dense algal blooms near the surface of water bodies may resemble a layer of green or blue green or red paint (Plate 1.1). These natural phenomena result from the addition of plant nutrients (principally phosphorus and nitrogen) to water. Nutrients may be added to waters intentionally, as in the case of ponds that are fertilized or where fish are fed to augment fish production. In many cases, algal blooms are the unwanted results of unintentional nutrient additions. Nutrients may also reach to the ponds through runoff from fertilized lawns or pastures and from the wastes of livestock or poultry. Poorly functioning septic systems are another common source. Not all algae problems result from human actions. Some soils and waters are naturally rich in plant nutrients.

Plate 1.1: The algal blooms in freshwater body

The geographic distributions as well as the intensity of algal blooms have increased (Hallegraeff *et al*., 1988). Among different classes of algae, euglenophytes and blue-green algae often become the dominant types of algae in nutrient rich waters during winter (Kim and Boo, 2001; Park and Chung, 1996) or summer and spring (Jewel *et al*., 2006; Jahan *et al*., 2010). Based on their effects, there are three major categories of algal bloom frequently observed in fresh water and marine environment. One: algal blooms like euglenophytes which produce harmless water discolouration but under certain conditions blooms can grow so dense that cause mass mortality of fish due to oxygen depletion as the bloom decay. Two: algal blooms which produce potent toxic compounds causing huge mortality of fish and other aquatic animals (Brown and Boyd, 1982 and Armstrong *et al*., 1986) and these toxic compounds can also find their way through the food chain to human body causing a variety of

gastrointestinal and neurological illness. Three: algal blooms which are non-toxic to human in most cases but harmful to fishes especially in intensive aquaculture system by intoxication, damaging or clogging of gills.

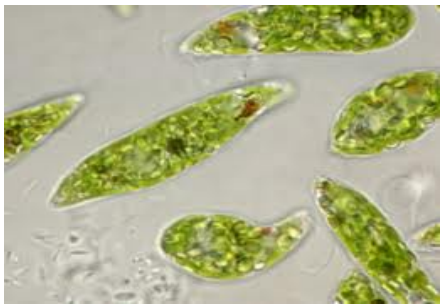
1.5. Euglenophytes: Phylogeny, morphology and biology

Euglenophytes are unique unicellular or single-celled organisms with both plant and animal features. Typified as a member of the protozoan order, Euglenida is classified as a member of the algal division, Euglenophyta. The systematic classification of Euglenida is given below.

Domain: Eukaryota
Kingdom: Excavata
Superphylum: Discoba
Phylum: Euglenozoa
Class: Euglenoidea
Order: Euglenales
Family: Euglenaceae
Genus: *Euglena* Ehrenberg, 1830
Trachelomonas Ehrenberg, 1835
Phacus Dujardin, 1841

Euglenoids possess elongated cell with one nucleus, pigmented chloroplasts, a red eyespot, a contractile vacuole and flagella (Plate 1.2). Several species produce breathing vesicles. Euglenoids ingest food into the gullet. Euglenoids are elliptical bodied with pear-shaped anterior and slender posterior. They lack a cell wall (an outer membrane containing cellulose). Instead, it has a pellicle made up of a protein layer supported by a substructure of microtubules, arranged in strips spiraling around the cell. The action of these pellicle strips sliding over one another gives *Euglena* its exceptional flexibility and

contractility (Moselio, 2011). The pellicle enables them to retain their shape. The nucleus is prominent in euglenoid cells and often readily visible in living individuals in the center or posterior of the cell.



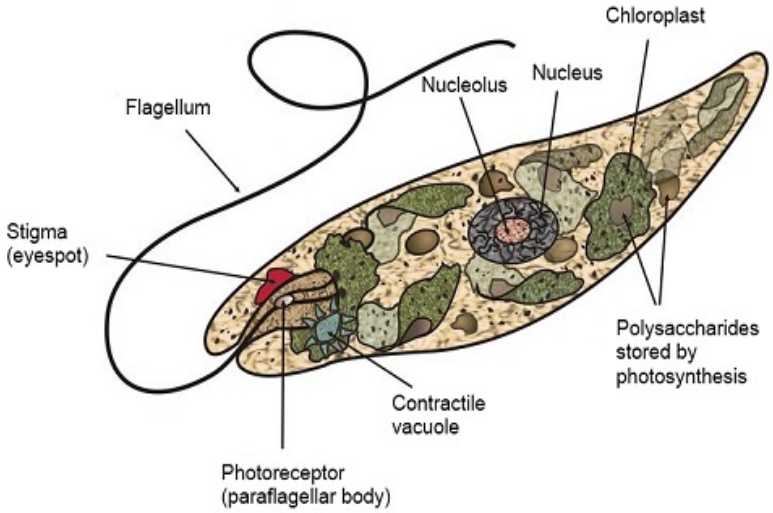


Plate 1.2: The diagram of *Euglena* sp. (Source: Encyclopedia)

Like plants, *Euglena* to derive their characteristic green tint from the chloroplasts present in their cells. The chloroplasts are discoid-lenticular, shield-shaped or ribbon like and contain pyrenoids, used in the synthesis of paramylon, a form of starch energy storage enabling *Euglena* to survive periods of light deprivation. The presence of pyrenoids is used as an identifying feature of the genus, separating it from other euglenoids, such as *Lepocinclis* and *Phacus* (Marin *et al*., 2003).

Euglenoids are both autotrophic as well as heterotrophic. When feeding as a heterotroph, they surround a particle of food and consume it by phagocytosis. When there is sufficient sunlight for it to feed by phototrophy, it uses chloroplasts containing the pigments chlorophyll-a and chlorophyll-b to produce sugars by photosynthesis (Henze *et al*., 1995).

The red eyespot of euglenoids composed of carotenoid pigment granules. It is not thought to be photosensitive rather it filters the sunlight that falls on a light-detecting structure at the base of the flagellum (paraflagellar body), allowing only certain wavelengths of light to reach it. As the cell rotates with respect to the light source, the eyespot partially blocks the source, permitting them to find the light and move toward it, process known as phototaxis (Moselio, 2011).

The cells of euglenoid are naked except in those genera (e.g., *Trachelomonas*) in which a lorica is present. Except when they are encysted or in a palmella phase, euglenoids are flagellate having two or several flagella rooted in basal bodies located at the front of the cell. In *Euglena*, one flagellum is very short and does not protrude from the cell while the other is relatively long and often easily visible with light microscopy. In some species, the longer and emergent flagellum is used to help the organism swim.

Euglenoids reproduce through mitosis cell division. The cell splits into halves that lead to the formation of another *Euglena*. Euglenoids require adequate amounts of food to reproduce. They have a star shaped structure at the rear end of its cell, which is basically orange in color called contractile vacuole which assists in excretion. Certain genera of euglenoids have the capacity to encyst and thus to withstand unfavorable environmental conditions. When they encyst, the cells become spherical and surrounded by a gelatinous sheath within which they may undergo movement and revolve (Harold and Michael, 1979).

1.6. Distribution of euglenophytes

The occurrence of euglenophytes has been reported in many countries of the world like Tadzhikistan, Ukraine, Vietnam, Indonesia, Israel (Hisoriev, 1995). This group of algae is also abundant in eutrophic water bodies in Korea (Kim and Boo, 1996 and 1998), India (Munawar, 1972; Duttagupta *et al*., 2004) and Bangladesh (Wahab *et al*., 1991; Rahman *et al*., 2005 and 2007). They prefer polluted water with high organic matter. *Euglena*-assemblages are known to be widely distributed in highly eutrophicated shallow ponds (Wild *et al*., 1995). At daytime, with high solar radiation, this group of algae occupies the surface of waters. But at night or at cloudy day, they may distribute to all water layers ranging from bottom to surface.

The species composition of euglenoids varies depending on the nutrient availability and differences in geographical locations (Hisoriev, 1995). About 40 genera and 800 spp of euglenoids have been described (Hallick *et al*., 1993). Three are three genera of euglenoids such as *Euglena*, *Phacus* and *Trachelomonas* occurred in the fresh water bodies of Bangladesh and among these genera, *Euglena* is the most dominant in fish ponds (Rahman *et al*., 2005, 2007; Jewel *et al*., 2006). Most species of *Phacus* grow in fresh water but a few occur in marine waters (Harold and Michael, 1979).

1.7. Euglenophytes bloom: Effects, management and exploitation

Among different classes of freshwater algae, euglenophytes form spectacular blooms in nutrient rich water bodies. They form coloured sticky scums on the upper surface of the water. The colour of scums looks brownish in the morning and become red or red mud (due to the presence of hemathochrome granules in the cell) with daylight and again pale reddish before sunset. Euglenophytes bloom is a common phenomenon in fish ponds of Bangladesh (Plate 1.2). These algal blooms have received much attention due to their mass occurrence on the surface of ponds and lakes throughout the country.

In freshwater fish ponds, the nutrient enrichment by decomposition of organic matters and by the addition of fertilizers and supplementary feeding, leads to eutrophication, thereby frequently developing dense bloom of euglenophytes. Euglenophytes bloom is conducive to higher concentrations of nitrogen and phosphorus nutrients (Kim and Boo, 2001, Duttagupta *et al*., 2004; Rahman *et. al*., 2007), heavy metals (Hutchinson and Nakatsu, 1984; Duttagupta *et al*., 2004) and acidic pH (Zakrys and Walne, 1994; Olaveson and Nalewajko, 2000).

Excessive algal blooms can have a negative effect causing serious economic losses to aquaculture (Hallegraeff, 1993). Dense algal bloom is known to negatively affect water quality in fish ponds. They collapse periodically leading to decomposition of dead algae resulting in fish kills due to anoxia (Boyd *et al*., 1975 and Barica, 1975) and due to high level of ammonia (Tucker *et al*., 1984). Euglenophytes bloom can cause problems through biomass effects, shading of submerged vegetation, disruption of food web structure and oxygen depletion. Dense bloom of euglenophytes can collapse the gills and cause breathe difficulty of fish resulting lose their weight markedly (Xavier *et al*., 1991). This algal bloom often leads to environmental degradation that hampered fish growth (Rahman *et al*., 2007) even caused mass mortality due to severe oxygen depletion (Rahman *et al*., 2005). Therefore, the bloom of these algae in aquaculture pond should be managed or controlled to minimize its negative impacts on fish production.

Several chemical methods are employed to control algal blooms in tropical water bodies (Yin *et al*., 1989; Jhingran, 1995), but they are either expensive or have residual effects in the aquatic food chain in the long run. Continuous and excessive use of algaecides can kill fish or affect their growth. Algaecides can destroy water quality and add new toxic sediment to the bottom which interfere fish growth (Lembi, 2000). On the other hand, uses of herbicides to control algal bloom are not environment friendly and have negative effects on fish growth (McIntosh an Kavern, 1974).

Direct grazing by filter-feeding fish is another method for algal bloom management (Liu *et al*., 2009). Though, filter-feeding fish are selective phytoplankton grazers that can suppress phytoplankton directly through ingestion but they can also be enhanced algal bloom indirectly by suppressing herbivorous zooplankton and by increasing nutrient availability (Drenner *et al*., 1987). In fact, the use of the filter-feeding fish to reduce algal biomass in lakes and reservoirs is still controversial (Radke and Kahl, 2002). In addition, if the algal bloom in the fish pond is controlled without eliminating the cause (the nutrients), the algal bloom will quickly return. Therefore, environment friendly and effective management systems should be found out to minimize the noxious effects of euglenophytes bloom in fish pond.

Moreover, the presence of cheaper high quality feed is one of the important factors for fish culture (Cho and Slinger, 1979). For fish culture, farmers conventionally use fish meal, mustard oil cake and soybean meal as fish feed. But, due to high cost and uncertain availability of fishmeal, and the presence of antinutritional factors in mustard oil cake and soybean meal, the farmers are now compelled to search for cheaper alternative protein sources. Therefore, algal meals are alternative plant feedstuffs that are increasingly being used in aqua feeds because of their nutritional quality, low cost and availability (Mustafa and Nakagawa, 1995; Hassim and Maat-Saat, 1992). Algal genera like *Spirulina*, *Chlorella, Scenedesmus, Dunaliella* etc. are widely used as aquaculture feeds for their high nutritional value (Avron and Ben-Amotz, 1992; Yamaguchi, 1997). Like other algae, the cells of euglenophytes contain high quality protein, polyunsaturated fatty acids and vitamin (Hayashi *et al*., 1993a; Becker, 1994). Continuously increasing demand for fish feed, pressures the consideration of every possible natural resource as potential ingredient in fish feed. Therefore, these locally available algae can be used as a feed ingredient for formulation of cheaper alternative and nutritive fish feed.

1.8. Study area: Rajshahi, North-west part of Bangladesh

The part of greater Rajshahi, Dinajpur, Rangpur and Bogra District of Bangladesh and the Indian territorial Maldah District of West Bengal are geographically identified as Barind Tract (Plate 1.3). Barind tract, the north-west region of Bangladesh covers about 3.5 million ha. The Rajshahi Barind tract is located in between 24º 23′ to 25º15′ North Latitudes and 88 º 2′ to 88 º 57′ East Longitudes. The hard red soil of this region is very significant in comparison to that of the other parts of the country. A typical dry climate with comparatively high temperature prevails in this region except for the wet season beginning from mid June to October. Rainfall is comparatively low in this region, with the long-term average being about 1,250 mm in the west and 2,000 mm in the northeast, occurring mainly from late April to October. With a variable rainfall and temperature ranging from 25.0 to 35.0 ºC (frequently exceeding 40.0 ºC) in the monsoon season, the area is consisted semiarid and drought-prone (Charles, 2008).

Rajshahi is one of the greater districts of northwest region of Bangladesh covers 2407.01 sq. km of area (Plate 1.4). Open water capture fisheries of this district comprise the major river, the Padma (The Bangladesh portion of the Ganges), the Shivbaronai and the network of lesser rivers and tributaries. The Rajshahi district has vast fisheries resources, covering 43861 ha of total water areas of which about 8838 ha. of rivers and canals, 6728 ha. of beels and 12,733 ha. of floodplains and 9882.80 ha of ponds.

There are about 154 species of fin fishes belonging to 12 orders, 32 family and 73 genera (Bhuiyan *et al*., 1992). The total fish production of this area was estimated as 50256.40 mt of which open water contributed to 20.16% and culture fishery produced 79.84% for the year 2011. Among the estimated fish production from different water bodies in Rajshahi (Table 1.1.), the ponds contributed the highest fish production (73.35%), followed by floodplains

(8.20%), beels (7.60%), semi-closed water bodies (6.47%), rivers and canals (4.35%). The capture fishery has been declined whereas the culture fishery especially pond culture of this region has been increased gradually.

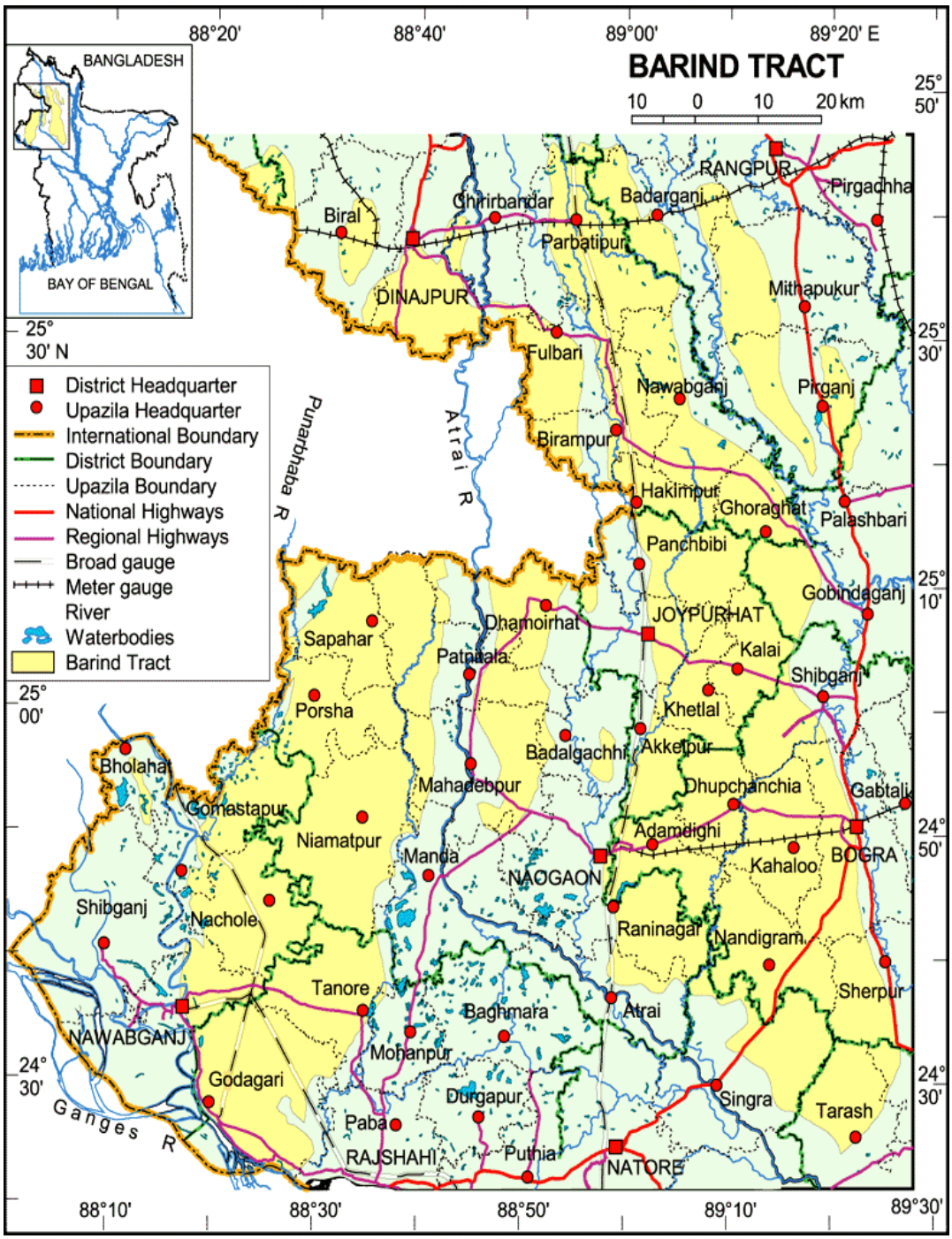


Plate 1.3: The map of the Barind tract, Bangladesh (Source: Banglapedia)

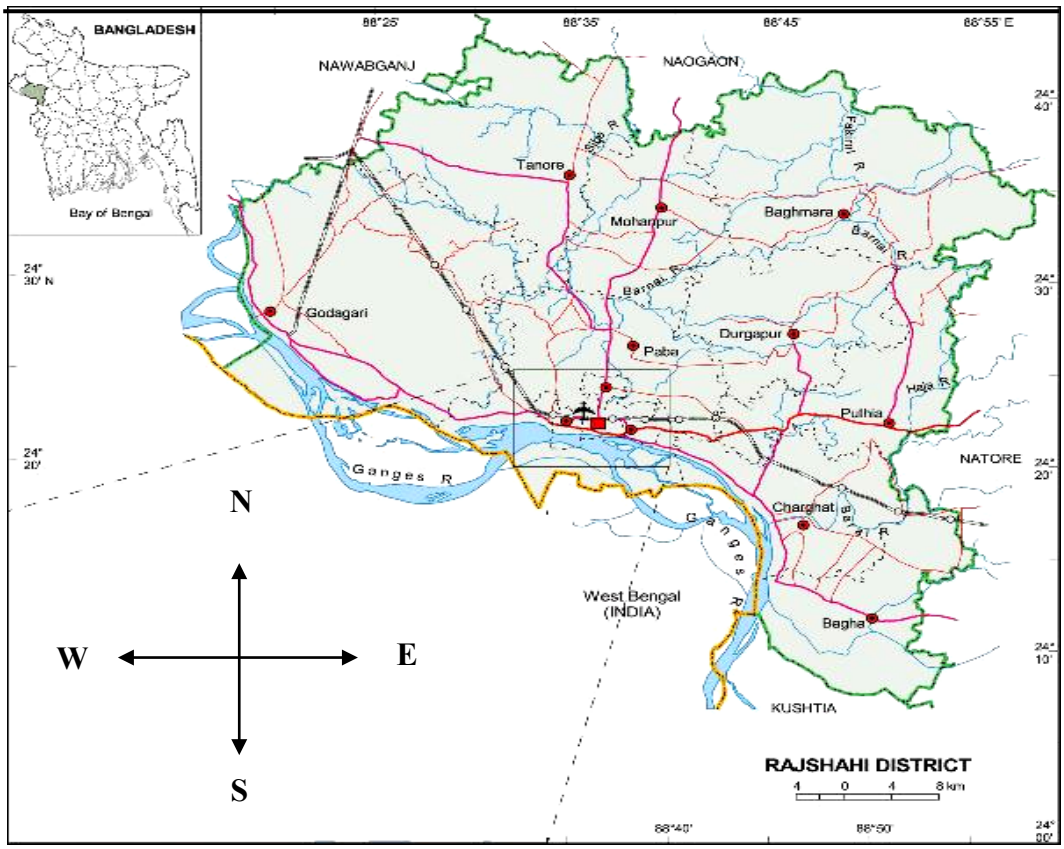


Plate 1.4: The map of Rajshahi district, Bangladesh (Source: Banglapedia)

Table 1.1: Fish production of different water bodies in Rajshahi (Source: DoF)

Inland water	Area (ha.)	%	Production (mt)	%
Rivers	8838.00	20.15	2188.30	4.35
Beels	6728.66	15.34	3817.00	7.60
Floodplains	12733.00	29.03	4122.40	8.20
Semi-closed waters	5643.60	12.87	3253.50	6.47
Ponds	9882.80	22.53	36859.00	73.35
Paddy-fish culture	35.84	0.08	16.20	0.03
Total	**43861.90**	**100.00**	**50256.40**	**100.00**

The survey report from the DoF (Department of Fisheries, Bangladesh) showed that open water area in the greater Rajshahi including rivers, numerous beels and floodplains, is gradually declining because of flood control, drainage and irrigation project as well as the Farakkah barrage impact. The increasing population growth and the faster rate of expansion of agricultural, domestic, irrigation and industrial activities pose threats to the development of the fisheries sector in this region. Therefore, emphasis has been given to culture based fisheries (Aquaculture) in different inland water bodies of this region.

Although aquaculture production is increasing gradually, but, the fish farmers of this drought prone area have been facing various problems due to eutrophication and algal bloom. Among various environmental problems, eutrophication and algal bloom are the more common problems for fish culture in Rajshahi. Each year, fisheries extension personnel receive numerous calls regarding ponds with odd-looking scums, unusual colours or mats of algae. Problem algal blooms are more frequent in times of drought which lead to huge mortality of fish causing severe economic losses. Appropriate management measures can minimize the impacts of harmful algal bloom and can ensure economic fish production in the bloom ponds. Moreover, utilization of euglenophytes algae as a fish feed ingredient can include an alternative low cost and nutritive feed stuff in the fish feed industry.

1.9. Research efforts made on management of algal bloom and exploitation of algae as fish feed ingredient

A number of research works have already been done concerning the relationship of algal bloom to environmental factors, management or control of algal bloom and dietary value of different algae to various fish species in different parts of the world including Bangladesh (Table 1.2).

Table 1.2: Research efforts on ecology and management of algal bloom, and exploitation of algae as fish feed ingredient

Researchers	Major thrust	Remarks
Rahman *et al.* (2007)	Euglenophytes bloom in experimental fish ponds	No. emphasis on farmers' fish ponds
Duttagupta *et al.* (2004)	Euglenophytes bloom in floodplain	No. emphasis on fish culture ponds
Kim and Boo (2001)	Ecology of euglenoids in river	No. emphasis on fish culture ponds
Xavier *et al.* (1991)	Euglenophytes bloom in fish breeding tank	No. emphasis on fish culture ponds
Lynch (2009)	Managing of algal bloom	No. emphasis on euglenophytes bloom
Lembi (2003)	Managing of blue-green algal bloom	No. emphasis on euglenophytes bloom
McGregor (2002)	Controlling of blue-green algal bloom	No. emphasis on euglenophytes bloom
Tongsiri *et al.* (2010)	Evaluation dietary value of algae, *Spirulina* sp.	No. emphasis on euglenophytes algae
Soler-Vila *et al.* (2009)	Evaluation dietary value of algae, *Porphyra* sp.	No. emphasis on euglenophytes algae
Tartiel *et al.* (2008)	Evaluation dietary value of algae, *Chlorella* sp.	No. emphasis on euglenophytes algae

1.10. Indications from the earlier research efforts

Earlier researches indicated that

I. Most of the researches have been done on ecology of algal bloom in experimental tanks/ponds or rivers, emphasis not given in fish culture ponds.

II. In case management of algal bloom, emphasis has been given on blue-green algae not on euglenophytes algae in fish culture ponds.

III. For evaluation of dietary value of algae, most of the researches have been done on such types of algae that their availability may be the major constrain in using them. Moreover, the production costs of such micro algae are quite expensive making them almost unaffordable in developing country like Bangladesh. Emphasis has not been given on naturally available algae.

Based on the aforementioned indications, some research questions are raised as follows:

I) What are the environmental factors which influencing the euglenophytes bloom in fish pond?

II) What are the effective management measures to minimize the euglenophytes bloom in fish pond?

III) Have any dietary effects of euglenophytes algae as a feed ingredient on the growth and carcass composition of fish?

1.11. Research need to develop management systems for euglenophytes bloom and to exploit these algae as feed ingredient

Aquaculture has greater potentials in nutrient supply, employment generation, poverty reduction and socio-economic improvement in the rural areas of Bangladesh. On the other hand, aquatic environmental intensification and diversification are practiced for higher aquaculture production which leads to environmental degradation through formation of noxious euglenophytes bloom.

Due to frequent occurrences of dense bloom of euglenophytes in fish pond at Rajshahi, farmers have been faced various problems for fish production. Only, appropriate environment friendly aquaculture technology can contribute to overcome such problems based upon the availability of useful information. Unfortunately, no sufficient researches based on the algal bloom which can contribute well to overcome the euglenophytes bloom related problems. In addition, investigations for cheaper alternatives feed stuffs as protein and energy source for fish diets have become a priority in order to produce low cost feeds available for the small-scale fish farmers. But, investigations based on the exploitation of available algal biomass as an alternative feed stuff to produce low cost feed for fish farmers in Rajshahi even in the country have not yet been done.

1.12. Research objectives

Considering the indications and questions raised from the earlier research efforts on algal bloom, it is necessary to find out environment friendly and effective management measures to minimize euglenophytes bloom related problems and to exploit these available algae as a fish feed ingredient by research. Therefore, the present research was undertaken on "Management of euglenophytes bloom in fish ponds and its effect on the growth of common carp (*Cyprinus carpio* L.) as an algal meal" with a view to the following objectives:

1. To study the relationships of euglenophytes bloom to environmental factors in fish pond.
2. To study the management of euglenophytes bloom in fish pond.
3. To study the effects of euglenophytes algae supplemented feeds on the growth and carcass composition of common carp.
4. Finally, to recommended a suitable management measure for minimizing euglenophytes bloom in fish pond and an optimum dietary inclusion of euglenophytes algae in feed for common carp.

CHAPTER TWO

Relationships of Euglenophytes Bloom to Environmental Factors in Fish Pond

Chapter Two

RELATIONSHIPS OF EUGLENOPHYTES BLOOM TO ENVIRONMENTAL FACTORS IN FISH POND

2.1. Introduction

Planktonic algae are the first link in most food chains in aquatic ecosystem and are essential for the functioning of higher trophic levels. They are sensitive to the environment in which they live and any alteration in them leads to change in the algal communities in terms of abundance, diversity and dominance. They are the basic members in the aquatic ecosystem and hence changes in algal population have a direct link with the change of water quality in any aquatic medium. The dynamic features of a water body such as colour, clarity, trophic state, zooplankton and fish production depend to a large degree on the planktonic algae (Goldman and Horne, 1983).

The consideration of the physico-chemical factors in the study of limnology is basic to the understanding of trophic dynamics of the water body. Each factor does play its individual role but at the same time the final effect is the actual result of the interaction of these factors. The planktonic algal community on which whole aquatic population depends is largely influenced by the interaction of a number of physico-chemical factors such as temperature, dissolved oxygen, pH, nutrients, trace elements etc.

Water temperature is one of the important factors for growth of algae. Most of the algae are mesophilic. Certain algae exhibit seasonal pattern which is in part temperature controlled (Imai *et al*., 2008). Algae are one of the major sources of dissolved oxygen in the pond water (Dupree and Huner, 1984). But, excessive algae in the pond can lead to oxygen deficit (Boyd *et al*., 1975). The oxygen deficits condition is helpful to trigger the oxygen-iron-phosphate complex,

releasing larger quantities of phosphorus and iron which enhaned the proliferaion of euglenophytes (Munawar, 1972). Several studies have shown that variations in pH influence the algal abundance and species distribution (Goldman and Shapiro, 1973). Variation in pH can also change the distribution of carbon dioxide and alter the availability of essential nutrients and trace elements (Boyd, 1979).

Nutrients are one of the most important factors that influence algal growth (Okaichi *et al.,* 1989). As a general principle, algae require a supply of inorganic nutrients. The primary inorganic nutrients required for algal growth are nitrogen, phosphorus and carbon in different chemical forms. Many other elements are needed for algal growth in lesser or often trace amounts and are collectively referred to as micronutrients. The micronutrients required for growth and enzymatic activity of algae include iron, zinc, manganese, copper, sulfur, calcium, magnesium, sodium, potassium and cobalt (Goldman and Horne, 1983).

Most aquaculturists assume that elevated soil organic matter concentrations, high rates of microbial respiration and anaerobic conditions at the soil–water interface are closely associated. There is increasing evidence that the amount of organic matter in bottom soil of the pond and the exchange of nutrients between soil and overlaying water strongly influence water quality and concentration of nutrients available to algae (Boyd, 1995). Thus, concentration of soil organic matter in the pond plays an important role for algal growth.

However, favourable environmental conditions help to enhance higher density of algae referred to as algal bloom. There are various types of algal bloom both toxic and non-toxic developed in freshwater and marine water environments. The geographic distributions as well as the intensity of algal blooms have increased (Hallegraeff *et al*., 1988). In freshwater aquaculture pond, the nutrients enrichment by the addition of fertilizers and supplementary feeding, leads to eutrophication, thereby frequently developing dense algal blooms (Padmavathi and Prasad, 2007).

In aquaculture pond, moderate level of algal bloom is beneficial. But, excessive algal blooms can have a negative effect causing serious economic losses to aquaculture (Hallegraeff, 1993). Dense algal bloom is known to negatively affect water quality in fish pond in at least three ways. First, it can lead to chronic oxygen deficiency. Second, algal blooms collapse periodically leading to decomposition of dead algae resulting in fish kills due to anoxia (Boyd *et al*., 1975; Barica, 1975) and higher ammonia (Seymour, 1980; Tucker *et al*., 1984). Third, the algae can exude toxic chemicals that causing off-flavour and mortality of fish (Brown and Boyd, 1982; Armstrong *et al*., 1986; Jewel *et al*., 2003; Padmavathi and Prasad, 2007).

Among different classes of freshwater algae, euglenophytes members such as *Euglena, Phacus* and *Trachelomonas* are commonly abundant in fish pond as eutrophic genera (Kim and Boo, 2001). These algae formed spectacular water bloom in the fish pond throughout the country. If we look at our traditional or commercial fish ponds then only by observing the colour we can easily understand their occurrence and intensity. These algae are abundant at high organic loading rates (Phang and Ong, 1988), high concentration of nutrients (Kim and Boo, 2001, Duttagupta *et al*., 2004; Rahman *et. al*., 2007) and at acidic pH value (Zakrys and Walne, 1994; Olaveson and Nalewajko, 2000).

Euglenophytes algae are thought to be non-toxic. But, they form thick red scum on the surface of the fish pond (Plate 2.1) responsible for water quality problems, the most severe of which being the oxygen depletion leading to mass mortality of fish (Rahman *et al*., 2005). Their bloom often leads to algal die off and environmental degradation that hampered growth of fish (Rahman *et al*., 2007). Furthermore, this bloom has a blanketing effect on the pond, thereby preventing the entry of sunlight into water that hampered growth of other algae through hampering photosynthesis process. The blooms of *Euglena elastica, E. gracilis* and *Trachelomonas charkoweinis* have a significant effect in reducing the number of other algal species in fish pond (Hosmani, 1988).

Plate 2.1: The euglenophytes bloom in fish pond

Understanding the environmental factors which influence the density of euglenophytes in fish pond will help to manage the bloom of these problematic algae. However, concerning the effect of environmental factors on the growth of euglenophytes algae in laboratory conditions or in experimental pond, a number of findings have been reported in some countries of the world including Bangladesh (Xavier *et al*., 1991; Zakrys and Walne, 1994; Kim and Boo, 2001; Spackova *et al*., 2009; Rahman *et al*., 2007; Jahan *et al*., 2010). But, the dynamics of euglenophytes bloom in relations to environmental factors in farmer managed fish pond has been poorly understood in Rajshahi, Bangladesh. Therefore, the present study was conducted to investigate the relationships of euglenophytes bloom to environmental factors in the fish pond with a view to the following specific objectives.

1. To monitor the environmental factors in the bloom pond.
2. To determine the soil organic matter in the bloom pond.
3. To study the status of planktonic algal community in the bloom pond.
4. To study the seasonal variation in density of euglenophytes algae.
5. To study the relationships between euglenophytes density and environmental factors in bloom pond.

2.2. Review of literature

Review of related literature is a necessity in the sense that it provides as scope for reviewing the stock of knowledge and information relevant to the proposed research. These knowledge and information give a guideline in designing the future research problem and validating new findings. However, environmental factors play an important role for algal growth, their density and seasonal succession as well as for bloom formation. There are many published reports on ecology, seasonal successions and bloom of algae in different water bodies with their effects on fish growth in various parts of the world. But, very little reports are available on ecology, seasonal successions and bloom of euglenophytes algae in the fish pond of Bangladesh. However, some research findings relevant to the present study are reviewed hereunder.

Jaworska and Zdanowski (2012) conducted a study on the patterns of seasonal dynamics of phytoplankton in a lake (Lake Kortowskie, northern Poland). The basis of their study was the analysis of long-term seasonal changes in the taxonomic structure and the estimation of the intensity of algal community development in the lake. They observed intensive algal growth in spring lasted until late autumn and the highest phytoplankton biomass was always recorded in summer which related to increased blue green algae domination.

Shams *et al.* (2012) carried out an investigation on seasonal variations in phytoplankton communities in Zayandeh-Rood Dam Lake (Isfahan, Iran). They identified a total of 53 phytoplankton genera belonging to 6 divisions Bacillariophyta, Chlorophyta, Cyanophyta, Euglenophyta, Dinophyta and Chrysophyta. They observed the highest phytoplankton diversities and densities in November and the lowest in May with the density ranged from 470 to 150,470 cells/cm^3. Regarding physico-chemical analysis and phytoplankton composition, they concluded that some species of phytoplankton can be used as indicators for evaluating water quality.

Wirasith and Traichaiyaporn (2012) carried out a study on water quality variation and algal succession in commercial hybrid catfish production ponds in Bang Pa-In district, Ayutthaya province, Thailand. Their study covered two fish crops, May-August and September-December. They observed dramatic changed in physico-chemical parameters of water in the ponds over the study period. During the first crop period, they observed that algae samples contained 83 species belonging to the Chlorophyta (34 spp.), Cyanophyta (28 spp.), Euglenophyta (12 spp.), Bacillariophyta (6 spp.), Chrysophyta (1 sp.), Pyrrhophyta (1 sp.) and Cryptophyta (1 sp.) whereas the second crop contained 60 species of Chlorophyta (28 spp.), Cyanophyta (16 spp.), Euglenophyta (10 spp.) and Bacillariophyta (6 spp.). In their study, Cyanophyta was the most abundant, followed by Chlorophyta, Euglenophyta, Bacillariophyta, Chrysophyta, Cryptophyta and Pyrrhophyta. Their study concluded that physico-chemical parameters of water may account for algal proliferation resulting in algal blooms and influence algal succession.

Jahan *et al.* (2010) conducted a study to analyze the mechanisms and contributing factors related to the seasonal dynamic of algal blooms in a shallow eutrophic pond in Bangladesh. They recorded two conspicuous events simultaneously throughout the study period: high concentration of phosphate–phosphorus (>3.03 mg/l) and permanent cyanobacterial blooms. They also recorded cyanobacterial blooms characterized by three abundance phases, high nitrate–nitrogen (>2.35 mg/l) associated with higher abundance phase, low nitrate–nitrogen (0.36 mg/l) associated with moderate abundance phase and extremely low NO_3–N/PO_4–P ratio (>3.55) negatively correlated with all blooming taxa. Their study showed that cyanobacterial blooms positively correlated with water temperature ($r = 0.35$; $p = 0.05$) and pH ($r = 0.84$; $p = 0.05$) and negatively correlated with water transparency ($r = -0.96$; $p=0.01$).

Spackova *et al.* (2009) studied the seasonal succession of epipelic algae on a mesotrophic pond in a temperate climate. They reported that the composition of epipelic algal assemblages changed over time in both higher taxonomic groupings and species representations. Spring and autumn were characterized by a dominance of diatoms; euglenophytes had their maximum in June and cyanobacteria were typical for the summer season. The occurrence of algal species correlated with water temperature.

Padmavathi and Prasad (2007) conducted a study on the algal bloom disasters in carp culture ponds to investigate the effect of algal blooms on the water quality, plankton diversity and density, and fish production. In this study, they selected three carp culture ponds in the west Godavari district, Andhra Pradesh, India with *Microcystis, Oscillatoria* and *Anabaena* blooms, respectively. They recorded lower yield of fish with concomitant fish mortalities in the pond with *Microcystis* bloom followed by the ponds with *Anabaena* and *Oscillatoria* blooms.

Rahman and Khan (2007) conducted an investigation on the noxious euglenophytes bloom in fertilized fish ponds at Bangladesh Agricultural University, Mymensingh, Bangladesh. In their study, temperature, pH, phosphate, nitrate and phytoplankton populations were monitored. They recorded three genera of euglenophytes such as *Euglena*, *Phacus* and *Trachelomonas* and significantly higher density of these algae in the chicken manure treated ponds at pH around 6.5 with higher phosphate (1.37 mg/l) and nitrate (1.47 mg/l) concentrations. Their study concluded that euglenophytes density showed positive correlation with phosphate-phosphorus and nitrate-nitrogen concentration while negative with pH value.

Rahman *et al.* (2007) carried out a study to asses the impacts of euglenophytes bloom on the growth of fish. They monitored some water quality parameters

viz., water temperature, dissolved oxygen, pH, PO_4-P and NO_3-N concentration, algal population and growth of fish. They recorded heavy bloom of euglenophytes in late August in the ponds with lower growth of fish than verified for those in the ponds where the bloom did not occur. In conclusion they stated that acidic environment and nutrient enrichment enhanced bloom of euglenophytes which hampered the growth of beneficial algae (chlorophytes and bacillariophytes) and growth of fish.

Jewel *et al.* (2006) monitored the occurrence and abundance of cyanobacterial bloom in a lake at Bangladesh Agricultural University, Mymensingh, Bangladesh. In their study, environmental parameters viz., water temperature, pH and nutrients (NO_3-N and PO_4-P) were monitored and their relationship with the bloom of cyanobacteria was analyzed. They recorded 5 species of cyanobacteria of which *Microcystis aeruginosa* was the dominant during the bloom period. They concluded that the initiation and persistence of natural bloom of cyanobacteria was found to be controlled by relatively high temperature (>25 ^{0}C) and nutrients enrichment, especially high concentration of NO_3-N (3.8 mg l^{-1}).

Queiroga *et al.* (2006) conducted a study on a combination of enclosure nutrient enrichment to identify the factors controlling seasonal dynamics and competition of the phytoplankton community in the Curonian lagoon. In their study, changes in chlorophyll-a concentrations, inorganic nutrient concentrations and plankton cell density were monitored. They reported that phytoplankton development in the lagoon is strongly affected by ambient environmental factors and nutrient limitation plays an important role in seasonal succession mechanisms showing quite distinct seasonal development patterns.

Sen and Sonmez (2006) carried out a study on the seasonal variations of algae in fish ponds. They reported that algal flora of the ponds consisted of Bacillariophyta, Chlorophyta, Cyanophyta and Euglenophyta among which

diatoms were the most noticeable algae showed their best growths in winter and spring whilst they occurred in low numbers in the other seasons. Chlorophyta, Cyanophyta and Euglenophyta were other groups of algae in fish ponds occurring generally in summer and autumn.

Affan *et al.* (2005) conducted a study on the seasonal changes of phytoplankton community in aquaculture ponds of Bangladesh. They identified total 27 phytoplankton genera of which 13 belong to Cyanophyceae, 6 to Chlorophyceae, 5 to Bacillariophyceae and 3 to Euglenophyceae. They observed the highest phytoplankton abundance in spring followed by early autumn, summer and the lowest in winter. The annual succession of Cyanophyceae was characterized by spring and early autumn, Chlorophyceae was by rainy season, Bacillariophyceae was by winter and Euglenophyceae was by late autumn and *Euglena* sp. was the dominant species.

Rahman *et al.* (2005) observed fish mortality associated with euglenophytes bloom in a polyculture fish pond at Bangladesh Agricultural University campus, Bangladesh. They observed reddish brown water colour with dead mucilaginous bloom in the pond and phytoplankton community mostly dominated by *Euglena* comprised more than 95.0% of the phytoplankton. On the day of mortality, relatively higher water temperature (surface 31.0º and bottom 30.0º), lower dissolved oxygen (surface 0.68 mg/l and bottom 0.34 mg/l) lower concentrations of nutrients (PO_4-P: surface 0.26 mg/l and bottom 0.28 mg/l; NO_3-N: surface 0.24 mg/l and bottom 0.27) and acidic pH (surface 6.10 and bottom 6.08) were recorded. Their study concluded that fish mortality possibly due to gill clogging and lower dissolved oxygen induced by death and decomposition of settle dead euglenophytes in the pond bottom.

Duttagupta *et al.* (2004) studied the euglenoid blooms in the floodplain wetlands of Barak Valley, Assam, North eastern India. They reported that

euglenoids bloom found to be induced by high concentrations of NO_3-N, NH_3-N, Fe, Mg and to some extent, PO_4, Cu and Zn in the water. They also reported that the trace elements rapidly accumulated by the bloom organisms to high levels, whereby their concentrations in the water declined, leading to a collapse of the bloom, which tended to reappear as decomposition again led to the release of the nutrients.

Jewel *et al.* (2003) observed mass mortality of fishes in a farmer's pond of Mymensingh, Bangladesh. At the time of fish kill, they observed a massive bloom of cyanobacteria (210.5×10^3 cells ml^{-1}) and the gills of dead fishes were pale-white with large number of *Aphanizomenon* and *Microcystis* cells. During mortality event, they recorded high concentration of PO_4-P (9.5 mg l^{-1}), high water temperature (31^0C) and lower dissolved oxygen (0.95 mg l^{-1}). Their study concluded that the fish mortality was possibly caused either by oxygen deficiency or toxins secreted by cyanobacteria or by combination of both.

Kim and Boo (2001) undertook an investigation on the morphological variation and density of the *Euglena viridis* cells related to environmental factors in the urban drainage of Korea. The results of their study showed that all *E. viridis* cells were same with single star-cluster of chloroplast lobes and included two morphotypes. The morphotype I cells agreed well with the typical form of *E. viridis* commonly occurred in most of waters and bloomed with 5386 cells/ml whereas the morphotype II cells were characterized by having randomly scattered cytoplasm granules beneath pellicle and uneven margined lobes of chloroplasts. They stated that density of morphotype-I positively correlated with ammonium (r=0.80) and nitrite (r=0.68), while negatively correlated with nitrate whereas the density of the morphotype-II positively correlated with nitrate (r=0.98) while negatively with ammonium and nitrite. They did not found any significant relation of cell density with inorganic phosphate, temperature and pH of water.

Kim and Boo (2001) carried out a study to know the relationship of green euglenoid to environmental variable in Jeonjucheon, Korea. They recorded 5 genera of euglenoid and 71 species throughout the year, increasing in the early summer (35 to 42 taxa) and decreasing in the winter (below 20 taxa). They observed a typical bimodal pattern of total density of the green euglenoids, being maximal in the winter and in the early summer in which the winter peak was a result of active growths of *E. caudata*, *E. geniculata* and *E. viridis*, each of which positively correlated with the phosphate whereas the early summer peak was attributed to *E deses*, *Lepocinclis ovum* and *Phacus trypanon*, each of which positively correlated with ammonium and nitrate. They concluded that the complete bimodal spectrum of species number and density of green euglenoids provides a sensitive image in detecting the changes of environmental variables in polluted water bodies.

Olaveson and Nalewajko (2000) carried out an investigation on the effects of acidity on the growth of two *Euglena* species. Their study separated the effects of elevated protons (at pH <3) and elevated metals (Al, Cd, Cu, Fe, Ni, Zn) on the growth of *E. mutabilis* and *E. gracilis*. They reported that both species were acid tolerant, growing optimally at pH 2.5-7.

Kim and Boo (1998) conducted a field survey to know the morphology, population size and environmental factors of two morphotypes in *Euglena geniculata*. They separated two morphotypes of the species based on detailed features and observed that the population size of the morphotype-1 showed positive correlation with nitrate concentration while negative with ammonium concentration. In contrast, the morphotype-2 showed less significant relationship with the surrounding nutrients factors.

Kim and Boo (1996) studied the seasonal changes of the euglenoid species and biomass in the Kumgang River from May 1994 to September 1996 in Napo and

Jangam. They found a typical seasonality that related to the seasonal change of species number in Jangam but not in Napo. They also observed seasonal change of euglenoid biomass which was the maximum with 1190-1720 cells/ml in February and the minimum with 160-200 cells/ml in April, 1996, differed from the change of water temperature.

Park and Chung (1996) carried out a survey to investigate the population dynamics of Euglenophyceae. They recorded total 44 taxa within 6 genera and total standing crops varied from minimum 3,665cell/ml to maximum 165,920 cells/ml during winter and the significant factors for population development were DO, BOD, phosphate and ammonia. They stated that population density of Euglenophyceae showed its peak at low water temperature.

Nwankwo (1995) investigated the growth of euglenoid in six polluted stream water channels in Lagos mainland. He reported that the presence of high oxidizable organic matter favoured euglenoid growth and most euglenoid species were recorded from November through February when water temperature, nutrient values and BOD were high.

Wild *et al.* (1995) conducted a study on the phycological and hydrological properties of two small and shallow eutrophic ponds in an urban area of Wuezburg, Germany. Based on nutrient status, they identified two distinct algal assembles in two different ponds as polytrophic *Aphanizomenon/Scenedesmus*-pond and eutrophicated *Euglena* pond. They reported that *Euglena* assemblages are widely distributed in eutrophicated shallow ponds at elevated temperature.

Mishra and Saksena (1993) carried out an investigation on the phytoplankton composition of sewage polluted Morar River in Gwalior, Madhya Pradesh, India. They reported that the density of Euglenophyceae and Cyanophyceae

were greater compared to Bacillariophyceae and Chlorophyceae in polluted water areas.

Tripathi and Shukla (1993) have been made an investigation on the significance of euglenoids as indicator of organic pollution in the river of Ganga at Kanpur, India. They reported that the majority of the euglenoids species were found in water with high organic contents and most species of *Euglena* and *Phacus* can tolerate varying degrees of organic pollution.

Xavier *et al.* (1991) investigated the *Euglena sanguinea* bloom in a fish breeding tank at the Estacao de Piscicultura, Brazil. During bloom of *E. sanguinea*, they registered the data of environmental parameters as water temperature 27.0 ^{0}C, pH 6.9, dissolved oxygen 4.29 mg/l, oxygen saturation 59%, electric conductivity 69.0 µs/cm, ammonia 0.77 mg/l, nitrate 0.15 mg/l, phosphate 0.35 mg/l and silica 4.0 mg/l. They reported that *Euglena sanguinea* bloom developed in chicken manure treated tank caused breath difficulty of fish through trapping of algae in the gill and lower dissolved oxygen resulting the fish eat less and loss their weight markedly.

Olaveson and Stokes (1989) conducted a study on the growth of three axenic strains of *Euglena mutabilis* over pH range of 1.5 to 9.0 under a defined medium. They reported that *Euglena mutabilis* grew best under acidic pH (pH <5.5) with highest growth rate at pH 3.4.

Phang and Ong (1988) undertook a study on the algal biomass production in digested plam oil mill effluent and reported that *Chlorella* dominant at low organic loading rate while *Phacus* and *Euglena* were abundant at high organic loading rates.

Hutchinson and Nakatsu (1984) have been made an investigation on the mutualism of *Euglena mutabilis* and *Cryptococcus* sp. They reported that both *Euglena mutabilis* and *Cryptococcus* sp. grew much at higher concentrations of Fe, Al, Zn, Mn and Cu.

Munawar (1972) investigated the ecology of Euglenineae in certain polluted and unpolluted environments of Hyderabad, India. He reported that higher concentrations of free CO_2 favourable for euglenoid growth in sewage pond and higher values of percent Cl + NO_3 ratio responsible for the luxuriant growth of these flagellates. He also reported that inorganic nitrogen might be more important in their ecology. He concluded that the temperature range 27°–39°C favourable for their growth and had a direct relationship between higher concentrations of oxidizable organic matter and euglenoid population in sewage pond.

2.3. Methodology

The research tools and equipments, the methods for sample collection and analysis, and the methods for data collection and analysis used in the present study are described below.

2.3.1. Duration and location of the study

The study was conducted on relationship of euglenophytes bloom to environmental factors for a period of twelve months from July 2010 to June 2011 in nine fish ponds at three stations of Rajshahi district, Bangladesh. Among these ponds, six were euglenophytes bloom ponds and three were non-bloom ponds. Among the bloom ponds (BP), three ponds were located at Raighati of Mohanpur Upazila (BP-R) and another three bloom ponds were located at Yusufpur of Charghat Upazila (BP-Y). The non-bloom ponds (NBP) were located at Meherchandi of Motihar Thana. The non-bloom ponds were selected to compare the environmental factors, algal community and density in the bloom ponds. The location of study areas are shown in Plate 2.2. The plan works for this study is shown in Chart 2.1.

2.3.2. Study ponds

The ages of the ponds were more or less 10-15 years. The ponds were more or less rectangular in shape with area range was 2.5 to 3.5 dec. and well exposed to sunlight. The embankments of the ponds were well protected. The main sources of water in the ponds were rainfall. During the study period, water level in the ponds varied between 3.0 and 5.5 feet. The ponds were not interconnected and had no outlet. Semi-intensive culture system was practiced in these ponds. The photograph of the study ponds are shown in Plate 2.3, 2.4 and 2.5.

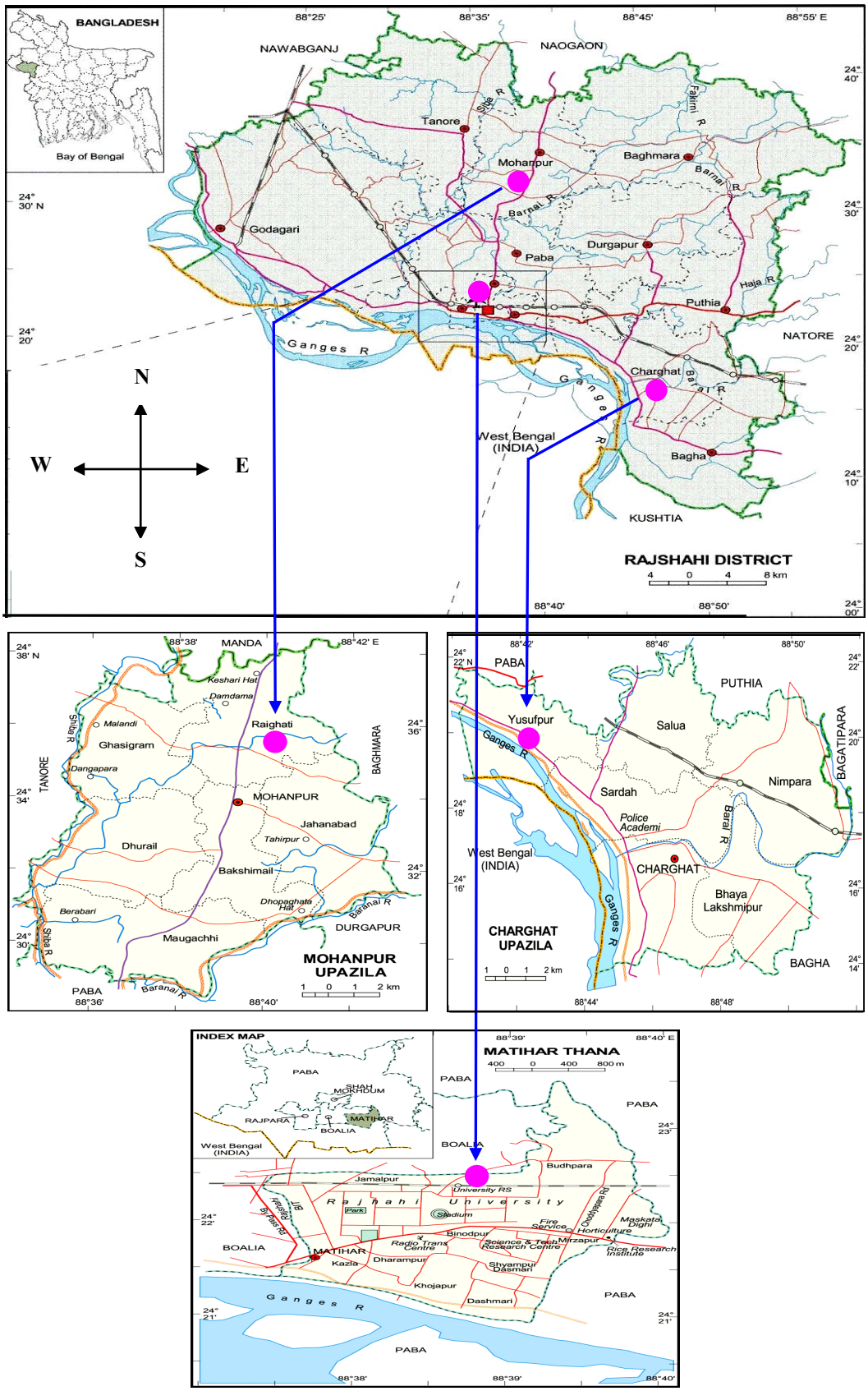


Plate 2.2: The location of the study area

Plate 2.3: The bloom ponds at Raighati, Mohanpur Upazila (BP-R)

Plate 2.4: The bloom ponds at Yusufpur, Charghat Upazila (BP-Y)

Plate 2.5: The non-bloom ponds at Meherchandi, Motihar Thana (NBP)

2.3.3. Pond management

Initially the ponds were limed with quick lime (CaO) at the rate of 1kg/dec. Fertilization of the ponds was done with both organic (cow-dung and poultry manure) and inorganic fertilizers (urea and TSP). The initial and periodic doses of both organic and inorganic fertilizers are shown in Table 2.1. The manures were applied into the ponds as slurry on wet weight basis and applied by spreading all over the pond water. Inorganic fertilizers were also applied by spreading all over the pond water after dissolving in water. After four to seven days of fertilization, the ponds were stocked with fingerlings of common polyculture species such as rohu (*Labeo rohita*), catla (*Catla catla*), mrigel

(*Cirrhina mrigala*), silver carp (*Hypophthalmichthys molitrix*) and silver barb (*Puntius gonionotus*) at the rate of 60-75 fingerlings per decimal. Rice bran and mustard oil cake were applied to the ponds as supplementary feed (1:1) once in a day in small ball form.

Table 2.1: Doses of fertilizers applied in the bloom ponds and non-bloom ponds

Ponds	Fertilizers	Initial dose (/dec.)	Periodic dose (/dec. /15 days)
BP-R	Cow-dung	6-7 kg	2-4 kg (Irregular)
	Poultry manure	2-4 kg	---
	Urea	100-200 g	50-100 g
	TSP	100-200 g	50-100 g
BP-Y	Cow-dung	5-6 kg	2-3 kg (Irregular)
	Poultry manure	2-3 kg	---
	Urea	100-150 g	50-100 g
	TSP	100-150 g	50-100 g
NBP	Cow-dung	6 kg	1-2 kg
	Poultry manure	2 kg	2-3 kg (Irregular)
	Urea	100 g	50 g
	TSP	100 g	50 g

2.3.4. Monitoring of environmental factors

The environmental factors such as water temperature, pH, dissolved oxygen (DO), nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), phosphate-phosphorus (PO_4-P), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) concentrations in the water samples were monitored monthly.

2.3.4.1. Sample collection

Some environmental factors were monitored on the spot. For laboratory analysis, water samples were collected from different points of each pond from surface to a depth of 50 cm in 500 ml sample bottles. .

2.3.4.2. Sample analysis

Collected water samples were analyzed in the laboratory of Department of Fisheries, University of Rajshahi, Rajshahi and SRDI (Soil Resource Development Institute) Laboratory, Rajshahi, Bangladesh. The methods used for analyzing different environmental factors are mentioned below.

a) **Water temperature:** Water temperature was determined on the spot using a Celsius thermometer.

b) **Dissolved oxygen:** Dissolved oxygen was determined by the aid of a water quality test kit (HACH kit FF-2, USA). The estimated concentration of dissolved oxygen was expressed in milligram per litter (mg/l) of water.

c) **pH:** A digital pH meter (HANNA, Model: HI-9142) was used to measure pH of water on the spot.

d) **Nutrients:** The nutrients such as NO_3-N, NH_4-N and PO_4-P concentrations of water samples were determined by using a direct reading of HACH kit (model, Odyssey, DR-2500) with Nitrover and Phosver powder pillows. The estimated concentrations of nutrients were expressed in milligram per litter (mg/l) of water.

e) **Heavy metal:** The heavy metals such as iron, zinc, manganese and copper concentrations of water samples were analyzed by Atomic Absorption Spectrophotometer (Model-3310). The estimated concentrations of heavy metals were expressed in milligram per litter (mg/l) of water.

2.3.5. Determination of soil organic matter

To determine the organic matter of bottom soil in the study ponds, the soil samples were collected and analyzed monthly.

2.3.5.1 Collection of bottom soil sample

An amount of bottom soil with the sediment was collected from each pond with the help of scoop from 6 selected places. After collection of soil sample, each time, it was kept in the plastic bucket and mixed homogeneously in the bucket. Later, it was spread on the polythene paper with the help of bamboo stick. Then half of the samples were thrown out. Again the next half of the sample was spread and then half of the samples were thrown out. At last the remaining soil samples were taken and kept at room temperature in the laboratory for air-drying (one month). After drying, the samples were grinded to make powder and sieved. Afterward about 500 g of sample had been sent to SRDI Laboratory, Shympur, Rajshahi, Bangladesh.

2.3.5.2. Sample analysis

Soil organic carbon was determined by the Walkley-Black method (sulfuric acid-potassium dichromate oxidation). Organic matter of soil was determined by multiplying the percentage of organic carbon with conventional Van-Bemmelen's factor of 1.724 (Piper, 1949).

2.3.6. Study of planktonic algae

2.3.6.1. Sample collection and preparation

For quantitative and qualitative study of planktonic algae, water samples were collected from different depth of each pond. A known volume (10 L) of water was collected in a plastic bucket and passed through plankton net of 25 μm mesh size. The concentrated algae samples were preserved in plastic vials with 5% buffered formalin for subsequent studies.

2.3.6.2. Enumeration and counting

For identification and quantification, 1 ml of concentrated algae sample was taken by a dropper and then put on the S-R (Sedgewick-Rafter) cell. The S-R cell

is a special type of slide having a counting chamber with 1 ml of volume. The counting chamber is equally divided into 1000 fields. After pouring the sample, counting chamber was covered with a cover slip so as to eliminate the air bubbles and left to stand for a few minutes to allow the algae to settle down. After placing the S-R cell under a binocular microscope (Olympus, M-4000D), the algae were identified and counted. The identification of algae was done up to generic level according to Prescott (1964), Belcher and Swale (1978), APHA (1992) and Bellinger (1992). Quantification of the algae was done according to following formula. The number of planktonic algae was expressed numerically per liter of water (cells/l).

$$N = \frac{Ax1000xC}{VxFxL} \quad \text{(Stirling, 1985)}$$

Where, N = No. of algae cells per liter;

A = Total no. of algae counted;

C = Volume of final concentrated sample in ml;

V = Volume of a field in cubic milliliter;

F = Number of the fields counted; and

L = Volume of original water in liter.

2.3.7. Statistical analysis

For statistical analysis of the collected data, one way analysis of variance (ANOVA) was performed using computer software SPSS (Statistical Package for Social Science, version 16.0). Significance was assigned at the 0.05 level. The mean values were compared to see the significant difference from the DMRT (Duncan Multiple Range Test). Correlation analyses were performed to determine relationships between euglenophytes density and environmental factors by using computer software SPSS.

Plan of works for the Study on Relationships of Euglenophytes Bloom to Environmental Factors in Fish Pond

(1st Experiment)

Activities	Month (July 2010 to July 2011)												
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Ma	Jun	Jul
Literature collection													
Ponds selection													
Monitoring of environmental factors													
Analysis soil organic matter													
Enumeration and counting of algae													
Data analysis													

Chart 2.1: Plan of works for the study on relationships of euglenophytes bloom to environmental factors in fish pond

2.4. Results

During the study period, environmental factors, soil organic matter, planktonic algal community and density, correlation between euglenophytes density and environmental factors, and correlation of euglenophytes with other algae were analyzed. The results of these parameters are presented below.

2.4.1. Environmental factors

The environmental factors viz., water temperature, DO, pH, NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu concentrations were monitored monthly and the variations in these factors are shown in Figure 2.1, 2.2 and 2.3. The mean values and ranges of these factors are shown in Table 2.2. The environmental factors (except water temperature) in the bloom ponds (BP-R and BP-Y) showed significant difference from the non-bloom ponds (NBP) but, between BP-R and BP-Y, these factors did no show any significant difference.

2.4.1.1. Water temperature

During the study period, water temperature showed a seasonal trend and it was over 32.0 ºC in the summer and below 18.0 ºC in the winter (Figure 2.1). The values of water temperature varied from 17.21 to 32.16, 17.30 to 32.09 and 17.39 to 32.19 ºC in BP-R, BP-Y and NBP, respectively. There was no significant difference in water temperature among the study ponds.

2.4.1.2. Dissolved oxygen

Dissolved oxygen concentrations were found to be ranged from 4.07 to 5.52, 4.06 to 5.50 and 5.52 to 5.98 mg/l in BP-R, BP-Y and NBP, respectively. The maximum concentration (5.98 mg/l) was recorded in NBP in April and the minimum (4.06 mg/l) in BP-Y in November (Figure 2.1). The mean concentration was significantly lower in BP-R and BP-Y compared to NBP (Table 2.2 and Figure 2.4).

Table 2.2: Mean values (±SD) and ranges of environmental factors in the bloom ponds and non-bloom ponds at different stations of Rajshahi

Parameters	Study ponds		
	BP-R	BP-Y	NBP
Temperature (^{0}C)	26.29 ± 4.47^a (17.21-32.16)	26.37 ± 4.42^a (17.30-32.09)	26.39 ± 4.52^a (17.39-32.19)
DO (mg/l)	4.96 ± 0.47^b (4.07-5.52)	4.99 ±0.44^b (4.06-5.50)	5.72 ± 0.38^a (5.52-5.98)
pH	6.30 ± 0.39^b (5.94-6.60)	6.34 ± 0.41^b (5.99-6.65)	7.84 ± 0.39^a (7.67-8.03)
NO_3-N (mg/l)	1.22 ± 0.28^a (0.96-1.76)	1.24 ± 0.29^a (0.95-1.81)	0.48 ± 0.11^b 0.29-0.54)
NH_4-N (mg/l)	1.05 ± 0.26^a (0.68-1.43)	1.08 ± 0.27^a (0.70-1.49)	0.23 ± 0.07^b (0.19-0.28)
PO_4-P (mg/l))	1.17 ± 0.35^a (0.88-1.86)	1.19 ± 0.32^a (0.93-1.80)	0.41 ± 0.10^b (0.27-0.48)
Fe (mg/l)	0.50 ± 0.15^a (0.35-0.78)	0.53 ± 0.13^a (0.39-0.75)	0.18 ± 0.05^b (0.11-0.24)
Zn (mg/l)	0.25 ± 0.09^a (0.17-0.38)	0.28 ± 0.10^a (0.18-0.42)	0.09 ± 0.03^b (0.04-0.11)
Mn (mg/l))	0.26 ± 0.07^a (0.16-0.36)	0.24 ± 0.07^a (0.15-0.33)	0.11 ± 0.03^b (0.06-0.14)
Cu (mg/l)	0.25 ± 0.05^a (0.20-0.31)	0.26 ± 0.07^a (0.17-0.37)	0.10 ± 0.03^b (0.07-0.13)

***BP-R:** Bloom ponds at Raighati, Mohanpur; **BP-Y:** Bloom ponds at Yusufpur, Charghat; and **NBP:** Non-bloom ponds at Meherchandi, Motihar.

*Values of environmental factors are mean of triplicate determination. Values in the same row with different superscripts are significantly different ($P<0.05$)

2.4.1.3. pH

pH values were varied from 5.94 to 6.60, 5.99 to 6.65 and 7.67 to 8.03 in BP-R, BP-Y and NBP, respectively. The maximum value (8.03) was recorded in NBP in September and the minimum (5.94) in BP-R in December (Figure 2.1). Significantly lower mean value was recorded in BP-R and BP-Y compared to NBP (Table 2.2 and Figure 2.4).

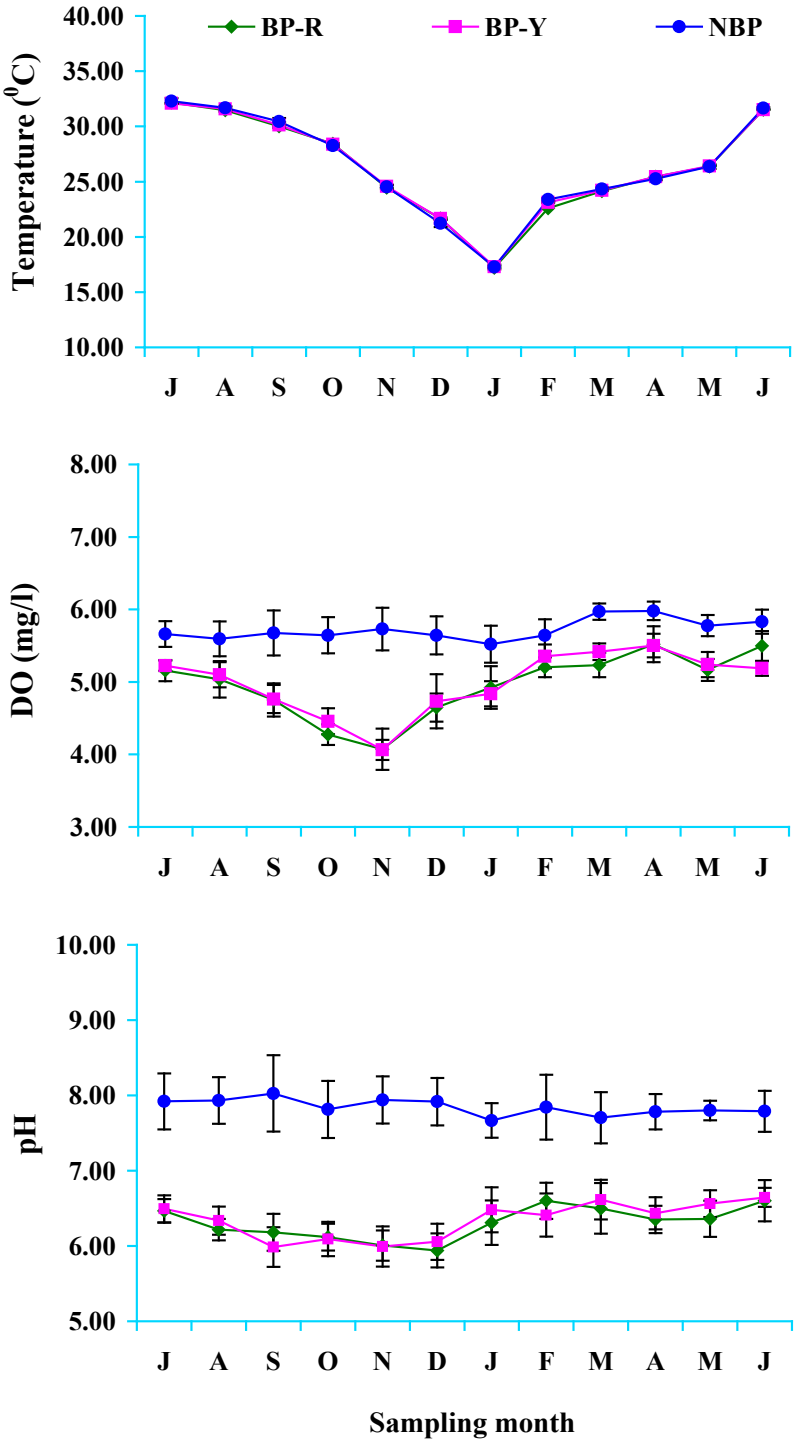


Figure 2.1: Monthly variations in water temperature, DO and pH in BP-R, BP-Y and NBP

2.4.1.4. Nitrate-nitrogen

The concentrations of NO_3-N varied from 0.96 to 1.76, 0.95 to 1.81 and 0.29 to 0.54 mg/l in BP-R, BP-Y and NBP, respectively. The maximum concentration (1.81 mg/l) was recorded in BP-Y in November and the minimum (0.29 mg/l) in NBP in July (Figure 2.2). During the study period, the concentration of this nutrient was almost over 1.0 mg/l in BP-R and BP-Y and increased up to 1.81 mg/l in BP-Y whereas in NBP, it was below 0.55 mg/l. Significantly higher mean concentration was recorded in BP-R and BP-Y compared to NBP (Table 2.2 and Figure 2.4.).

2.4.1.5. Ammonium-nitrogen

During the study period, the concentration of NH_4-N in BP-R and BP-Y was over 0.65 mg/l and increased up to 1.49 mg/l in BP-Y whereas in NBP, it was below 0.30 mg/l. The concentrations of this nutrient were found to be ranged from 0.68 to 1.43, 0.70 to 1.49 and 0.19 to 0.28 mg/l in BP-R, BP-Y and NBP, respectively. The maximum (1.49 mg/l) concentration was recorded in BP-Y in November and the minimum (0.19 mg/l) in NBP in May (Figure 2.2). The mean concentration was significantly high in BP-R and BP-Y compared to NBP (Table 2.2 and Figure 2.4.).

2.4.1.6. Phosphate-phosphorus

The concentrations of PO_4-P varied from 0.88 to 1.86, 0.93 to 1.80 and 0.27 to 0.48 mg/l in BP-R, BP-Y and NBP, respectively. The maximum concentration (1.86 mg/l) was recorded in BP-R in December and the minimum (0.27 mg/l) in NBP in July (Figure 2.2). The concentration of this nutrient in BP-R and BP-Y was over 0.81 mg/l and increased up to 1.86 mg/l in BP-R whereas in NBP, it was below 0.50 mg/l. Like other nutrients, significantly higher mean concentration was recorded in BP-R and BP-Y compared to NBP (Table 2.2 and Figure 2.4).

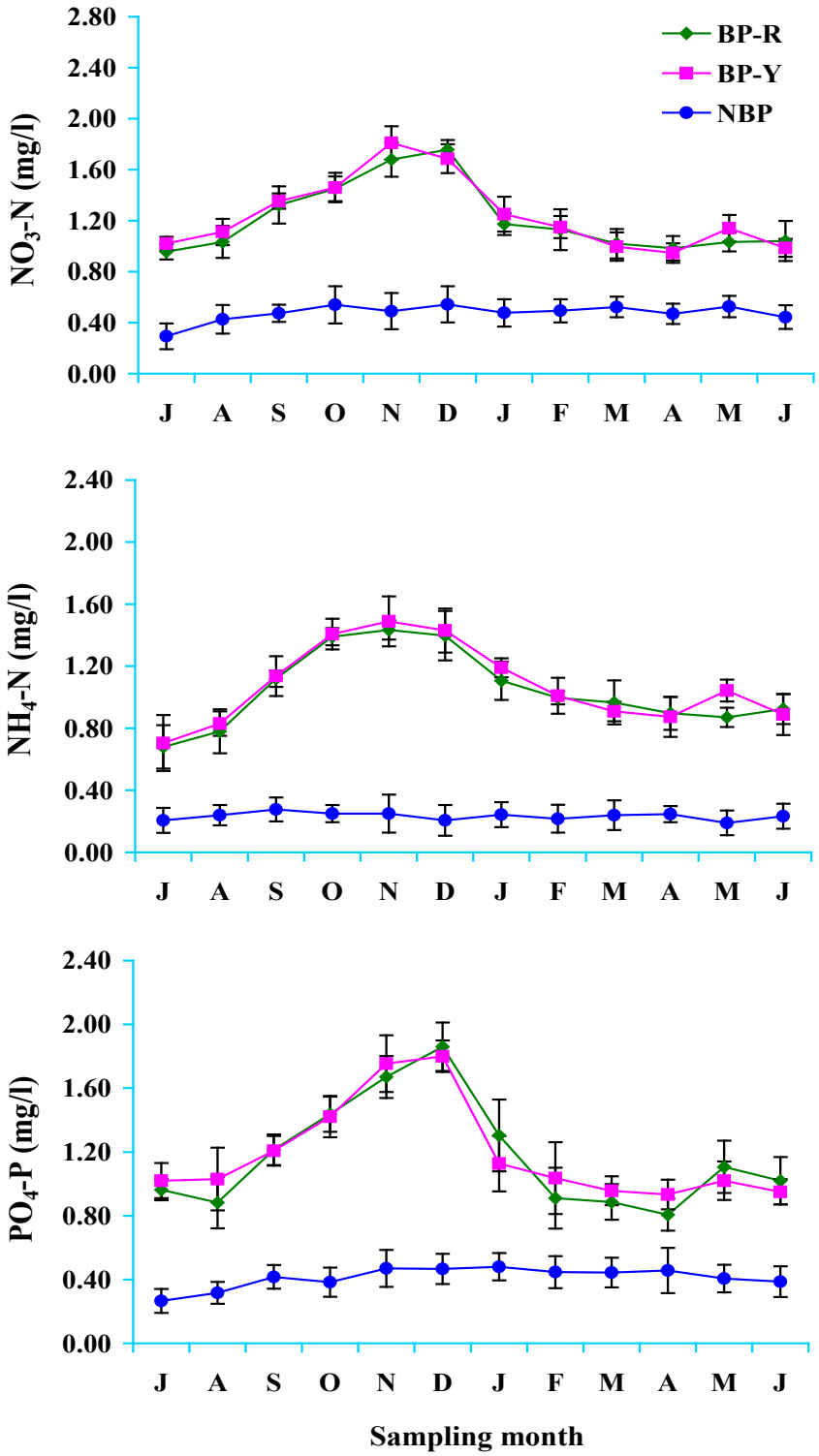


Figure 2.2: Monthly variations in NO_3-N, NH_4-N and PO_4-P concentrations in BP-R, BP-Y and NBP

2.4.1.7. Iron

The concentrations of Fe varied from 0.35 to 0.78, 0.39 to 0.75 and 0.11 to 0.24 mg/l in BP-R, BP-Y and NBP, respectively. The maximum concentration (0.78 mg/l) was recorded in BP-R in November and the minimum (0.11 mg/l) in NBP in July (Figure 2.3). Significantly higher mean concentration was recorded in BP-R and BP-Y compared to NBP (Table 2.2 and Figure 2.4).

2.4.1.8. Zinc

The concentrations of Zn were found to be ranged from 0.17 to 0.38, 0.18 to 0.42 and 0.04 to 0.11 mg/l in BP-R, BP-Y and NBP, respectively. The maximum concentration (0.42 mg/l) was found in BP-Y in November and the minimum (0.04 mg/l) in NBP in August (Figure 2.3). The mean concentration of this heavy metal was significantly high in BP-R and BP-Y but in NBP, it was quietly low (Table 2.2 and Figure 2.4).

2.4.1.9. Manganese

The concentrations of Mn varied from 0.16 to 0.36, 0.15 to 0.33 and 0.06 to 0.14 mg/l in BP-R, BP-Y and NBP, respectively. The maximum concentration of this heavy metal (0.36 mg/l) was recorded in BP-R in November and the minimum (0.06 mg/l) in NBP in July (Figure 2.3). Significantly higher mean concentration was recorded in BP-R and BP-Y compared to NBP (Table 2.2 and Figure 2.4).

2.4.1.10. Copper

The concentrations of Cu varied from 0.20 to 0.31, 0.17 to 0.37 and 0.07 to 0.13 mg/l in BP-R, BP-Y and NBP, respectively. The maximum concentration was recorded in BP-Y in November and the minimum in NBP in July (Figure 2.3). The mean concentration of this heavy metal was significantly high in BP-R and BP-Y compared to NBP (Table 2.2 and Figure 2.4).

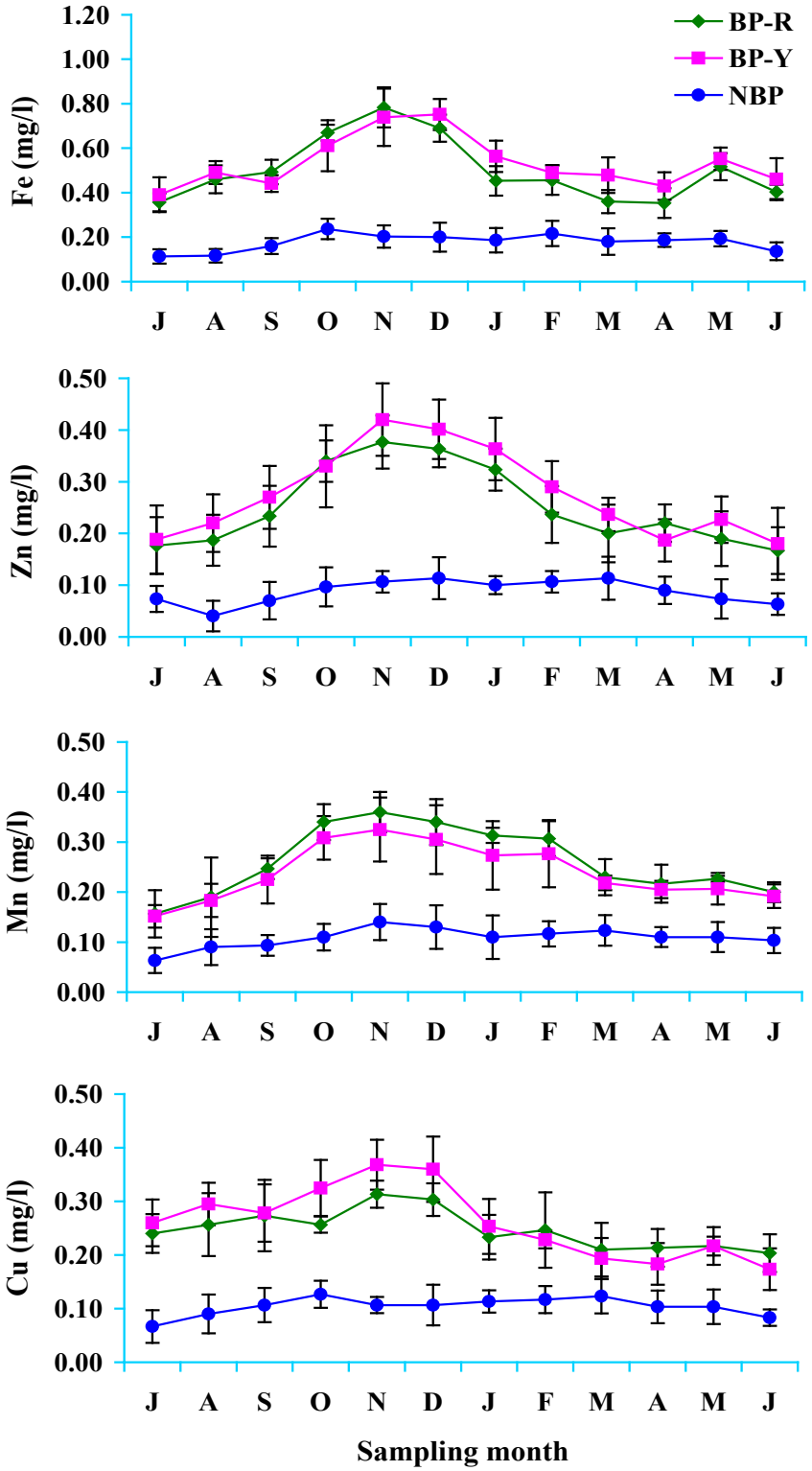


Figure 2.3: Monthly variations in Fe, Zn, Mn and Cu concentrations in BP-R, BP-Y and NBP

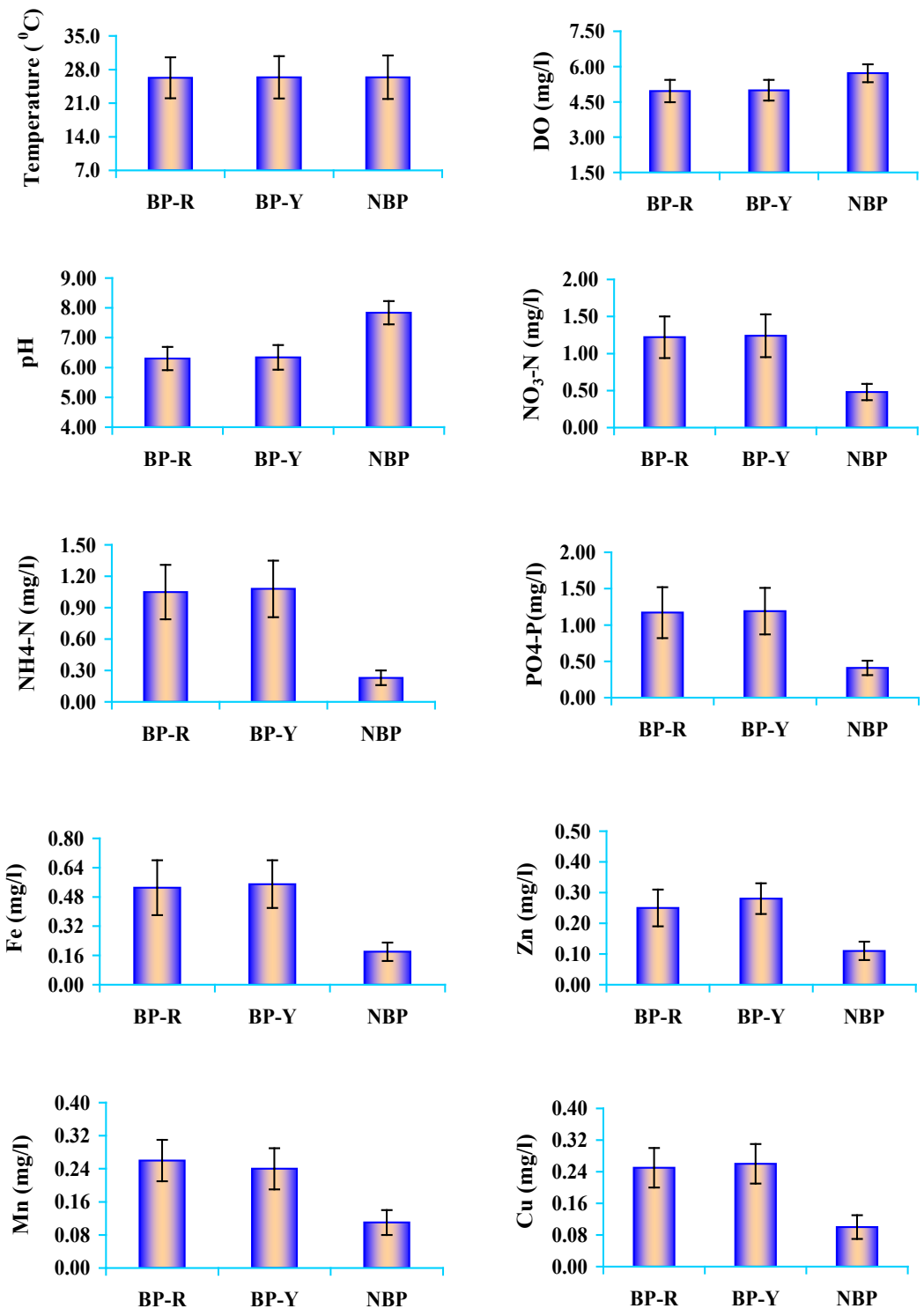


Figure 2.4: Variations in mean values of environmental factors in BP-R, BP-Y and NBP

2.4.2. Soil organic matter

Monthly variations in organic matter of bottom soil in BP-R, BP-Y and NBP are shown in Figure 2.5. Significantly higher soil organic matter was recorded in BP-R and BP-Y compared to NBP ($p<0.05$) and the values varied from 5.06 to 7.98, 5.26 to 7.64 and 2.88 to 3.55% in BP-R, BP-Y and NBP, respectively. The variation in mean values of soil organic matter in BP-R, BP-Y and NBP is shown in Figure 2.6.

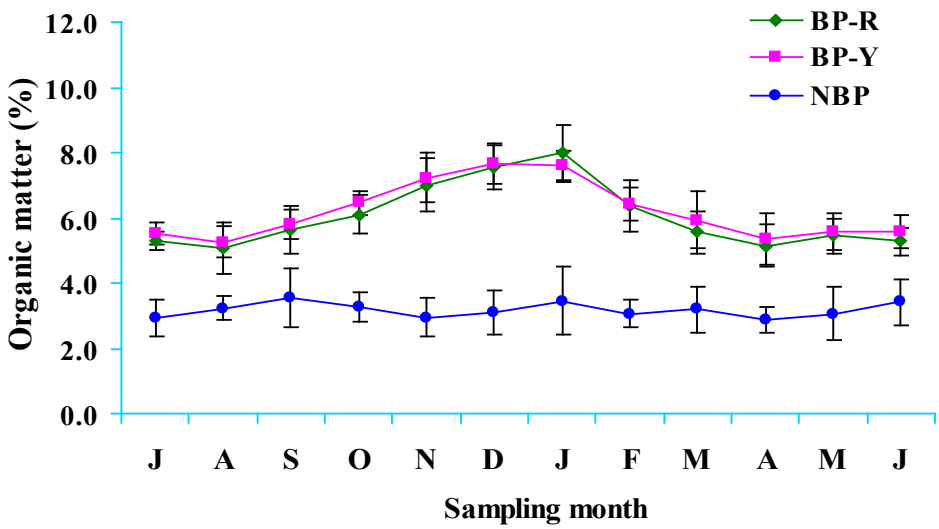


Figure 2.5: Monthly variations in soil organic matter in BP-R, BP-Y and NBP

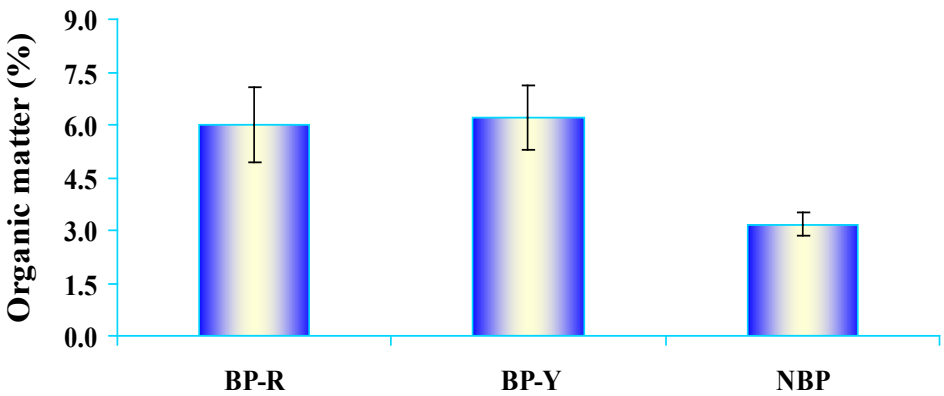


Figure 2.6: Variations in mean values of soil organic matter in BP-R, BP-Y and NBP

2.4.3. Planktonic algal community

During the entire study period, total 28 genera of planktonic algae belonging to euglenophytes, cyanophytes, chlorophytes and bacillariophytes were recorded from the study ponds (Table 2.3). Monthly variations in number of planktonic algal genera in BP-R, BP-Y and NBP are shown in Figure 2.7. The number of planktonic algal genera varied from 13 to 22, 12 to 23 and 21 to 27 in BP-R, BP-Y and NBP, respectively. Relatively higher number of algal genera was recorded in NBP compared to BP-R and BP-Y. In the bloom ponds, the maximum number (23) was recorded in BP-Y in August and the minimum number (12) was also in BP-Y in January whereas in the non-bloom ponds, the maximum number (27) was recorded in May and the minimum number (21) in January. Among four groups of planktonic algae, chlorophytes had the maximum number of genera (11) and euglenophytes had the minimum number of genera (3). Only euglenophytes genera were occurred in each sampling month in all the study ponds throughout the study period.

Table 2.3: Generic status of planktonic algae in the bloom ponds and non-bloom ponds at different stations of Rajshahi

Algal group	Genera under each group
Euglenophytes	*Euglena, Phacus* and *Trachelomonas*
Cyanophytes	*Anabaena, Apanizomenon, Aphanocapsa, Chroococcus, Gomphospheria*, *Oscillatoria* and *Microcystis*
Chlorophytes	*Botryococcus, Chlorella, Closterium, Pediastrum, Scenedesmus, Spirogyra*, *Staurastrum, Teraedon, Ulothrix*, *Volvox* and *Zygnema*
Bacillariophytes	*Asterionella, Cyclotella, Fragilaria, Navicula, Nitzschia, Synedra* and *Tabellaria*

2.4.4. Density of planktonic algae

2.4.4.1. Density of total planktonic algae

Monthly variations in density of total planktonic algae in BP-R, BP-Y and NBP are shown in Figure 2.8. The density of total planktonic algae were found to vary from 15.36 to 36.52, 15.21 to 41.90 and 14.25 to 20.84 x 10^4 cells/l in BP-R, BP-Y and NBP, respectively. The maximum density (41.90 x 10^4 cells/l) was recorded in BP-Y in December and the minimum (14.25 x 10^4 cells/l) in NBP in February. The mean density of total planktonic algae was significantly high in BP-R and BP-Y compared to NBP (Table 2.4 and Figure 2.11.a).

2.4.4.2. Density of euglenophytes

Monthly variation in density of euglenophytes in BP-R, BP-Y and NBP is shown in Figure 2.8. The density of this group of algae varied from 8.36 to 31.88, 8.12 to 38.79 and 2.24 to 3.67 x 10^4 cells/l in BP-R, BP-Y and NBP, respectively. Monthly percent contributions of these algae in BP-R and BP-Y were always higher than NBP (Figure 2.10). Significantly higher mean density was recorded in BP-R and BP-Y compared to NBP (Table 2.4 and Figure 2.11.b). The average percent contributions of these algae were 68.03, 69.79 and 17.69% in BP-R, BP-Y and NBP, respectively (Figure 2.12).

In BP-R and BP-Y, these algae occupied the most dominant group in respect of density. Their density was relatively low in July and August but started to increase in September and formed a peak bloom in November and December with the maximum density (38.79 x 10^4 cells/l) in BP-Y. The density was started to decrease from January and it was quietly low in February, March and June, although a light increase was observed in May. But in NBP, their density showed no significant variation in monthly observations and it was quietly low throughout the study period compared to BP-R and BP-Y.

Table 2.4: Mean density (± SD) of different groups of planktonic algae in the bloom ponds and non-bloom ponds at different stations of Rajshahi

Group of algae	Study ponds		
	BP-R	BP-Y	NBP
Total algae (x 10^4 cells/l)	23.16 ± 7.65[a] (15.36-36.52)	24.91 ± 9.02[a] (15.21-41.90)	16.67 ± 2.80[b] (14.25-20.84)
Euglenophytes (x 10^4 cells/l)	15.76 ± 8.11[a] (8.36-31.88)	17.39 ± 10.30[a] (8.12-38.79)	2.95 ± 1.12[b] (2.24-3.67)
Cyanophytes (x 10^4 cells/l)	4.53 ±2.06[b] (2.99-8.37)	4.52 ± 2.20[b] (1.63-8.95)	9.45 ± 2.25[a] (7.57-13.34)
Chlorophytes (x 10^4 cells/l)	2.65 ± 0.76[b] (1.57-3.38)	2.76 ± 0.85[b] (1.03-3.48)	3.60 ± 0.63[a] (2.74-4.34)
Bacillariophytes (x 10^4 cells/l)	0.23 ± 0.11[b] (0.08-0.40)	0.25 ± 0.10[b] (0.09-0.36)	0.67± 0.13[a] (0.44-0.86)

* **BP-R:** Bloom ponds at Raighati, Mohanpur; **BP-Y:** Bloom ponds at Yusufpur, Charghat; and **NBP:** Non-bloom ponds at Meherchandi, Motihar.

*Values of algal density are mean of triplicate determination. Density values in the same row with different superscripts are significantly different ($p<0.05$)

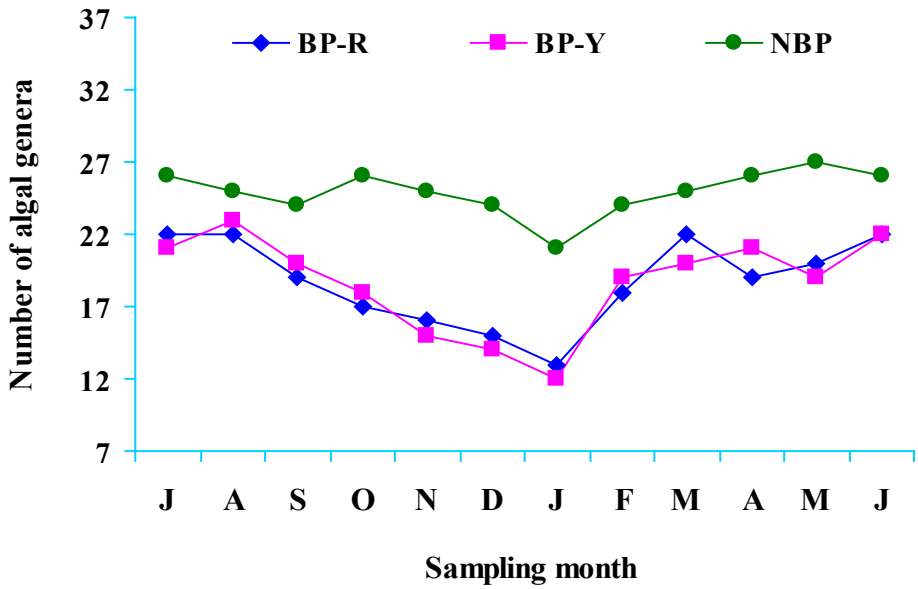


Figure 2.7: Monthly variations in number of planktonic algal genera in BP-R, BP-Y and NBP

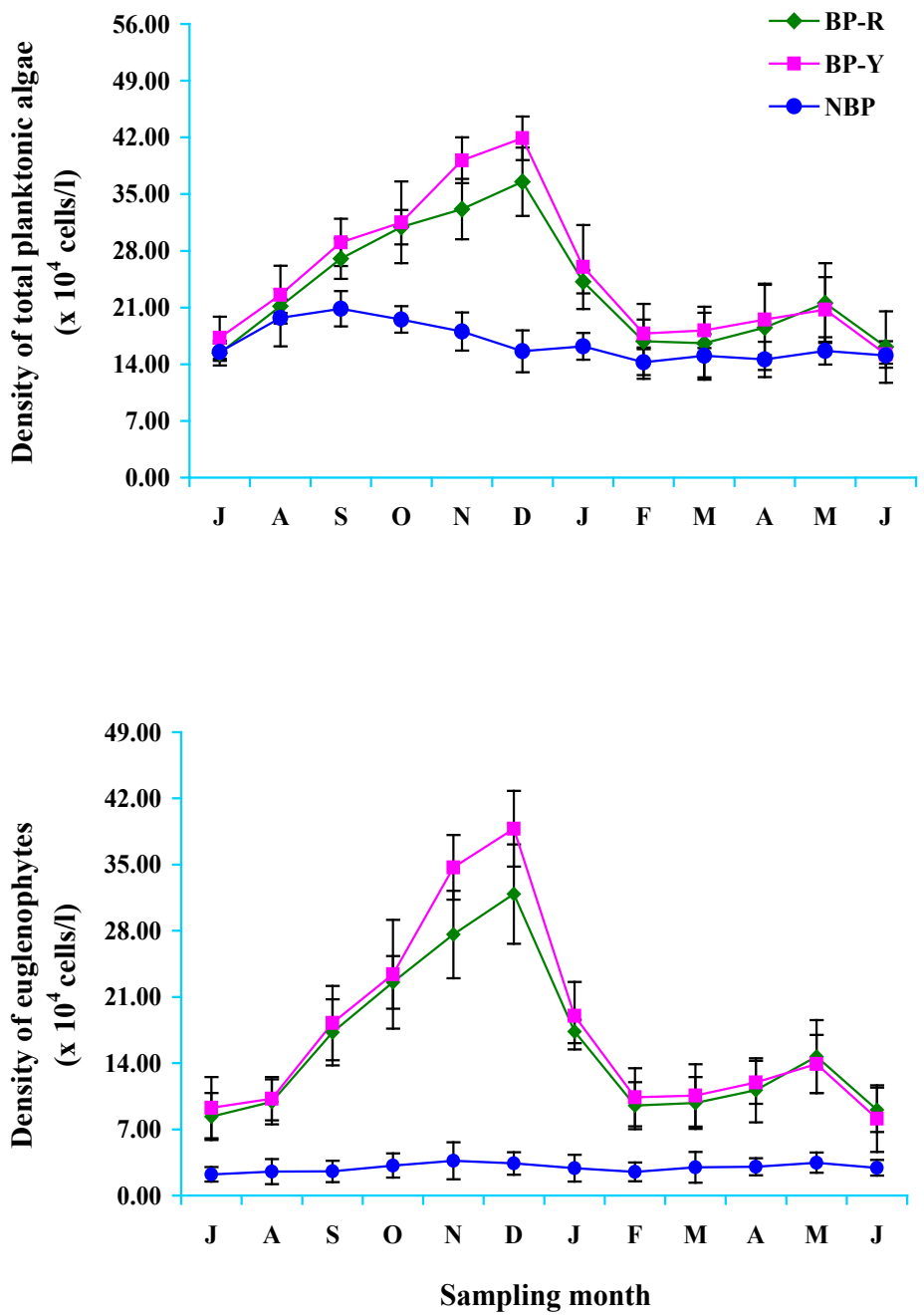


Figure 2.8: Monthly variations in density of total planktonic algae and euglenophytes in BP-R, BP-Y and NBP

2.4.4.3. Density of cyanophytes

Cyanophytes was the second abundant group of algae in BP-R and BP-Y but in NBP, it was the most abundant group of algae. Monthly variations in density and in percent contributions of this group of algae are shown in Figure 2.9 and 2.10. Significantly higher mean density was recorded in NBP compared to BP-R and BP-Y (Table 2.4 and Figure 2.11.c). The maximum density (13.34 x 10^4 cells/l) was recorded in NBP in September and the minimum (1.63 x 10^4 cells/l) was in BP-Y in December. The average percent contributions of cyanophytes in the total algae were 19.55, 18.13 and 56.72 % in BP-R, BP-Y and NBP, respectively (Figure 2.12).

2.4.4.4. Density of chlorophytes

Monthly variations in density and in percent contributions of chlorophytes are shown in Figure 2.9 and 2.10. Significantly higher mean density was recorded in NBP compared to BP-R and BP-Y (Table 2.4 and 2.11.d). The maximum density (4.34 x 10^4 cells/l) was recorded in NBP in August and the minimum density (1.03 x 10^4 cells/l) in BP-Y in November. The average percent contributions of chlorophytes in the total algae were 11.43, 11.09 and 21.59% in BP-R, BP-Y and NBP, respectively (Figure 2.12).

2.4.4.5. Density of bacillariophytes

Bacillariophytes was the least abundant group of algae but its mean density was significantly higher in NBP compared to BP-R and BP-Y (Table. 2.4 and Figure 2.11.e). Month variations in density and in percent contributions of these algae are shown in Figure 2.9 and 2.10. The maximum density (0.86 x 10^4 cells/l) was recorded in NBP in October and the minimum (0.08 x 10^4 cells/l) in BP-R in December. The average percent contributions of bacillariophytes in the total algae were 0.99, 0.98 and 4.00 % in BP-R, BP-Y and NBP, respectively (Figure 2.12).

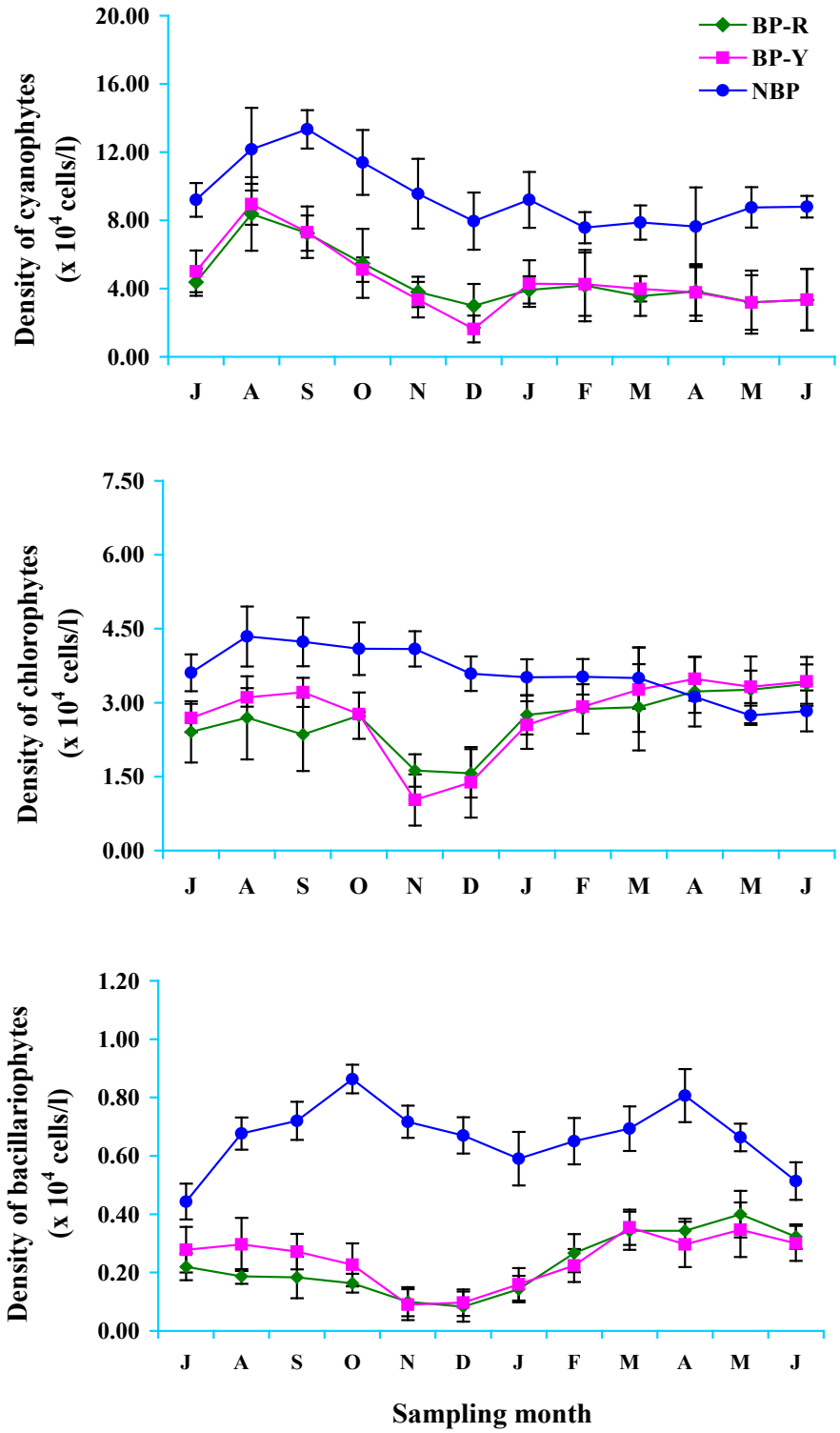


Figure 2.9: Monthly variations in density of cyanophytes, chlorophytes and bacillariophytes in BP-M, BP-Y and NBP

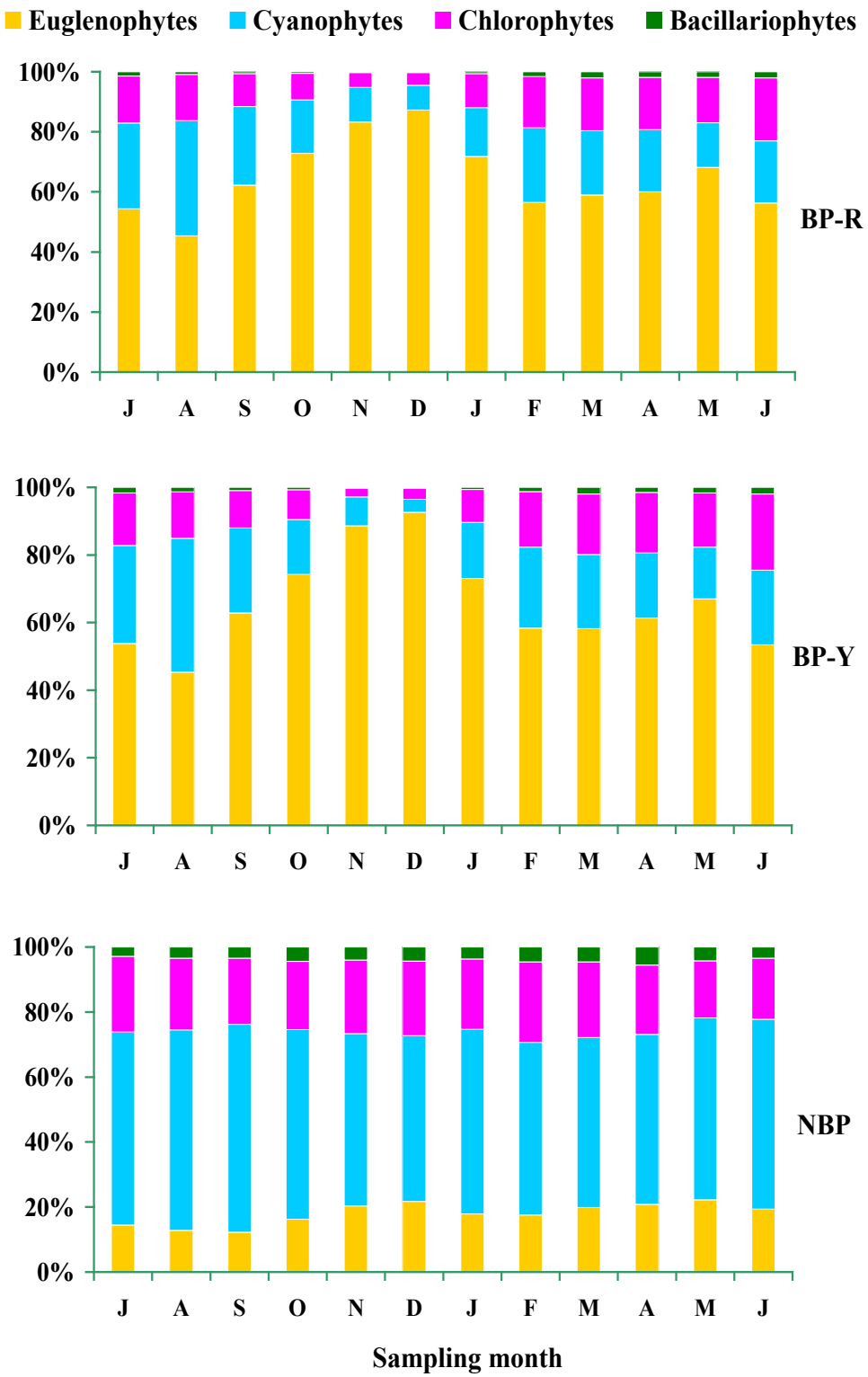


Figure 2.10: Monthly variations in percent contributions of different algal groups in BP-R, BP-Y and NBP

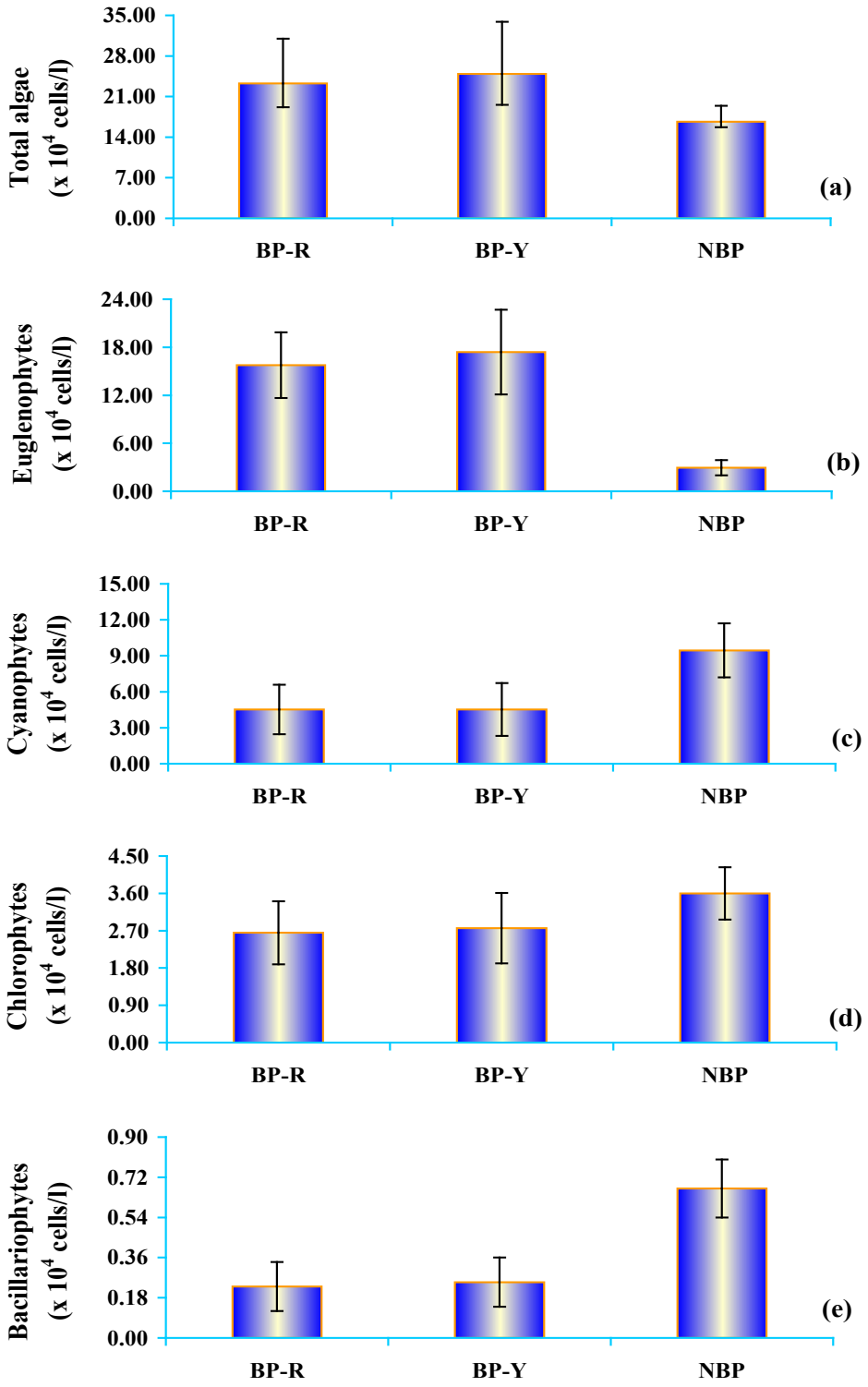


Figure 2.11: Variations in mean density of (a) total algae, (b) euglenophytes (c) cyanophytes (d) chlorophytes and (e) bacillariophytes in BP-R, BP-Y and NBP

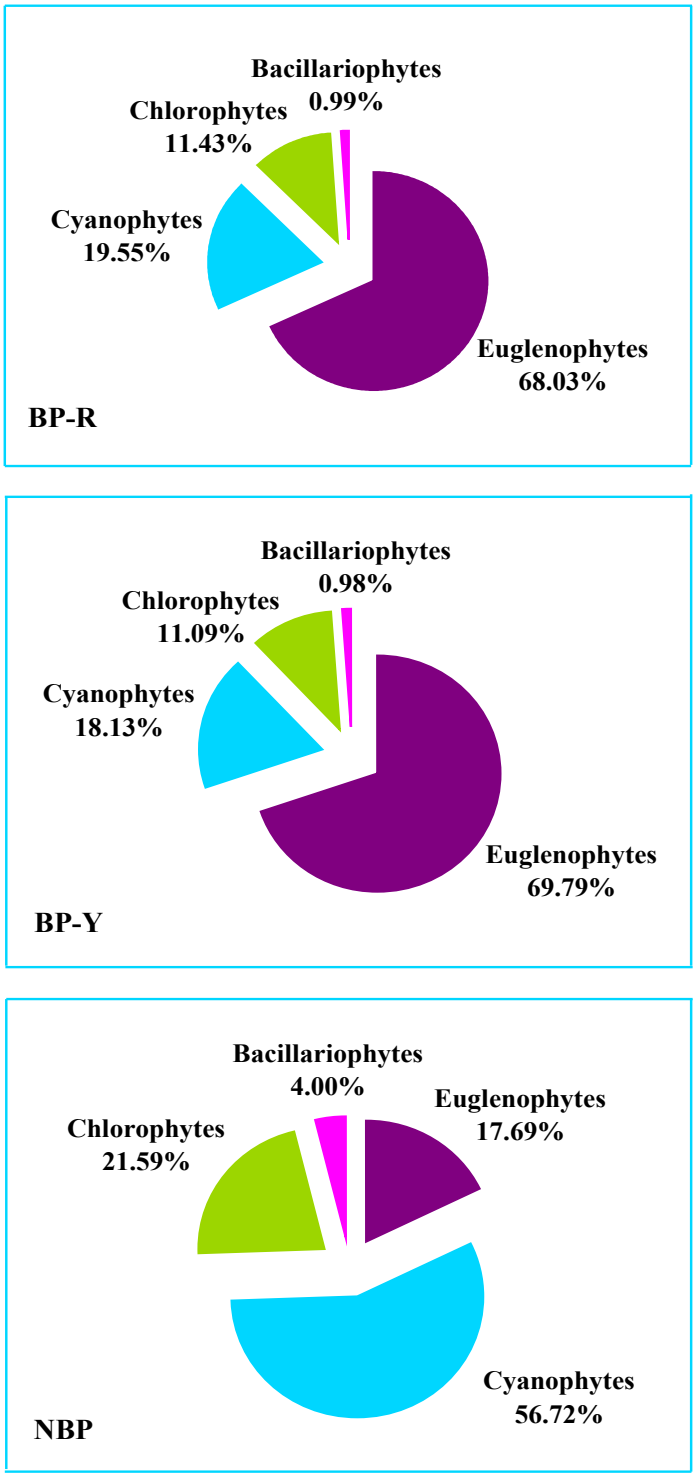


Figure 2.12: Average percent contributions of different groups of algae in BP-R, BP-Y and NBP

2.4.5. Density of euglenophytes genera

In the present study, only euglenophytes genera were included further analyses because emphasis has been given on this group of algae due to its higher density. Euglenophytes algae in the study ponds were occurred by three genera, *Euglena, Phacus* and *Trachelomonas* (Plate 2.6). Monthly variations in density of euglenophytes genera are shown in Figure 3.13. Mean density and ranges of these three genera are shown in Table 2.5.

2.4.5.1. Genus: *Euglena*

Among three genera of euglenophytes, *Euglena* was the most dominant genus based on the density. The density of *Euglena* varied from 7.16 to 28.30, 6.25 to 34.34 and 2.17 to 3.50 x 10^4 cells/l in BP-R, BP-Y and NBP, respectively. The maximum density value (34.34 x 10^4 cells/l) was recorded in BP-Y in December and the minimum density value (2.17 x 10^4 cells/l) was in NBP in July (Figure 2.13). Significantly higher mean density of this genus was recorded in BP-R and BP-Y as compared to NBP (Table 2.5). In the total euglenophytes, average percent contributions of *Euglena* were 83.21, 83.50 and 95.52% in BP-R, BP-Y and NBP, respectively (Figure 2.14).

2.4.5..2. Genus: *Phacus*

Significantly higher mean density of *Phacus* was recorded in BP-R and BP-Y compared to NBP (Table 2.5) and its maximum density value (4.38 x 10^4 cell/l) was recorded in BP-Y in November. In NBP, the occurrence of this genus was very little in number compared to BP-R and BP-Y and its minimum density value (0.02 x 10^4 cell/l) was recorded in July. The density of this genus varied from 0.81 to 3.56, 1.02 to 4.38 and 0.02 to 0.14 x 10^4 cells/l in BP-R, BP-Y and NBP, respectively. In the total euglenophytes, average percent contributions of *Phacus* were 13.53, 13.37 and 3.07% in BP-R, BP-Y and NBP, respectively (Figure 2.14).

Table 2.5: Mean density (±SD) and ranges of euglenophytes genera in the bloom ponds and non-bloom ponds at different stations of Rajshahi

Euglenophytes genera	Study ponds		
	BP-R	BP-Y	NBP
Euglena (x 10^4 cells/l)	13.11 ± 6.93^a 7.16-28.30	14.52 ± 9.07^a 6.25-34.34	2.82 ± 0.48^b 2.17-3.50
Phacus (x 10^4 cells/l)	2.13 ± 0.98^a 0.81-3.56	2.32 ± 1.16^a 1.02-4.38	0.09 ± 0.04^b 0.02-0.14
Trachelomonas (x 10^4 cells/l)	0.52 ± 0.17^a 0.33-0.72	0.54 ± 0.19^a 0.35-0.78	0.04 ± 0.03^b 0.02-0.08

* **BP-R:** Bloom ponds at Raighati, Mohanpur; **BP-Y:** Bloom ponds at Yusufpur, Charghat; and **NBP:** Non-bloom ponds at Meherchandi, Motihar.

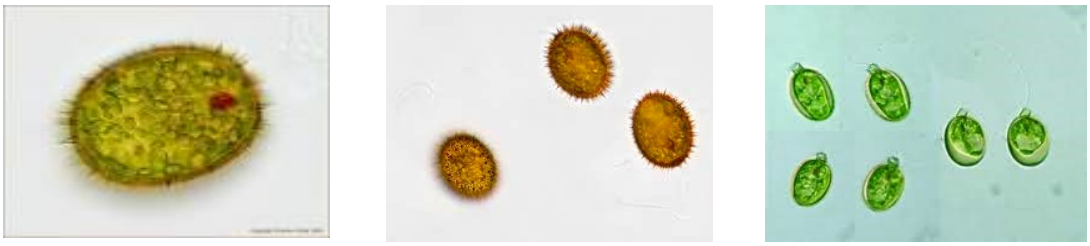
* Values of algal density are mean of triplicate determination. Density values in the same row with different superscripts are significantly different ($P<0.05$)

2.4.5.3. Genus: *Trachelomonas*

The genus, *Trachelomonas* was occurred less in number compared to other two genera. Density of this genus varied from 0.33 to 0.72, 0.35 to 0.78 and 0.02 to 0.08 x 10^4 cells/l in BP-R, BP-Y and NBP, respectively. The maximum density value (0.78 x 10^4 cell/l) was recorded in BP-Y in November and the minimum density value (0.02 x 10^4 cells/l) was in NBP in October (Figure 2.13). Significantly higher mean density was recorded in BP-R and BP-Y compared to NBP (Table 2.5). In the total euglenophytes, average percent contributions of this genus were 3.27, 3.13 and 1.41% in BP-R, BP-Y and NBP, respectively (Figure 2.14).

Genus: *Euglena*

Genus: *Phacus*



Genus: *Trachelomonas*

Plate 2.6: The genera of euglenophytes

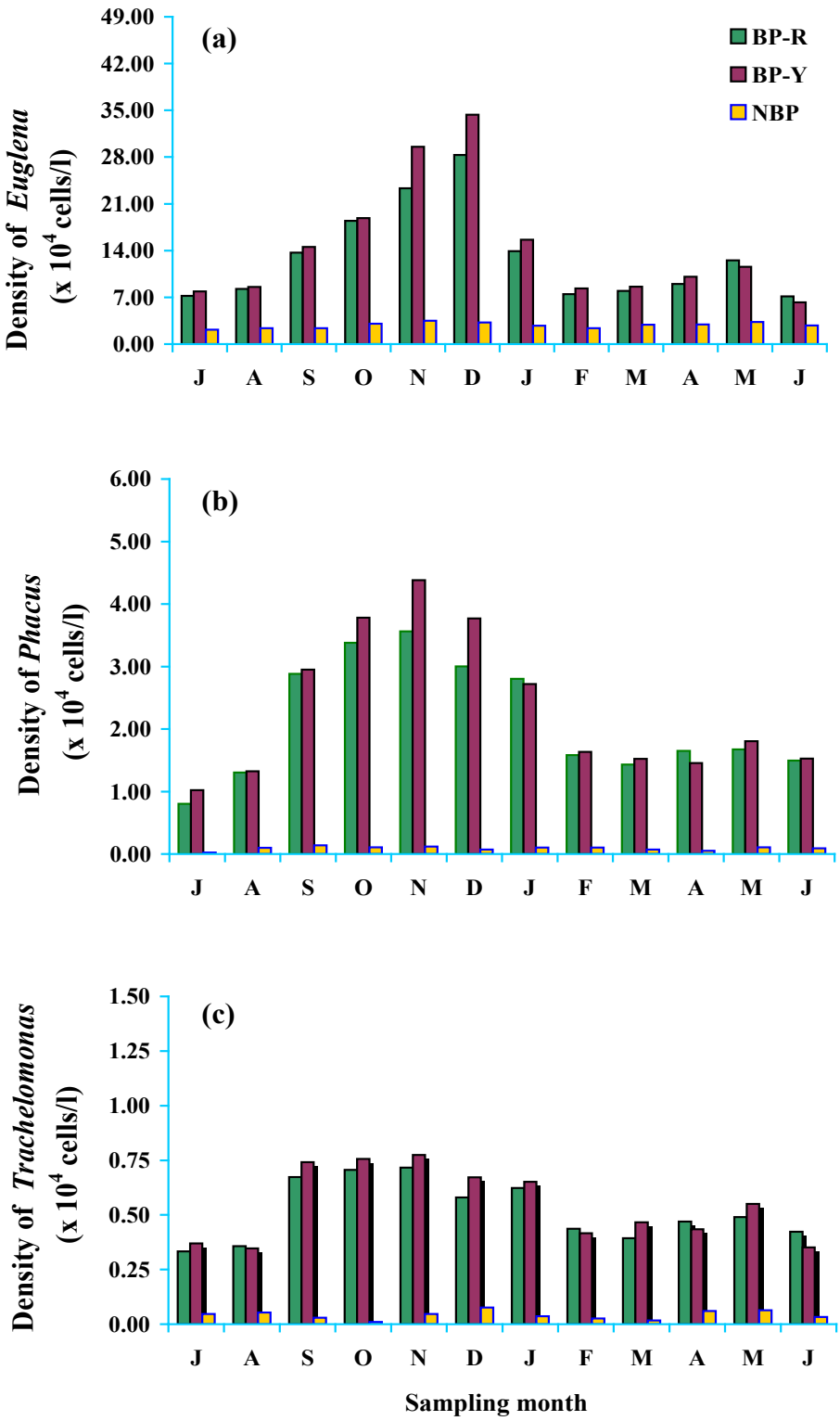


Figure 2.13: Monthly variations in density of (a) *Euglena* , (b) *Phacus* and (c) *Trachelomonas* in BP-R, BP-Y and NBP

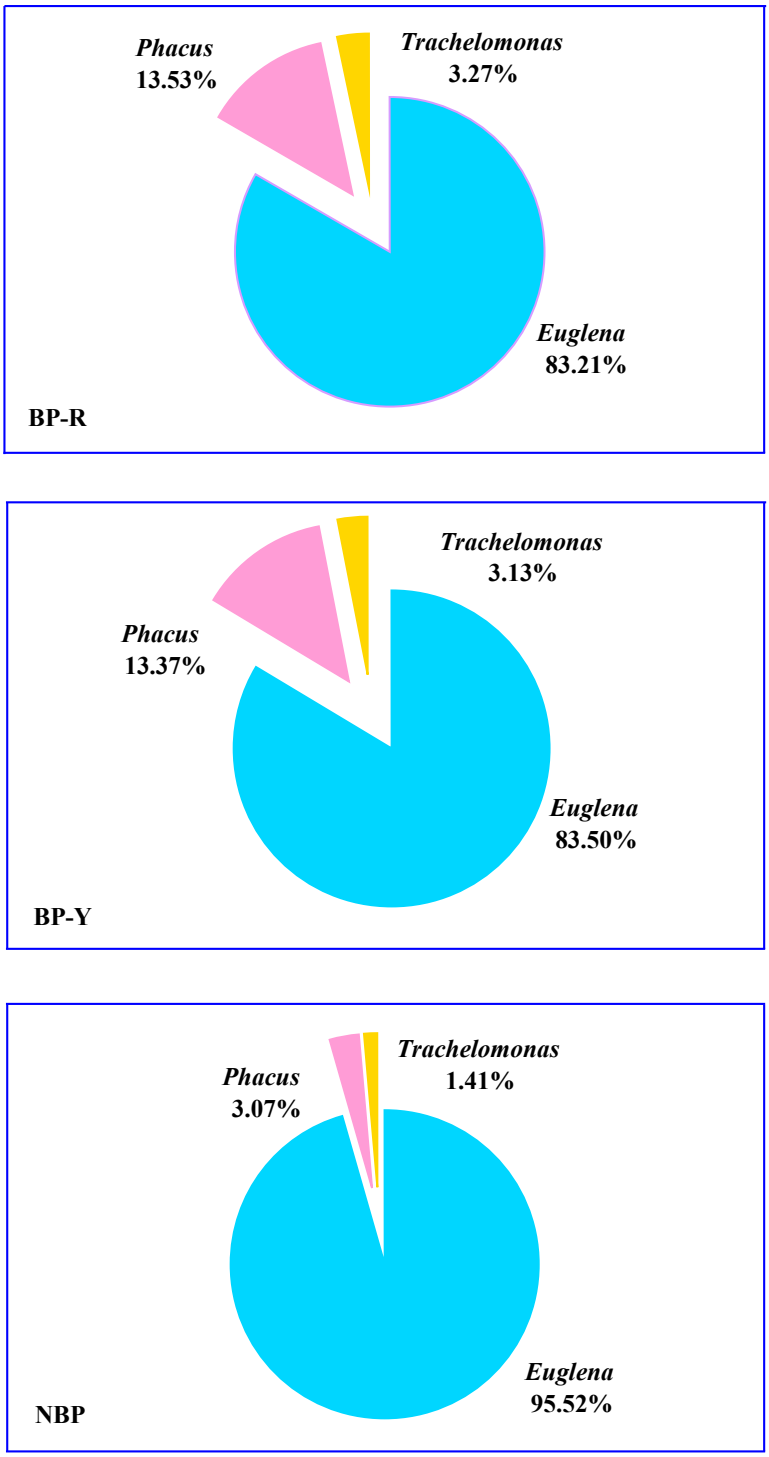


Figure 2.14: Average percent contributions of different genera of euglenophytes in BP-R, BP-Y and NBP

2.4.6. Correlations between euglenophytes density and environmental factors

By correlation analysis, it was observed that euglenophytes density in the blooms ponds (BP-R and BP-Y) was negatively correlated with water temperature ($r = -0.407$ and -0.432; $P<0.05$), dissolved oxygen ($r = -0.807$ and -0.806; $P<0.05$) and pH ($r = -0.905$ and -0.868; $P<0.05$) whereas the density was positively correlated with NO_3-N ($r = 0.949$ and 0.914; $P<0.05$), NH_4-N ($r = 0.793$ and 0.815; $P<0.05$) and PO_4-P ($r = 0.793$ and 815; $P<0.05$). Euglenophytes density was also positively correlated with Fe ($r = 0.886$ and 0.868; $P<0.05$), Zn ($r = 0.902$ and 0.895; $P<0.05$), Mn ($r = 0.809$ and 0.813; $P<0.05$) and Cu ($r = 0.782$ and 0.824; $P<0.05$).

The relationships between euglenophytes density and environmental factors are shown graphically in Figure 2.15, 2.16 and 2.17. It was observed that euglenophytes density was increased with decreasing water temperature, dissolved oxygen and pH, and with increasing nutrients and heavy metal concentrations whereas the density showed a declining trend with increasing temperature, dissolved oxygen, pH, and with decreasing nutrient and heavy metal concentrations.

2.4.7. Correlations of euglenophytes with other algae

In the present study, the density of euglenophytes in the bloom ponds (BP-R and BP-Y) showed negative correlation with the density of cyanophytes ($r = -0.024$ and -0.092, insignificant, $p>0.05$), chlorophytes ($r = -0.492$ and -0.650, $p<0.01$) and bacillariophytes ($r= -0.725$ and -0.853, $p<0.05$). The relationships of euglenophytes density with other groups of algae are shown in Figure 2.18. In relation analysis, it was observed that the density of cyanophytes, chlorophytes and bacillariophytes showed a decreasing trend when the density of euglenophytes was increased.

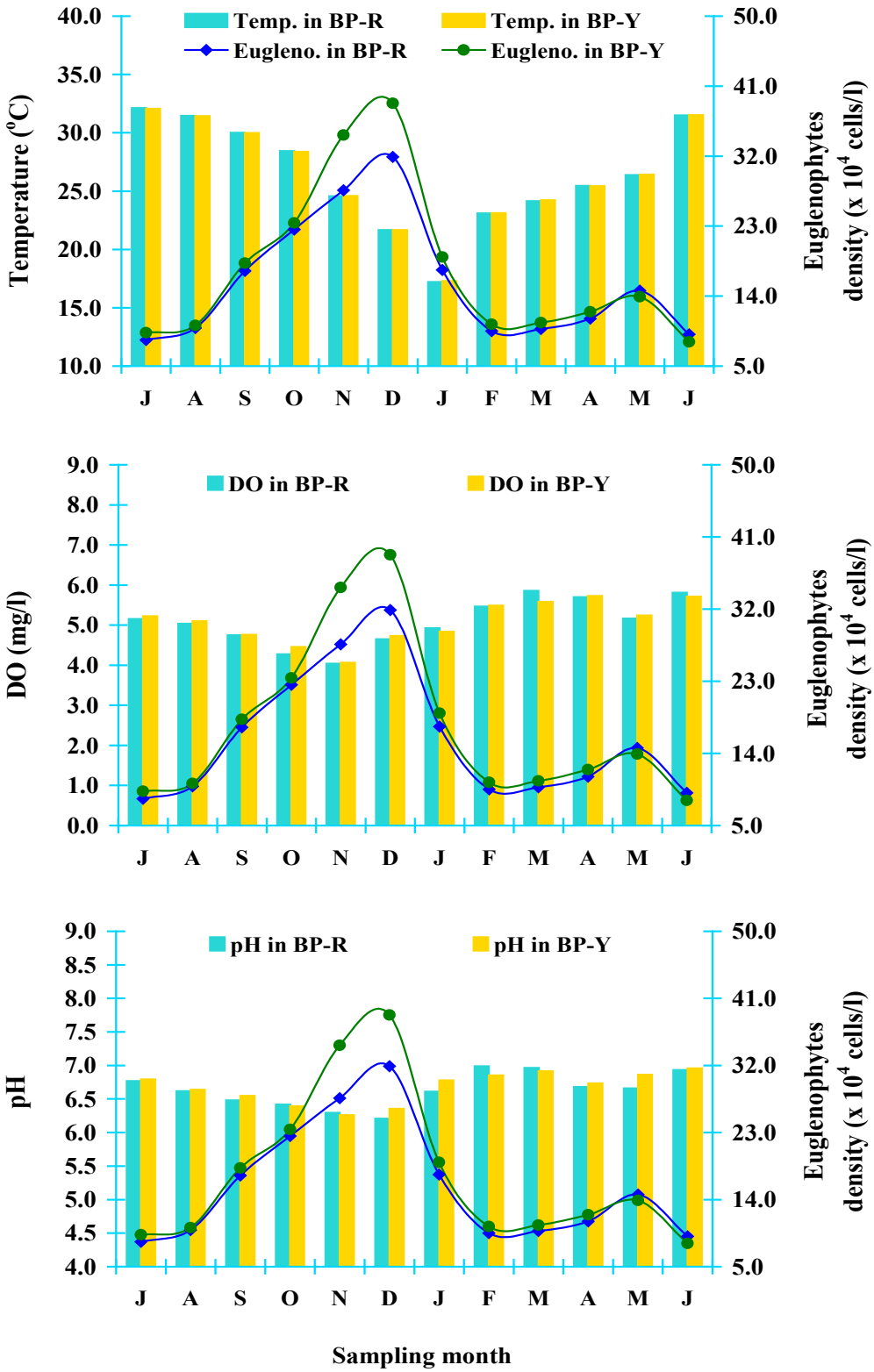


Figure 2.15: Relationships of euglenophytes density (line) with temperature, DO and pH (column)

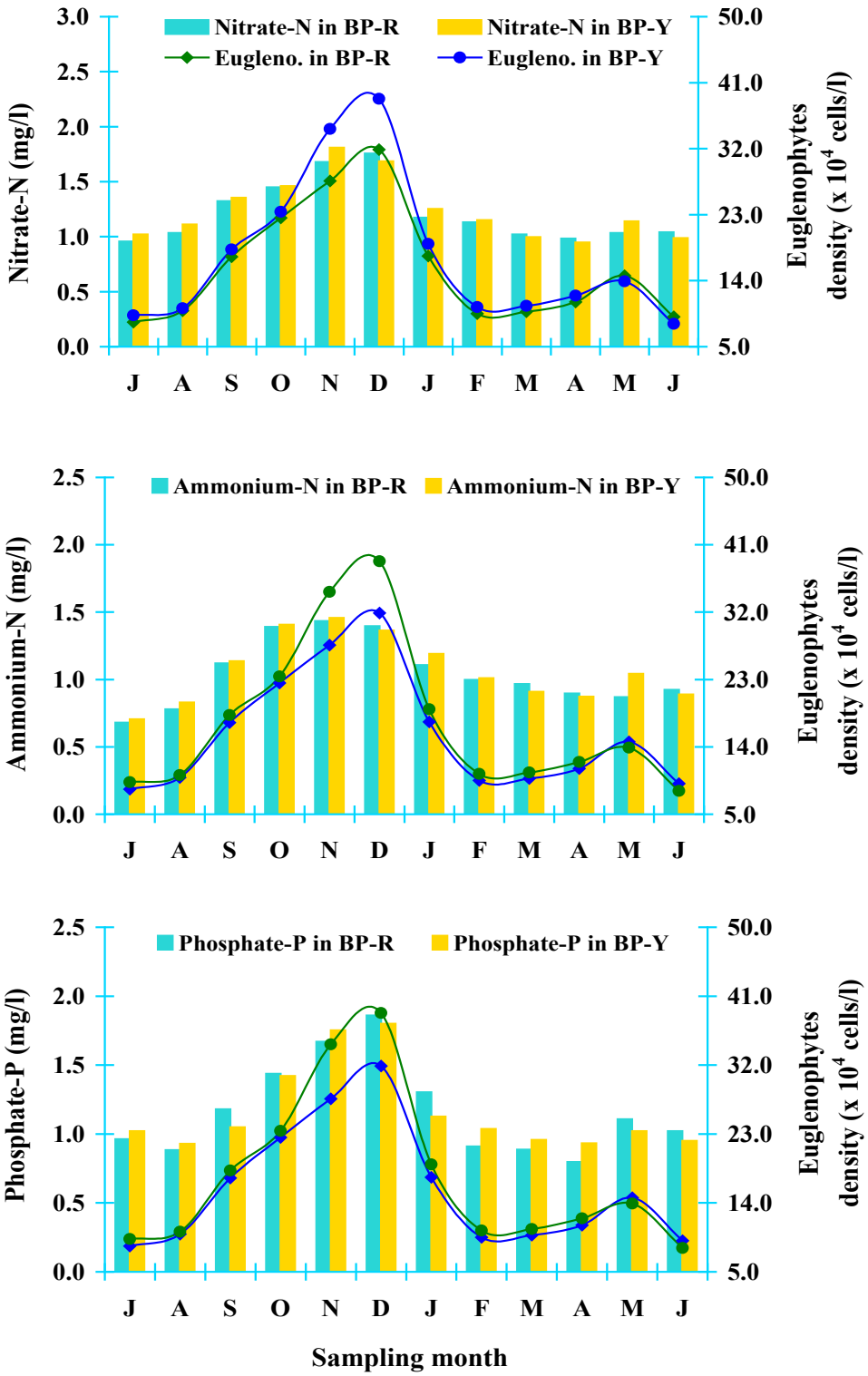


Figure 3.16: Relationships of euglenophytes density (line) with Nitrate-N, Ammonium-N and Phosphate-P concentrations (column)

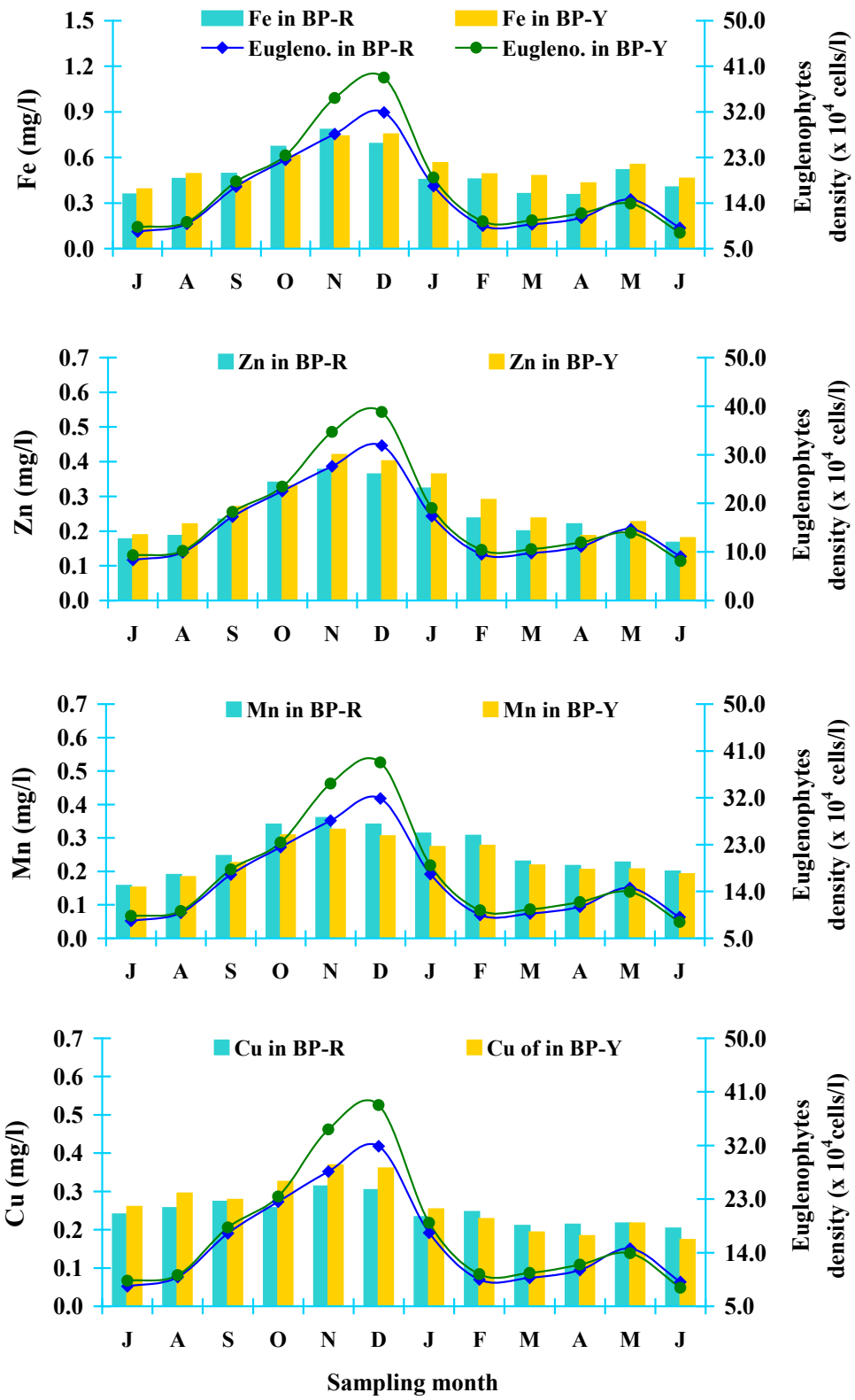


Figure 2.17: Relationships of euglenophytes density (line) with Fe, Zn, Mn and Cu concentrations (column)

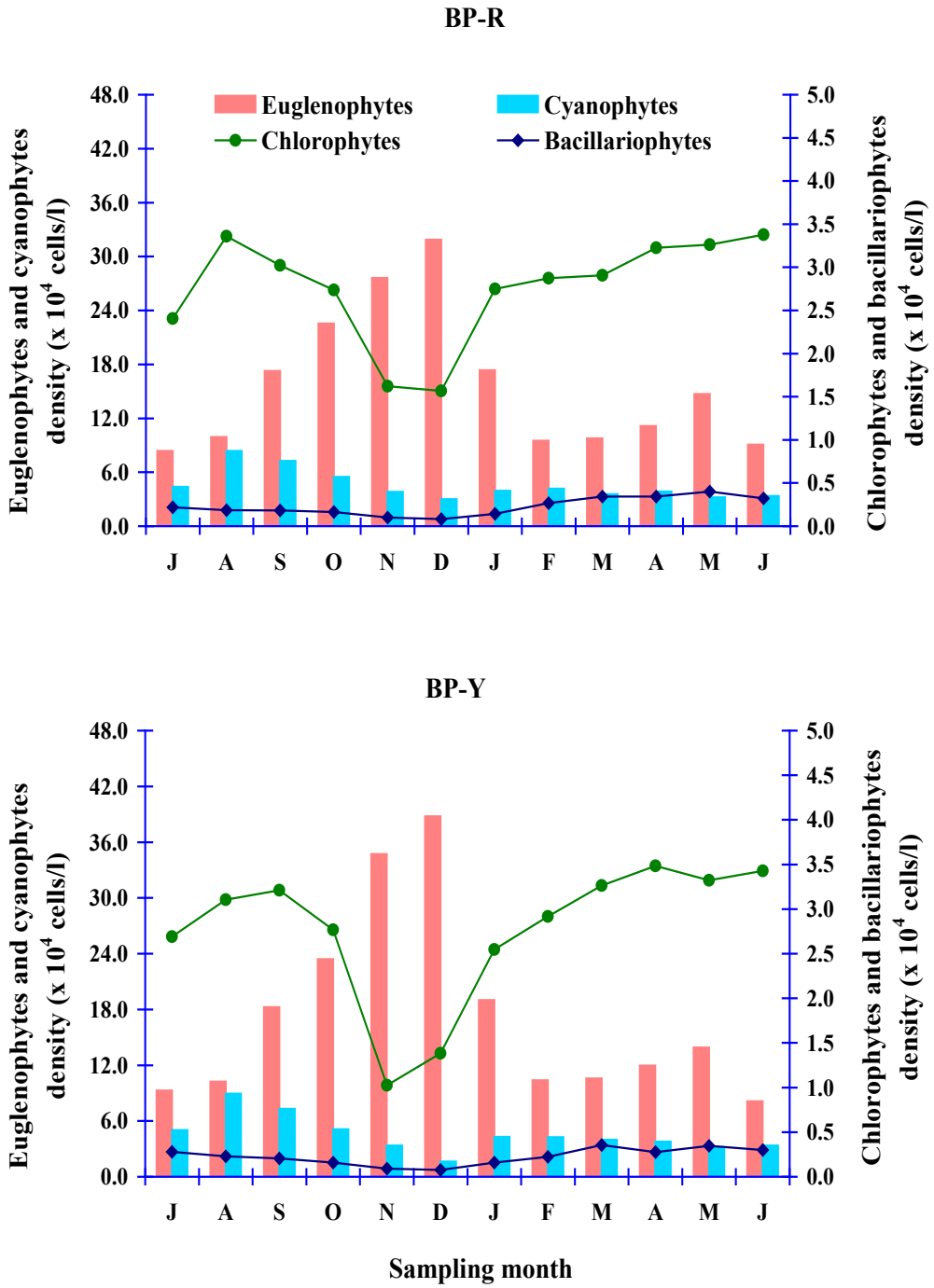


Figure 2.18: Relationships of euglenophytes density with cyanophytes, chlorophytes and bacillariophytes density in BP-R and BP-Y

2.5. Discussion

In this section, the results of environmental factors, planktonic algal community and density, density of euglenophytes and their seasonal variation, relationship between euglenophytes density and environmental factors, and relationship between euglenophytes density and other algal density are discussed and corroborated with the findings of previous related researches.

2.5.1. Environmental factors

The primary productivity of the water body depends on the physico-chemical and other factors of environment (Rahman, 1992). Suitable physico-chemical factors are the prerequisites for healthy aquatic environment. The results of the present study showed that physico-chemical factors other than temperature of the bloom ponds were significantly different from the non-bloom ponds ($p<0.05$). The values of water temperature in the bloom ponds and non-bloom ponds showed no significant difference. It was found to vary from 17.21 ºC to 32.29 °C which might be due to the changes of weather condition from winter to summer season. The recorded values of water temperature in the present study were within the productive range according to Jhingran (1991) who reported that water temperature range 18.5 to 37.5 ^{O}C is suitable for pond productivity.

Dissolved oxygen is considered to be the most important and critical one for all aquatic organisms. In nutrients rich water bodies, depletion of dissolved oxygen occurs occasionally due to high organic decomposition (Boyd and Tucker, 1998). In the present study, significantly lower dissolved oxygen concentration was recorded in the bloom ponds (average value below 5.0 mg/l) as compared to the non-bloom ponds (average value more than 5.5 mg/l). This might be due to heavy bloom of euglenophytes. This assumption is in conformity with the findings of previous studies (Boyd *et al*., 1975 and Rahman *et al*. 2007) that excessive algal bloom can lead to oxygen deficiency by decomposition of dead algae or by hampering photosynthesis of other algae.

pH regulates the productivity of water body and the variation in water pH can change the distribution of carbon dioxide and alter the availability of essential nutrients and trace elements (Boyd, 1979 & 1990; Boyd and Tucker, 1998). Estimated pH value in the bloom ponds was significantly low (average value around 6.30) as compared to the non-bloom ponds (average value around 7.8). Lower pH value in the bloom pond might be due to lower dissolved oxygen and higher free carbon dioxide concentrations. Supportive evidence can be drawn to this assumption from the previous reports (Tucker, 1984; Sipauba-Tavares *et al*., 2003) that pH in water has a direct relation with dissolved oxygen and an inverse relation with free carbon dioxide concentration.

Central to all definitions of eutrophication (Nixon 1995; Jorgensen and Richardson, 1996) is the concept that the primary cause is an increase in nutrients loading. In the present study, the bloom ponds received constant excess nutrients from various sources which might be the reasons for year-round eutrophication. Estimated nutrients (nitrate-nitrogen, ammonium-nitrogen and phosphate-phosphorus) and heavy metals were significantly abundant in the bloom ponds throughout the study period compared to the non-bloom ponds. Though nutrients and heavy metals originated from the same sources (e.g., households wastes, fertilization, surface runoff etc.) throughout the year, it showed seasonality with the maximum values in autumn-winter and the minimum in summer- monsoon. The possible mechanisms leading to greater availability of nutrients and heavy metals in autumn-winter over other seasons are decomposition of organic materials, depletion in water level, diffusion from sediment etc. (Schwoerbel, 1987). Estimated soil organic matter was found higher in the bloom ponds (average value more than 6.0 %) which contributed higher concentration of nutrients in those ponds. Rosy *et al*. (1998) reported that higher organic matter provides higher nutrients especially nitrogen and phosphorus. This report is supportive to the present results.

2.5.2. Planktonic algal community and density

Planktonic algal community structure is regulated by environmental factors, growth rate of algal species and specific rate of loss attributed to grazing, sedimentation and dilution (Fogg, 1975). In the present study, a total of 28 genera of planktonic algae were recorded from the study ponds belonging to euglenophytes, cyanophytes, chlorophytes and bacillariophytes. The total numbers of planktonic algal genera recorded in the present study are more or less close to the findings of Wahab *et al*. (1995) and Affan *et al*. (2005) who recorded 26 and 27 genera of planktonic algae from the fish ponds in Bangladesh. Dewan *et al*. (1991) recorded 24 genera of planktonic algae belonging to euglenophytes, cyanophytes, chlorophytes and bacillariophytes from the fish ponds in Bangladesh. Again, Rahman and Khan (2007) recorded 34 genera of planktonic algae belong to euglenophytes, cyanophytes, chlorophytes and bacillariophytes from the experimental fish ponds in Bangladesh. The total numbers of planktonic algal genera recorded by the previous reports are diverged from the present study.

The result of the present study showed that the algal communities as the number of algal genera in the bloom ponds were low as compared to the non-bloom ponds (Figure 2.7). This might be due to the variation in ambient environmental factors as was confirmed by the results of environmental factors in the bloom ponds and non-bloom ponds. This assumption is consistent with the findings of Reynolds *et al*. (2000) who reported that aquatic environments are subject to high temporal variation with frequent reorganization of algal communities, as a result of interaction among physical, chemical and biological factors.

In the present study, euglenophytes algae were occurred with three genera such as *Euglena, Phacus* and *Trachelomonas*. They are commonly abundant as eutrophic genera (Kim and Boo, 2001). Previous phycological studies (Rahman *et al*., 2005 & 2007; Affan *et al*., 2005; Rahman and Khan., 2007) reported that

euglenophytes algae were occurred by three genera, *Euglena, Phacus* and *Trachelomonas*, among them *Euglena* was the most dominant genus in the eutrophic fish ponds of Bangladesh. These reports are fairly well supportive to the present study.

Algal densities differ in magnitude from one year to the other (Lancelot *et al*., 1987; Affan *et al*., 2005) depending on environmental factors, availability of nutrients and grazing (Rhee and Gotham, 1981a; Riegman *et al*., 1993; Queiroga *et al*., 2006). During the present investigation, significantly higher density of total planktonic algae was recorded in the bloom ponds as compared to the non-bloom ponds. This might be due to higher organic mater in bottom soil and higher dissolved inorganic nutrients in the bloom ponds. Supporting evidence to this assumption can be drawn from the previous studies (Quader, 1997; Rosy *et al*., 1998) which reported that organic matter provides higher nutrients especially nitrogen and phosphorus that enhanced the growth of planktonic algae.

2.5.3. Variation in euglenophytes density

Euglenophytes algae are cosmopolitan, inhabiting very wide range of water environments (Kim *et al*., 1998) and often predominant in eutrophic waters including high organic and inorganic contents (Munawar, 1972; Tripathi and Shukla, 1993; Kim and Boo, 1996). In the present study, euglenophytes was the most abundant group of algae on the basis of density followed by cyanophytes, chlorophytes and bacillariophytes in the bloom ponds. This finding is agreed with the report of Mishra and Saksena (1993) who stated that euglenophytes density are higher compared to other group of algae in eutrophic water bodies. Significantly higher concentration of soil organic matter and inorganic nutrients in the bloom ponds indicated that the blooms ponds were highly eutrophic which might be enhanced the density of euglenophytes. The present results accord with the previous reports (Phang and Ong, 1988; Nwankwo, 1995; Wild *et al*., 1995) that euglenophytes are abundant in locations rich in organic and inorganic matter.

Physico-chemical parameters of water may account for algal proliferation resulting in algal blooms and influence algal succession (Wirasith and Traichaiyaporn, 2012). Different planktonic algal species can tolerate certain levels of temperature, pH and nutrients concentration. These tolerance levels determine the dominance of different species within different seasons (Fogg, 1975). In the present study, the density of euglenophytes showed an increasing trend from autumn to winter and peaked in late autumn (November) and early winter (December) with the dominant genus *Euglena* (Figure 2.13) whereas in summer, monsoon and spring season, the density of these algae were relatively low. The present results are agreed with the report of Shams *et al*. (2012) who observed the highest density of phytoplankton in November and the lowest in May in a lake. The present results are also in conformity with the report of Dewan (1973) who stated that plentiful growth of euglenophytes occurred in the fish pond from September to December. Affan *et al*. (2005) recorded late autumn (November) dominance of euglenophytes in aquaculture ponds with *Euglena* as the dominant genus. This report is also accord with the present study. The present findings are also agreed fairly well with the opinion of Park and Chung (1996) who stated that euglenophytes density increased in winter. Kim and Boo (2001) reported bimodal pattern of euglenophytes density, being maximal in the winter and in the early summer. This report is partially supportive to the present study.

2.5.4. Relationships between euglenophytes density and environmental factors

The natural habitats are not consistent and make a diverse condition of environmental factors which in turn bring about the changes in algal density. Hence, the changes in algal density can be explained in terms of variations in environmental factors. The results of the present study showed that the variation in density of euglenophytes algae in the study ponds were related to some environmental factors especially water temperature, dissolved oxygen, pH, nutrients and heavy metal concentrations.

Seasonal change of euglenoid biomass differed due to change in water temperature (Kim and Boo, 1996). In the present study, dense bloom of euglenophytes with maximum density was recorded in November and December when water temperature was relatively low whereas the density was relatively low when temperature was relatively high (Figure 2.15). In correlation analysis, it was observed that euglenophytes density showed significant negative correlation with water temperature. This result is consistent with the previous report of Kim and Boo (2001) who found that some euglenoids showed positive relation to low temperature those are abundant in winter. The present result is also agreed with the report of Park and Chung (1996) who stated that the population of euglenophytes proliferate its peak especially at low temperature.

In a study, Xavier *et al*. (1991) recorded *Euglena sanguinea* bloom in fish breeding tank at temperature 27.0 ^{0}C. Rahman *et al*. (2007) observed dense bloom of euglenophytes in experimental fish pond at relatively higher water temperature. Suykerbuyk (1991) also observed euglenophytes assemblage in polytrophic and shallow ponds at elevated temperature. Findings of the previous reports (aforementioned) are contrasting to the present study. This might be due to the variation in responses of algal species to temperature changes or variation in geographical position or variation in other specific environmental factors.

According to the present study, euglenophytes density showed significant negative correlation with dissolved oxygen concentration and the maximum density was recorded at lower dissolved oxygen concentration (Figure 3.15). This result is consistent with the previous reports (Xavier, 1985; Xavier *et al*., 1991; Rahman *et al*., 2007) that euglenophytes proliferate in the environment poor in dissolved oxygen concentration. The oxygen deficits condition can be helpful to trigger the oxygen-iron-phosphate complex, releasing larger quantities of phosphorus and iron which might be enhaned the proliferaion of euglenophytes (Munawar, 1972).

Algal abundance is affected by pH of the environment (Goldman and Shapiro, 1973). According to the present study, euglenophytes density negatively correlated to pH values and significantly higher density was recorded at pH around 6.30. In relation to pH values, it was observed that the density increased at acidic pH (less than 6.5) and showed a declining trend with increasing pH values (Figure 3.15). This finding is more or less consistent to the report of Leavitt (1999) who found that algal abundance increased when the pH of water lowered from 6.6 to 5.0.

The result showed that pH value <6.5 was conducive for increasing density of euglenophytes. Zakrys and Walne (1994) stated that *Euglena gracilis* grow well at acidic pH. *Euglena mutabilis* and *Euglena gracilis* are acid tolerant, growing optimally at pH 2.5 to 7.0 (Olaveson and Nalewajko, 2000). In a different study, Olaveson and Stokes (1989) recorded the best growth of *Euglena mutabilis* under acidic pH (pH<5.5). The statements aforementioned are consistent to the present result. Nonetheless, it is obvious that euglenophytes can grow quietly less in number at alkaline pH (>8.0) but pH value less than 6.5 is suitable for their bloom formation.

Nutrients are the most important factors which influence the growth of algae (Okaichi *et al*., 1989). In the present study, the nutrients such as nitrate, ammonium and phosphate were significantly abundant in the bloom ponds throughout the study period as compared to the non-bloom ponds. The maximum concentrations of these nutrients in the bloom ponds were recorded in November and December with higher density of euglenophytes. In correlation analysis, it was observed that euglenophytes density positively correlated to nitrate, ammonium and phosphate concentrations. The present results indicated that euglenophytes favoured to a combination of higher concentrations of nitrate, ammonium and phosphate. The present results are conformity with the findings of some previous studies which reported that

euglenophytes become abundant in higher concentrations of nitrate-nitrogen (Munawar, 1972; Kilham and Kilham, 1978; Xavier, 1985; Kim and Boo, 1998; Duttagupta *et al*., 2004; Rahman *et al*., 2007), higher concentrations of ammonium-nitrogen (Munawar, 1972; Kim and Boo, 2001; Duttagupta *et al*., 2004) and higher concentration of phosphate-phosphorus (Barone and Flores, 1994; Kim and Boo, 2001; Rahman and Khan, 2007).

As like major nutrients, the heavy metal concentrations were significantly higher in the bloom ponds as compared to the non-bloom ponds. In correlation analysis, it was observed that euglenophytes density was positively correlated to heavy metal concentrations (Fe, Zn, Mn and Cu) and the density showed its maximum value when the concentrations of heavy metals were relatively high (Figure 2.17). The findings of the present study are agreement with the report of Duttagupta *et al*. (2004) who speculated that euglenophytes bloom found to be induced by higher concentrations of Fe, Mg, Cu and Zn in water and whereby their concentrations in the water declined leading to a collapse of the bloom. The present result is also consistent to the report of Hutchinson and Nakatsu (1984) who stated that *Euglena* density increased at higher concentrations of Fe, Zn, Mn, Al and Cu.

In relation to the role of the nutrients and heavy metals on euglenophytes density, it seems to be clear that the nutrients (nitrate, ammonium and phosphate) and heavy metals (Fe, Zn, Mn and Cu) constitute the important regulatory factors for their bloom formation, since the concentrations of these nutrients and heavy metals were quietly low in the ponds where bloom did not occur.

2.5.5. Relationships between euglenophytes density and other algal density

In the present study, it was observed that the density of planktonic algae (other than euglenophytes) in the bloom ponds showed a significant decreasing

tendency when the density of euglenophytes increased (Figure 2.18). From the results of correlation analysis, it was also observed that the density of euglenophytes showed negative correlation with the density of cyanophytes, chlorophytes and bacillariophytes which indicated that euglenophytes bloom have an effect in reducing the number of other algae. These results are fairly agreement with those obtained by Rahman *et al*. (2007) who reported that bloom of euglenophytes hampered the density of chlorophytes and bacillariophytes. The results of the present study are also consistent with the report of Hosmani (1988) who stated that the blooms of *Euglena elastica, E. gracilis* and *Trachelomonas charkoweinis* have a significant effect in reducing the number of other algal species in fish ponds. Similar phenomenon was also reported by Leupold (1988).

2.6. Conclusion

The overall study revealed that euglenophytes algae in the fish ponds showed a seasonal variation with higher density in autumn to winter. In summer, monsoon and spring season, their density dropped. Temperature, dissolved oxygen, pH, nutrients (nitrate, ammonium and phosphate) and heavy metals (Fe, Zn, Mn and Cu) contributed to the variation in density of these algae. Higher nutrients and heavy metals concentrations under lower water temperature, dissolved oxygen and acidic pH produce peak density attributed by the active growth of *Euglena* sp. Further study at the sampling frequency of several days in a month for several years would allow more accurate correlation of changes in the density of euglenophytes and environmental factors.

CHAPTER THREE

Management of Euglenophytes Bloom in Fish Pond

Chapter Three

MANAGEMENT OF EUGLENOPHYTES BLOOM IN FISH POND

3.1. Introduction

Eutrophication is the process whereby water bodies become enriched with nutrients particularly with phosphorus and nitrogen from both external and internal sources. It is considered as one of the most pressing environmental problems in both the developed and the developing countries (Harper, 1992; Ryding and Rast 1989). It is one of the major water-quality problems in aquaculture pond of Bangladesh. Decomposition of organic wastes and unutilized feeds plus direct application of fertilizers are the major sources of eutrophication in aquaculture pond. A common symptom of eutrophication in the aquaculture pond is the appearance of algal blooms. Although, algal bloom indicates high productivity of the water body concerned (Boyd and Tucker, 1998), but dense algal bloom causing severe economic losses to aquaculture and having environmental impacts (Boyd *et al*., 1975; Hallegraeff, 1993).

There are three major categories of algal species which are responsible for producing harmful bloom in fresh water and marine environments. One: bloom of species (e.g., *Euglana*, *Phacus, Gonyaulax* etc.) which produce harmless water discolouration but under certain conditions blooms can grow so dense that they cause mass mortality of fish due to severe oxygen depletion through decomposition of dead algae. Two: bloom of species (e.g., *Dinophysis, Microcystis, Chattonella* etc.) which produce potent toxic compounds causing huge mortality of fish and other aquatic animals. Three: bloom of species (e.g., *Prymnesium, Gyrodinium, Chaetoceros* etc.) which are non-toxic to human in most cases but harmful to fish especially in intensive aquaculture and invertebrates by intoxication, damaging or clogging of gills.

Toxic algal blooms directly related to mortality of fish and other aquatic animals. But, the major problem of non-toxic algal bloom is the algal die off which creates two problems. The first is lack of oxygen (Dahl *et al*., 1989). Algal die off adds increased amount of organic matter to the pond. With the added of excess organic matter, the total amount of decomposition increases and the decomposition process uses up oxygen. Algal die off can be so severe that most of the available oxygen in a pond can be used up in the decomposition process and aquatic life will start to die off (Rodger *et al*., 1994; Onodera *et al*., 1996). The second problem of algal die off and increased organic matter is nutrients. When the algae die off and are decomposed, nutrients are released back into the pond and algal blooms appear again.

Among various types of algal bloom, euglenophytes bloom is the most common phenomenon in fish ponds of Bangladesh including Rajshahi. These algae have received much attention due to their mass occurrence with red sticky scum on the surface of ponds and lakes throughout the country. This non-toxic algal bloom can cause problems through shading of submerged vegetation, disruption of food web structure and oxygen depletion as the blooms decay. It often leads to algal die off and water quality degradation that hampered fish growth (Rahman *et al*., 2007). Bloom of *Euglena sanguinea* affects growth of fish markedly (Xavier *et al*., 1991). This bloom even causes mass mortality of fish due to due severe oxygen depletion (Rahman *et al*., 2005). Therefore, it is very urgent to develop management or control systems to minimize the noxious effects of euglenophytes bloom on fish growth.

Several chemical methods are employed to control algal blooms in tropical water bodies (Yin *et al*., 1989; Jhingran, 1995) but, they are either expensive or have some residual effects in the aquatic food chain in the long run. It is well known that algaecides remove algal bloom but use of excessive algaecides can kill fish or affect their growth. Copper algaecides can destroy water quality and

add new toxic sediment to the bottom which interfere bacterial decomposition of sediments and fish growth (Lembi, 2000). Alum algaecides can leave an aluminum hydroxide flocculent on the bottom that also interfere fish growth. Algaecides do nothing to improve the health and growth of fish, nothing to reduce bottom organic sediment. Sometimes, fish farmers used herbicides to control algal bloom in fish ponds without knowing their toxicity and residual effects. But, most of the herbicides are known to have negative effects on fish growth and are not environment friendly (McIntosh an Kavern, 1974).

Biomanipulation is one of the important methods to control planktonic algal bloom using filter feeders, including zooplankton, silver carp and bighead carp (Liu *et al*., 2009). Though, filter-feeding fish are selective planktonic algae grazers that can suppress algae directly through ingestion but they can also be enhanced algae indirectly by suppressing herbivorous zooplankton and by increasing nutrient availability, ichthyic-eutrophication (Drenner *et al*., 1987). In fact, the use of filter-feeding fish to reduce planktonic algal bloom in lakes and reservoirs is still controversial (Domaizon and Devaux 1999b; Radke and Kahl 2002). Moreover, if the algal bloom in the fish pond is controlled without eliminating the cause, the higher concentrations of nutrients are still in the water, the algal bloom will quickly return. Therefore, effective and environment friendly management methods should be developed to minimize the euglenophytes bloom in fish pond.

According to previous reports, euglenophytes bloom is conducive to higher concentrations of nitrogen and phosphorus nutrients (Kim and Boo, 2001, Rahman *et al*., 2007), higher concentrations of heavy metal (Hutchinson and Nakatsu, 1984; Duttagupta *et al*., 2004) and acidic pH (Zakrys and Walne, 1994; Olaveson and Nalewajko, 2000; Rahman *et al*., 2007). Thus, the bloom of euglenophytes can be controlled by reducing nutrients and heavy metal concentrations, and by increasing pH levels in the environment. For this

purpose, duckweeds and lime can be used. Duckweeds (*Lemna minor* and *L. gibba*) have shown potential usefulness to reduce the nutrients (Bergmann *et al*., 2000; Korner *et al*., 2003; Ferdoushi *et al*., 2008; Lukkhana *et al*., 2008) and heavy metals (Landholt and Kandeler, 1987; El-Kheir *et al*., 2007; Obek, 2009; Sekomo *et al*., 2012) from the waste water systems. On the other hand, lime can improve aquatic environment by raising the pH, alkalinity and hardness to a level suitable for pond productivity (Ivahnenko *et al*., 1988; Boyd, 1990; Sipauba-Tavares *et al*., 2003).

However, concerning the management and control of algal bloom in different types of water bodies, a number of researches have been done in different countries of the world including Bangladesh (Datta and Jana, 1998; McGregor, 2002; Lembi, 2003; Lynch, 2009). But, researches on the management of euglenophytes bloom in fish pond in relation to water quality parameters have been poorly understood in Rajshahi, Bangladesh. Therefore, the present study was planned to manage the euglenophytes bloom in fish pond by using duckweed and lime with a view to the following specific objectives:

1. To investigate the effects of duckweed and lime on the water quality parameters in the euglenophytes bloom pond.
2. To investigate the effects of duckweed and lime on the density of euglenophytes and other planktonic algae in the euglenophytes bloom pond.
3. To investigate the effects of duckweed and lime on the growth of fish in the euglenophytes bloom pond.
4. To investigate the grazing effects of fish on the density of euglenophytes.
5. Finally to recommend the suitable management system for minimizing euglenophytes bloom in fish pond.

3.2. Review of literatures

Eutrophication is a growing problem in the aquaculture ponds of Bangladesh which is characterized by two prime factors: nutrients loading and algal blooms. Excess algal blooms create various problems in aquaculture ponds. Use of duckweed and lime in the fish ponds can improve water quality and reduce algal bloom through absorbing nutrients and increasing pH. Introduction of filter feeding fishes can also reduce algal bloom in fish ponds through direct ingestion of algae. However, there are many published reports on the use of duckweed, lime and filter feeding fishes for water quality improvement, reduction of nutrients loads and algal density from the eutrophicated water in different parts of the world. But, such types of research reports are very little in Bangladesh. However, some reports relevant to the present study are reviewed below.

3.2.1 Reduction of nutrients and algae by duckweed and lime

Vanitha *et al.* (2013) conducted a study on duckweed for improving pond water quality. In their study, various physiochemical parameters such as dissolved oxygen, temperature, nitrate, ammonia, phosphate, turbidity and pH were analyzed. They reported that *Duckweed* plant improves water quality and it efficiently removes 75% phosphate from the pond water.

Azeez and Sabbar (2012) investigated the efficiency of duckweed (*Lemna minor* L.) in phytotreatment of wastewater pollutants by measuring some of the physicochemical characteristics of the control and plant treatments and recorded the rates of reduction. They recorded highest rates of reduction of heavy metals 99.8%, 99.6%, 98.7% and 72% for copper, cadmium, lead and zinc, respectively, followed by turbidity and nitrate 64% and 57.1%, respectively. They also recorded reduction of BOD 49.6 %, COD 32.7%, soluble solids 48.9% oils and grease 43%, total alkalinity 41%, phenols 40%, sulfide 39.1%, suspended solids 38% and phosphate 30%. They concluded that duckweed can be successfully used for wastewater pollutants removal.

Christian *et al.* (2012) undertook an investigation on the heavy metal removal from wastewater. They used duckweed and algae ponds as post-treatment for textile wastewater operated at a hydraulic retention time of 7 days and under two different metal loading rates and light regimes (16/8 h light/darkness and 24 h light). They observed that Cr removal rate was 94% for the duckweed ponds and 98% for the algal ponds, indifferently of the metal loading rate and light regime. Zn removal proceeded well (70%) at a low metal loading rate under the 16/8 light regime, but dropped to below 40% at the higher metal loading rate whereas the removal efficiency rose back to 80% at the higher metal loading rate under 24 h light regimes. Pb, Cd and Cu removal efficiencies were 36% and 33% for Pb, 33% and 21% for Cd and 27% and 29% for Cu in the duckweed and algal ponds, respectively.

Iram *et al.* (2012) conducted a research on the treatment of wastewater by *Lemna minor* to study the performance of bio-treatment ponds at National Agricultural Research Center, Islamabad, Pakistan. During their study the physical parameters (colour, pH, EC, TDS, turbidity) and chemical parameters (Zn, Cu, Cd, Ni, Mn, Fe and Pb) were within the limits and not sub-lethal for fish rearing. They reported that *Lemna* accumulated higher concentration of heavy metals as compared to wastewater and best for phytoremediation purpose.

Obek (2009) studied the bioaccumulation of heavy metals from the secondary treated municipal wastewater by *Lemna gibba* L. He reported that *Lemna gibba* accumulated high levels of Cr, Cu, Zn, Pb, Cd and Ni in the first few days, but then some decreases in the accumulation levels due to its saturation level. He concluded that *Lemna gibba* shows promise for the removal of Cr, Cu, Zn, Pb, Cd and Ni from contaminated wastewaters, since it accumulated high concentrations of these elements.

Ferdoushi *et al.* (2008) conducted an investigation on the effects of two fish–edible floating aquatic macrophytes on water quality in aquaculture ponds. In their study, *Lemna* sp. and *Azolla* sp. were used in treatment-1 and treatment-2, respectively and treatment-3 remained as control. The results of their study showed that aquatic macrophytes had considerable effects on water temperature, dissolved oxygen, nitrate-nitrogen, phosphate-phosphorus, chlorophyll-a, alkalinity and plankton abundance. They recorded lowest concentration of PO_4-P (0.01 mg/L) and chlorophyll-a (26.99 µg/L) in treatment-1 and treatment-2 whereas lowest concentration of NO_3-N in treatment-2. Their results concluded that *Lemna* sp. and *Azolla* sp. appeared as a nutrient filter for absorption of nitrogen and phosphorus, and removed the excessive nutrients from the water body.

Gurtekin and Şekerdag (2008) investigated the effects of duckweed (*Lemna minor L.*) on the effluent water quality and settling characteristics in the secondary clarifier tank of a conventional biological treatment plant and compared the performances of the secondary clarifier with and without duckweed. They reported that the secondary clarifier tank with duckweed, COD, BOD, ammonium and phosphate removal efficiencies were higher by 15, 25, 35 and 45%, respectively.

Lukkhana *et al.* (2008) carried out a study on the nitrogen removal in duckweed-based ponds with effluent recirculation. They observed that average removal efficiencies were 72% for total nitrogen (TN), 72% for total kjeldahl nitrogen (TKN) and 73% for ammonia-nitrogen (NH_4-N). Their study indicated that the three main mechanisms for nitrogen removal in the duckweed-based ponds with effluent recirculation as duckweed uptake, nitrification-denitrification and sedimentation.

El-Kheir *et al.* (2007) undertook an investigation on the efficiency of duckweed (*Lemna gibba* L.) as an alternative cost effective natural biological tool in eliminating nutrients and soluble salts and its effect on phytoplankton and coliform bacteria in an outdoor aquatic system. They reported that total suspended solids, BOD, COD, nitrate, ammonia, phosphate, Cu, Pb, Zn and Cd decreased by 96.3%, 90.6%, 89.0%, 100%, 82.0%, 64.4%, 100%, 100%, 93.6% and 66.7%, respectively. They also reported that phytoplankton standing crop decreased by 94.8% and coliform bacteria decreased by 99.8%.

Korner *et al.* (2003) studied the growth rates of different duckweed species in wastewater and the mechanism of organic matter and nutrients removal. They reported that growth rates of duckweed in different types of wastewater varied considerably among different species and the degradation of organic material enhanced by duckweed through both additional oxygen supply and additional surface for bacterial growth. They concluded that duckweed could be used to treat wastewater containing very high total ammonia concentrations and the duckweed mat can reduce three-quarters of the total nitrogen (N) and phosphorus (P) in very shallow systems.

Sipauba-Tavares *et al.* (2003) conducted an experiment on the effect of liming management on the water quality in *Colossoma macropomum* ponds. They reported that concentrations of total phosphorous and ammonia levels tended to decrease in the treatment with the highest liming amount. They also reported that metals and organic matter in the sediment decreased and liming did not affect the limnological variables, with the exception of free CO_2 that tended to decrease with increasing liming concentration.

Cheng *et al.* (2002) undertook a study on the nitrogen and phosphorus removal from swine lagoon liquid by growing *Lemna minor* under in vitro and field conditions. The results of their study showed that the rates of nitrogen and

phosphorus uptake by the duckweed growing in the in vitro system were as high as 3.36 g m^{-2} day^{-1} and 0.20 gm^{-2} day^{-1}, respectively. The highest nitrogen and phosphorus removal rates in the field system were 2.11 $gm^{-2}day^{-1}$ and 0.59 $gm^{-2}day^{-1}$, respectively. The results of their study concluded that duckweed assimilation was the dominant mechanism for nitrogen and phosphorus removal from the swine lagoon liquid.

Bergmann *et al.* (2000) conducted a study on nutrient removal from swine lagoon effluent by duckweed. They reported that *Lemna minor* as an effective one in reducing total nitrogen, NH_3-N, total phosphorus, orthophosphate-P, total organic carbon, K, Cu and Zn from the eutrophic aquatic environment.

Perniel *et al.* (1998) studied the nutrients removal from a storm water detention pond using duckweed (*Lemna minor, Wolffia columbiana, Spirodela polyrhiza, L. minuta* and *L. trisulca*). They reported that *Lemna minor* monoculture consistently removed the largest amount of ammonia and had the largest biomass while polyculture of *L. minor* and *S. polyrhiza* was the most stable nutrient sink and removed the largest amount of phosphorus from storm water.

Boyd (1974) reported that the use of water-hyacinth in channel catfish pond covering 10% of the water surface area reduced the density of phytoplankton by removing enough nutrients from the water and finally decreased the probability of fish kill.

3.2.2. Control of algal bloom by filter feeding fishes

Zhou *et al.* (2011) conducted an enclosure experiment to assess the impact of silver carp (*Hypophthalmichthys molitrix*) on the spring phytoplankton community structure and water quality of the Three-Gorges Reservoir. They observed that stocking of silver carp into enclosures caused a change in pH, transparency, reduction of DO and phosphate while chlorophyll-a concentration

and turbidity increased. They also observed that some zooplankton (rotifer and copepoda) were significantly reduced, and some phytoplankton and protozoa were significantly increased. The results of their study concluded that silver carp was not suitable for clearing spring phytoplankton blooms in the Three-Gorges Reservoir.

Ke *et al.* (2009) undertook a study on the impacts of two fishes on the plankton abundance and water quality. In their study, silver carp and bighead carp were stocked to control the nuisance cyanobacterial blooms in Meiliang Bay of Lake Taihu. They recorded significant negative correlation between the N:P weight ratio and phytoplankton biomass. They reported that the size-selective predation by the two carps had no effect on the biomass of green alga *Ulothrix* sp. and the *Microcystis* domination in the water of fish pen effectively suppressed by the pen-cultured carps. Based on the results they concluded that silver and bighead carp are two efficient biomanipulation tools to control *Microcystis* blooms in the tropical/subtropical eutrophic waters.

Rahman *et al.* (2005) undertook an experiment in three fish ponds to investigate the fishes that fed frequently on euglenophytes with a view to utilize the bloom as a food source. Their study carried out by stocking of five species of fish (*Labeo rohita*, *Catla catla*, *Cirrhina mrigala*, *Hypophthalmichthys molitrix* and *Puntius gonionotus*) and monthly collection of fish sample for their gut content analysis. The results of their study showed that among the different groups of phytoplankton consumed by the fishes, the highest percentage of euglenophytes found in the gut contents of silver carp and Thai sarputi, moderate percentage in the gut contents of rohu whereas least percentage in catla and mrigal. The results of electivity analysis showed that silver carp and Thai sarputi have positive affinities to this group of algae whereas rohu, catla and mrigel have negative affinity. The results of their study concluded that silver carp and Thai sarputi could be used to control euglenophytes bloom in fish pond

Jana and Datta (2000) conducted a study on the managing of algal bloom in a eutrophic lake. During the study, they attempted to highlight the use of herbivorous fishes (Silver carp and Tilapia) in controlling algal bloom in terms of biomanipulation, grazing activity in situ and laboratory, selective grazing, defecation, *Microcystis* digestibility, growth efficiency and ichthyoeutrophication. The results of their study concluded that silver carp is suitable for cleaning *Microcystis* bloom in the long term, whereas Tilapia may be used for short-term clearance of *Microcystis* bloom from small ponds.

Datta and Jana (1998) conducted a field and laboratory experiments to examine the grazing efficiency of silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*) and tilapia (*Oreochromis niloticus*) for controlling *Microcystis* bloom in a eutrophic shallow lake in West Bengal, India. Their survey and experiment results showed that the introduction of twelve 3-month-old specimens of any of the species into a limnocorral (500 litres) led to a dramatic reduction (60-93%) of the *Microcystis* population from an initial density of $18.4\text{-}19.3\times10^5$/litre² within 3-7 days. They observed the efficiency of the fish species for clearance of bloom in the order: silver carp>bighead>tilapia, while the ichthyoeutrophic potential in the reverse order.

3.2.3. Feeding preference of fish to different groups of algae

Mondol (2000) studied the food and feeding habits of *Amblypharyngodon mola* (Mola), *Puntius gonionotus* (Thai sarpunti) and *Cyprinus carpio* (Common carp) in rice field. He reported that Bacillariophyceae was the most dominant in the gut contents of sarpunti and preferred food item among the phytoplankton followed by Chlorophyceae. Euglenophyceae was the least preferred food both in number and percentage of occurrence in the gut content of these fish.

Quadir (1997) undertook an experiment to study the feeding preference of Thai sarpunti (*Puntius gonionotus*). He stated that Thai sarpunti showed positive

response to Euglenophyceae, Bacillariophyceae and Chlorophyceae and low or negative response to Cyanophyceae.

Haider (1996) conducted an investigation on the food selectivity of common carp (*Cyprinus carpio*) and sarpunti (*Puntius gonionotus*). He reported that common carp and sarpunti showed positive electivity to Bacillariophyceae and Chlorophyceae whereas negative to Euglenophyceae and Cyanophyceae.

Ahmed (1993) conducted an investigation on the feeding preference of Catla (*Catla catla*) in fertilized ponds and reported that catla showed positive selection for Bacillariophyceae, Cyanophyceae, Euglenophyceae, Rhodophyceae, Crustacea and Rotifers while negative selection for Chlorophyceae and Xanthophyceae.

Sarker (1992) conducted a study on the feeding preference of rohu (*Labeo rohita*) by gut contents analysis. He reported that rohu showed positive electivity to Chlorophyceae, Cyanophyceae, Bacillariophyceae and hydrozoa whereas a little or neutral electivity to euglenophytes and rotifers.

3.3. Methodology

3.3.1. Location of the study

The study was carried out in twelve euglenophytes bloom forming fish ponds at Raighati, Mohanpur Upazila, Rajshahi, North-west part of Bangladesh.

3.3.2. Study ponds

The ages of the ponds were more or less 10-15 years. The study ponds were more or less rectangular in shape. The area range of the ponds was 2.0-3.5 dec. The main water source of the ponds was rainfall. During the study period, water level of the ponds varied between 4.0 and 5.5 feet. The ponds were not interconnected and had no outlet. The ponds were well exposed to sunlight and the embankments of the ponds were well protected. The over views of the study ponds under four treatments are shown in Plate 3.1, 3.2, 3.3 and 3.4).

3.3.3. Study design

The study was conducted for a period of five months from August to December, 2011 in twelve euglenophytes bloom forming fish ponds under four treatments viz., Treatment-1 (T1), Treatment-2 (T2), Treatment-3 (T3) and Treatment-4 (T4) with three replicates in each treatment.

T1: assigned to the ponds treated with duckweed (*Lemna* sp.);

T2: assigned to the ponds treated with lime (CaO);

T3: assigned to the ponds treated with both duckweed and lime; and

T4: assigned to ponds treated as control ponds (without duckweed and lime).

A complete lay out of the study design is shown in Table 3.1. The plan of works for the study of management of euglenophytes bloom in fish pond is shown in Chart 3.1.

Plate 3.1: The ponds under T1
(Duckweed treated ponds)

Plate 3.2: The ponds under T2
(Lime treated ponds)

Plate 3.3: The ponds under T3
(Duckweed and lime treated ponds)

Plate 3.4: The ponds under T4
(Without duckweed and lime)

3.3.4. Pond management

Aquatic weeds and undesirable species were completely removed from the study ponds by repeated netting. Initially, all the ponds were treated with lime at the rate of 1kg/decimal. Both organic and inorganic fertilizers were applied after seven days of liming. The initial doses of fertilizers both organic and inorganic were same in all the ponds (Table 3.1). The study ponds were stocked with the fish species comprising rohu (*Labeo rohita*), catla (*Catla catla*), silver carp (*Hypophthalmichthys molitrix*), silver barb (*Puntius gonionotus*) and mrigel (*Cirrhina mrigala*) at the rate of 60/dec. with the ratio of 12:7:13:18:10. The initial weights of rohu, catla, mrigel, silver carp and silver barb were 25.64±6.21, 29.27±6.94, 15.70±5.68, 17.82±4.78 and 4.20±0.86g, respectively. Rice bran and mustard oil cake were applied as supplementary feed (1:1) once in a day.

Table 3.1: Layout of the study design

Treatment	Imputes	Fertilization	
		Initial dose/dec	Periodic dose/dec
T1	Duckweed, *Lemna* (covered 1/4 of the water surface)	Urea-100 gm TSP-100 gm Cowdung-5.kg	Urea-100gm TSP-100 gm Cowdung-2.50kg
T2	Lime (250g/dec/15days)	Do	Do
T3	Duckweed + lime (covered 1/4 of the water surface + 250g/dec/15days)	Do	Do
T4	Control (without duckweed and lime)	Do	Do

3.3.5. Monitoring of water quality parameters

The water quality parameters such as water temperature, pH, dissolved oxygen (DO), nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), phosphate-phosphorus (PO_4-P), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) concentrations were monitored fortnightly.

3.3.5.1. Sample collection

Some water quality parameters were monitored on the spot. For laboratory analysis, water samples were collected in 500 ml black bottle from different points of each pond from surface to a depth of 50 cm.

3.3.5.2. Sample analysis

Collected water samples were analyzed in the laboratory of Department of Fisheries, University of Rajshahi, Rajshahi and SRDI (Soil Resource Development Institute) Laboratory, Rajshahi, Bangladesh. The methods used for analyzing different water quality parameters are mentioned below.

a) **Water temperature:** Water temperature was determined on the spot using a Celsius thermometer.

b) **Dissolved oxygen:** Dissolved oxygen was determined by the aid of a water quality test kit (HACH kit FF-2, USA). The estimated concentration of dissolved oxygen was expressed in milligram per litter (mg/l) of water.

c) **pH:** A digital pH meter (HANNA, Model: HI-9142) was used to measure the pH of water on the spot.

d) **Nutrients:** The nutrients such as NO_3-N, NH_4-N and PO_4-P concentrations of water samples were determined by using a direct reading of HACH kit (model, Odyssey, DR-2500) with Nitraver and Phosver pillows. The estimated concentrations of nutrients were expressed in milligram per litter (mg/l) of water.

e) **Heavy metal:** The heavy metal such as iron, zinc, manganese and copper concentrations of water samples were analyzed by Atomic Absorption Spectrophotometer (Model-3310). The estimated concentrations of heavy metals were expressed in milligram per litter (mg/l) of water.

3.3.6. Determination of soil organic matter

To determine the organic matter of bottom soil of the study ponds, the soil samples were collected and analyzed fortnightly.

3.3.6.1 Collection of soil sample

An amount of bottom soil with the sediment was collected from each pond with the help of scoop from 6 selected places. After collection of soil, each time it was kept in the plastic bucket and mixed homogeneously in the bucket. Later, it was spread on the polythene paper with the help of bamboo stick. Then half of the samples were thrown out. Again the next half of the sample was spread and then half of the samples were thrown out. At last the remaining soil samples were taken and kept at room temperature in the laboratory for air-drying (for one month). After drying, the samples were grinded to make powder. Afterward, the samples were sieved. For analysis about 500 g of sample had been sent to SRDI (Soil Resource Development Institute) Laboratory, Shympur, Rajshahi, Bangladesh.

3.3.6.2. Analysis of soil sample

Determination of soil organic carbon was made by the Walkley-Black method (sulfuric acid-potassium dichromate oxidation). Organic matter of soil was determined by multiplying the percentage of organic carbon with conventional Van-Bemmelen's factor of 1.724 (Piper, 1949).

3.3.7. Study of planktonic algae

For qualitative and quantitative study of planktonic algae in the study ponds, the water samples were collected and analyzed fortnightly.

3.3.7.1. Collection and preparation of the sample

Water samples were collected from different depth of each pond. A known volume (10 L) of water samples was collected in a plastic bucket and passed through plankton net of 25 µm mesh size. The concentrated algae samples were preserved in plastic vials with 5% buffered formalin.

3.3.7.2. Enumeration and counting

For identification and quantification, 1 ml of concentrated algae sample was taken by a dropper and then put on the S-R (Sedgewick-Rafter) cell. After placing the S-R cell under a binocular microscope (Olympus, M-4000D), the algae were identified and counted. The identification of algae was done up to generic level according to Prescott (1964), Belcher and Swale (1978), APHA (1992) and Bellinger (1992). Quantification of the algae was done according to following formula. The number of algae was expressed numerically per liter of water (cells/l).

$$N = \frac{Ax1000xC}{VxFxL} \quad \text{(Stirling, 1985)}$$

Where, N = No. of algae cells per liter;

A = Total no. of algae counted;

C = Volume of final concentrate of the sample in milliliter;

V = Volume of a field of S-R cell in cubic milliliter;

F = Number of the fields counted; and

L = Volume of original water in liter.

3.3.8. Monitoring of growth

To monitor the growth of fish, about 10% of stocked fishes from each pond were sampled at one month interval with the help of a seine net and kept in a bowl with water. After measuring weight, the fishes were released into the ponds. After five months of study, all the fishes were harvested by repeated netting. The weight (g) of individual fish was measured with the help of a portable electronic balance (Plate 3.5). The following parameters were monitored to evaluate the growth performance of fishes.

3.3.8.1. Mean weight gain (MWG)

Mean weight gain was calculated by the following formula.

MWG (g) = Mean final weight (g) – Mean initial weight (g).

3.3.8.2. Average daily weight gain (ADWG)

Average daily weight gain was computed by subtracting the initial body weight from the final body weight and then divided by the number of days of rearing. Average daily gain was computed by following formula.

ADWG (gbwd^{-1}) = {Mean final weight – Mean initial weight}/Number of days

3.3.8.3. Specific growth rate (SGR)

SGR is the instantaneous change in weight of fish calculated as the percentage increase in body weight per day over a given time interval. SGR of the fish was calculated from the following formula.

$$\textbf{SGR (\% bwd}^{-1}\textbf{)} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100 \text{ (Brown, 1957)}$$

Where, W_1 = Initial body weight (g) at time T_1 (day)

W_2 = Initial body weight (g) at time T_2 (day)

Rohu (*Labeo rohita*)

Catla (Catla *catla*)

Silver carp (*Hypophthalmichthys molitrix*)

Silver barb (*Puntius gonionotus*)

Mrigel (*Cirrhina mrigala*)

Plate 3.5: Measuring weight of the fishes

3.3.8.4. Survival rate

Survival rate was calculated on the basis of total number of fishes during harvesting using the following formula and expressed as percentage (%).

$$\textbf{Survival rate (\%)} = \frac{\text{Number of fishes harvested}}{\text{Total number of fishes stocked}} \times 100$$

3.3.9. Analysis of gut contents of fish

For the investigation of gut contents, at least three fish of each species from each of the pond were collected and analyzed monthly.

3.3.9.1. Sample collection and preparation

Immediately after capture, fish sample were preserved in plastic container with 10% buffered formalin. The containers were labeled according to name of the species and the number of pond and then brought to the laboratory for further studies. After washing with clean water and the body cavity of the fish was carefully opened and the alimentary canal was dissected out into a clean petridish. Then the gut was opened with the help of scissors and forceps. Finally, the gut contents were taken in a vial and made into a volume of 5 ml with distilled water and preserved with 5% buffered formalin until examined.

3.3.9.2. Enumeration and counting of planktonic algae found in the gut contents

Quantitative and qualitative study of algae found in the gut contents of the fish species were done with the help of a Sedgwick-Rafter (S-R) cell. One ml sub-sample was poured by a pipette to the S-R cell. Organisms found in ten squares of the S-R cell (chosen randomly) were identified and counted under a HP microscope. The identification of planktonic algae was done up to generic level according to Needham and Needham (1962), Prescott (1964), Belcher and Swale (1978), APHA (1992) and Bellinger (1992). Quantitative estimation of

the algae was done according to (Stirling, 1985). The identified algae found in the gut contents were calculated as percentage in number.

3.3.10. Determination of electivity index

To gain an idea of the proportion of plankton organism in the diet relative to the proportion calculated in the pond water and index of selective feeding, "Electivity Index" was calculated by using the following formula.

$$\mathbf{E} = \frac{\mathbf{P_g - P_w}}{\mathbf{P_g + P_w}} \quad \text{(Ivlev, 1961)}$$

Where, E = Electivity index value;

P_g = Relative content of any food item in the ration, expressed as percentage of total ration; and

P_w= Relative content of the similar item in the pond water.

The value of "E" ranges from -1.0 to +1.0. The positive value indicates the selection of a particular food material while negative for avoidance.

3.3.11. Statistical analysis

For the statistical analysis of collected data, one way analysis of variance (ANOVA) was performed using computer software SPSS (Statistical Package for Social Science, version 16.0). Significance was assigned at the 0.05 level. The mean values were also compared to see the significant difference from the DMRT (Duncan Multiple Range Test).

Plan of Works for the Study on Management of Euglenophytes Bloom in Fish Pond

(2nd Experiment)

Activities	Month (July 2011 to March 2012)								
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Review of literature collection									
Ponds preparation									
Fish fingerling collection and stocking									
Water quality monitoring									
Fish harvesting									
Gut contents analysis									
Data analysis									

Chart 3.1: Plan of works for the study on management of euglenophytes bloom in fish pond

3.4. Results

During the study period, water quality parameters, soil organic matter, planktonic algal community and density, growth and gut contents of fish were analyzed. The results of these parameters are presented below.

3.4.1. Water quality parameters

The water quality parameters viz., water temperature, pH, dissolved oxygen (DO), nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), phosphate-phosphorus (PO_4-P), iron (Fe), zinc (Zn), Manganese (Mn) and copper (Cu) concentrations in the ponds under four treatments were monitored fortnightly and the variations in these parameters are shown in Figure 3.1, 3.2 and 3.3. The mean values and ranges of water quality parameters are shown in Table 3.2 and Figure 3.4.

3.4.1.1. Water temperature

During the study period, the values of water temperature in four treatments showed no significant difference ($P>0.05$) and were found to vary from 19.28 to 32.51, 19.35 to 32.52, 19.36 to 32.49 and 19.41 to 32.50 ^{0}C in T1, T2, T3 and T4, respectively. It was over 32.0 ^{0}C in mid of August and below 19.5 ^{0}C in end of December (Figure 3.1).

3.4.1.2. Dissolved oxygen

The concentrations of dissolved oxygen varied from 5.10 to 6.30, 5.05 to 6.25, 5.20 to 6.33 and 4.09 to 5.45 mg/l in T1, T2, T3 and T4, respectively. The maximum concentration (6.33 mg/l) was recorded in T3 in mid of August and the minimum (4.09 mg/l) was in T4 in end of November (Figure 3.1). The mean concentration of DO in T1 and T3 was relatively high followed by T2 whereas in T4, it was significantly low (Table 3.2 and Figure 3.4).

Table 3.2: Mean values (±SD) and ranges of water quality parameters in the ponds under four treatments

Parameters	Treatments			
	T1	T2	T3	T4
Temperature (^{0}C)	28.37 ± 4.39^a (19.28-32.51)	28.38 ± 4.43^a (19.35-32.52)	28.37 ± 4.44^a (19.36-32.49)	28.36 ± 4.43^a (19.41-32.50)
DO (mg/l)	5.54 ± 0.44^a (5.10-6.30)	5.39 ± 0.43^a (5.05-6.25)	5.53 ± 0.35^a (5.20-6.33)	4.72 ± 0.51^b (4.09-5.45)
pH	7.08 ± 0.26^b (6.47-7.24)	7.49 ± 0.34^a (6.86-7.96)	7.69 ± 0.41^a (6.80-8.02)	6.21 ± 0.34^c (5.98-6.97)
NO_3-N (mg/l)	0.74 ± 0.19^c (0.57-1.16)	1.13 ± 0.17^b (0.96-1.24)	0.71 ± 0.24^c 0.48-1.17)	1.41 ± 0.26^a 1.02-1.76)
NH_4-N (mg/l)	0.57 ± 0.23^c (0.26-1.07)	0.87 ± 0.18^b (0.63-1.16)	0.54 ± 0.25^c (0.21-1.10)	1.34 ± 0.18^a (1.12-1.61)
PO_4-P (mg/l))	0.76 ± 0.20^c (0.58-1.12)	1.14 ± 0.22^b (0.91-1.39)	0.69 ± 0.25^c (0.47-1.21)	1.61 ± 0.39^a (1.18-2.19)
Fe (mg/l)	0.29 ± 0.09^c (0.20-0.42)	0.36 ± 0.07^b (0.28-0.44)	0.24 ± 0.10^c (0.14-0.43)	0.69 ± 0.08^a (0.55-0.75)
Zn (mg/l)	0.14 ± 0.05^c (0.10-0.23)	0.23 ± 0.05^b (0.20-0.29)	0.15 ± 0.04^c (0.11-0.25)	0.33 ± 0.06^a 0.28-0.40)
Mn (mg/l))	0.15 ± 0.05^c (0.11-0.22)	0.20 ± 0.04^b (0.16-0.23)	0.14 ± 0.05^c (0.10-0.20)	0.29 ± 0.05^a (0.22-0.36)
Cu (mg/l)	0.13 ± 0.03^c (0.10-0.18)	0.17 ± 0.04^b (0.12-0.22)	0.17 ± 0.05^b (0.11-0.24)	0.26 ± 0.03^a (0.24-0.28)

*__T1:__ Ponds treated with duckweed, **T2:** Ponds treated with lime, **T3:** Ponds treated with duckweed and lime, and **T4:** Control ponds (without duckweed and lime).

*Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different (P<0.05).

3.4.1.3. pH

pH values varied from 6.47 to 7.24, 6.86 to 7.96, 6.80 to 8.02 and 5.98 to 6.97 in T1, T2, T3 and T4, respectively. The maximum value (8.02) was recorded in T3 in end of October and the minimum (5.98) in T4 in end of December (Figure 3.1). The mean value was significantly high in T2 and T3 (P<0.05) followed by T1 whereas in T4, it was significantly low (Table 3.2 and Figure 3.4).

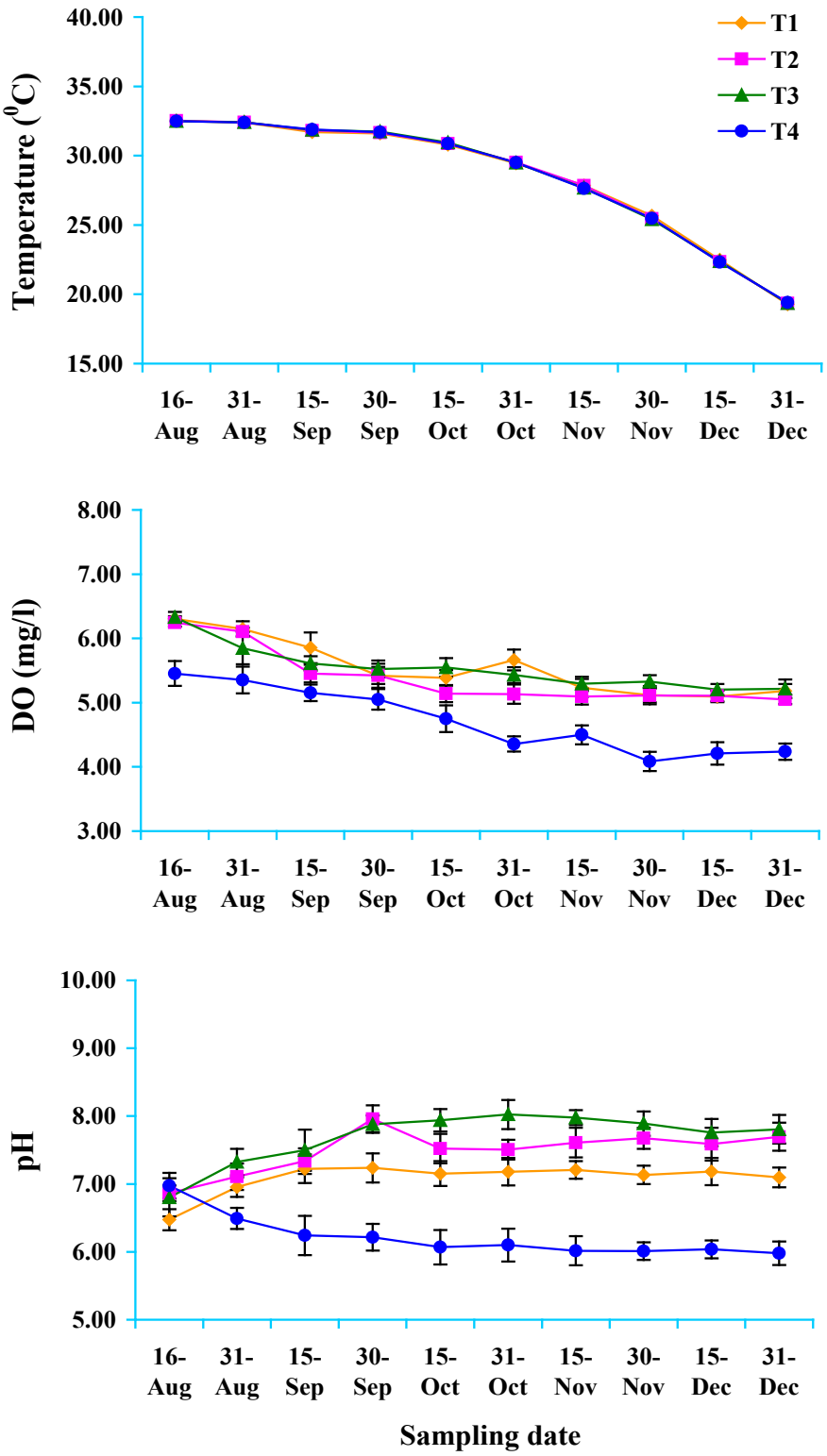


Figure 3.1: Fortnightly variations in water temperature, DO and pH in T1, T2, T3 and T4

3.4.1.4. Nitrate-nitrogen

The concentrations of NO_3-N were found to vary from 0.57 to 1.16, 0.96 to 1.24, 0.48 to 1.17 and 1.02 to 1.76 mg/l in T1, T2, T3 and T4, respectively. The concentrations of this nutrient showed a sharp decrease in T1 and T3 (Figure 3.2). The maximum concentration (1.76 mg/l) was recorded in T4 in end of November and the minimum (0.48 mg/l) was recorded in T3 in mid of December. The mean concentration of this nutrient was significantly high in T4 ($P<0.05$) followed by T2 whereas in T1 and T3, it was significantly low (Table 3.2 and Figure 3.4).

3.4.1.5. Ammonium-nitrogen

The concentrations of NH_4-N varied from 0.26 to 1.07, 0.63 to 1.16, 0.21 to 1.10 and 1.12 to 1.61 mg/l in T1, T2, T3 and T4, respectively. At the beginning of the study, concentrations of NH_4-N in four treatments were more or less similar, after that a sharp decrease was observed in T1 and T3 (Figure 3.2). The maximum concentration (1.61 mg/l) of this nutrient was recorded in T4 in end of November and the minimum (0.21 mg/l) was recorded in T3 in end of December. Significantly higher mean concentration was recorded in T4 ($P<0.05$) followed by T2 whereas in T1 and T3, it was significantly low (Table 3.2 and Figure 3.4).

3.4.1.6. Phosphate-phosphorus

The concentrations of PO_4-P were found to vary from 0.58 to 1.12, 0.91 to 1.39, 0.47 to 1.21 and 1.18 to 2.19 mg/l in T1, T2, T3 and T4, respectively. The maximum concentration (2.19 mg/l) was recorded in T4 in mid of November and the minimum (0.47 mg/l) was recorded in T1 in end of December (Figure 3.2). Like nitrate and ammonium, phosphate concentrations also showed a mark decrease in T1 and T3. Significantly higher mean concentration of this nutrient was recorded in T4 ($P<0.05$) followed by T2 and the lower concentration was recorded in T1 and T3 (Table 3.2 and Figure 3.4).

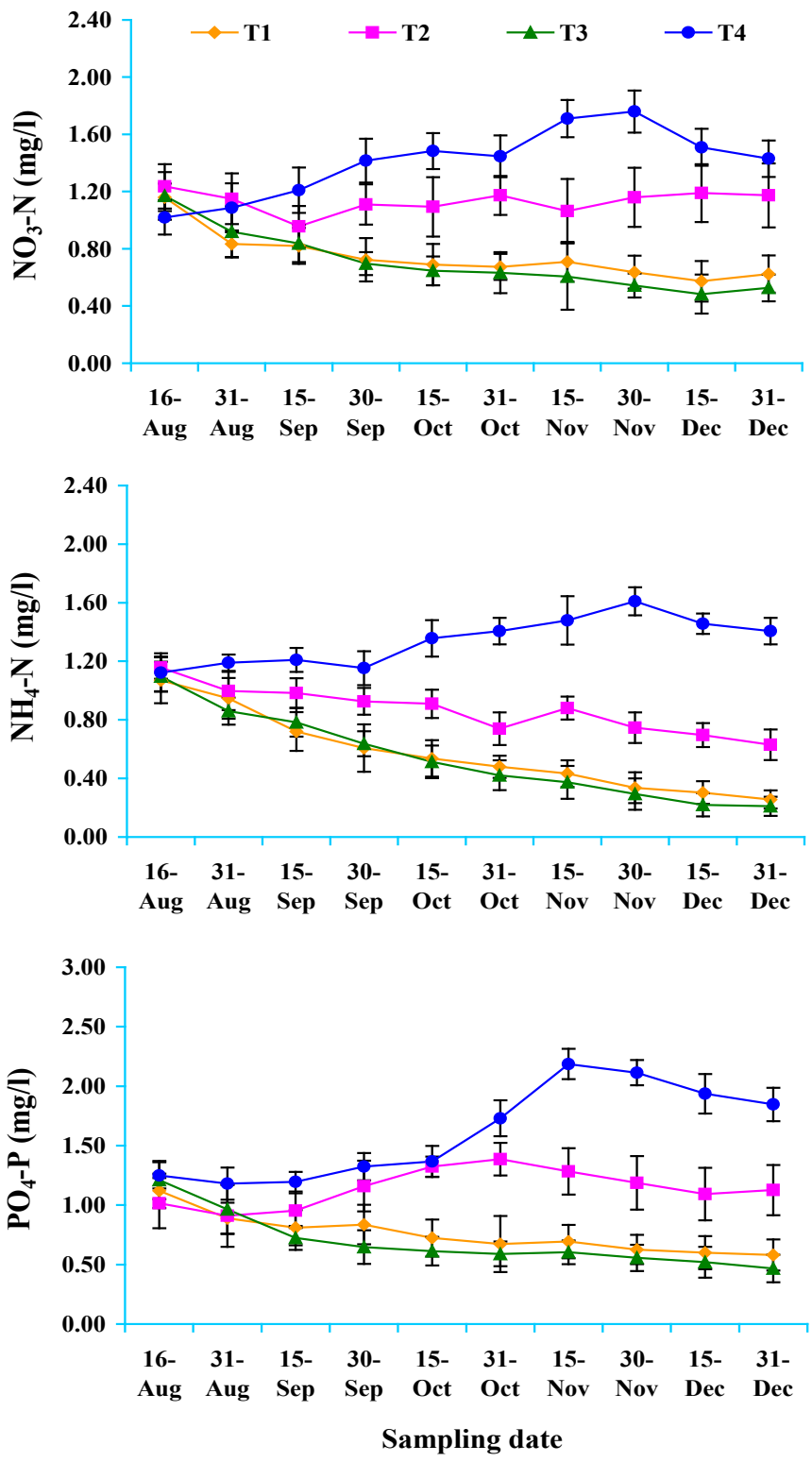


Figure 3.2: Fortnightly variations in NO_3-N, NH_4-N and PO_4-P concentrations in T1, T2, T3 and T4

3.4.1.7. Iron

Significantly higher mean concentration of Fe was recorded in T4 ($P<0.05$) followed by T2 whereas in T1 and T3, it was quietly low (Table 3.2 and Figure 3.4). The concentrations of this heavy metal were found to vary from 0.20 to 0.42, 0.28 to 0.44, 0.14 to 0.43 and 0.55 to 0.75 mg/l in T1, T2, T3 and T4, respectively. The maximum concentration (0.75 mg/l) was recorded in T4 in mid of November and the minimum (0.14 mg/l) in T3 in end of December.

3.4.1.8. Zinc

The concentrations of Zn varied from 0.10 to 0.23, 0.20 to 0.29, 0.11 to 0.25 and 0.28 to 0.40 mg/l in T1, T2, T3 and T4, respectively. The maximum concentration (0.40 mg/l) was recorded in T4 in mid of November and the minimum (0.10 mg/l) in T1 in end of December (Figure 3.3). Significantly lower mean concentration of this heavy metal was recorded in T1 and T3 ($P<0.05$) followed by T2 whereas in T4, it was quietly high (Table 3.2 and Figure 3.4).

3.4.1.9. Manganese

The concentrations of Mn varied from 0.11 to 0.22, 0.16 to 0.23, 0.10 to 0.20 and 0.22 to 0.36 in T1, T2, T3 and T4, respectively. Significantly higher mean concentration of this heavy metal was recorded in T4 ($P<0.05$) with the maximum (0.36 mg/l) in end of November and the lower concentration was in T1 and T3 with the minimum (0.10 mg/l) in T3 in mid of September (Figure 3.3 and 3.4).

3.4.1.10. Copper

Significantly higher mean concentration of Cu was recorded in T4 ($P<0.05$) with the maximum (0.28 mg/l) in mid of November and the concentration was quietly low in T1 with the minimum (0.10 mg/l) in end of November (Figure 3.3 and 3.4). The concentration of this heavy metal varied from 0.10 to 0.18, 0.12 to 0.22, 0.11 to 0.24 and 0.24 to 0.28 mg/l in T1, T2, T3 and T4, respectively.

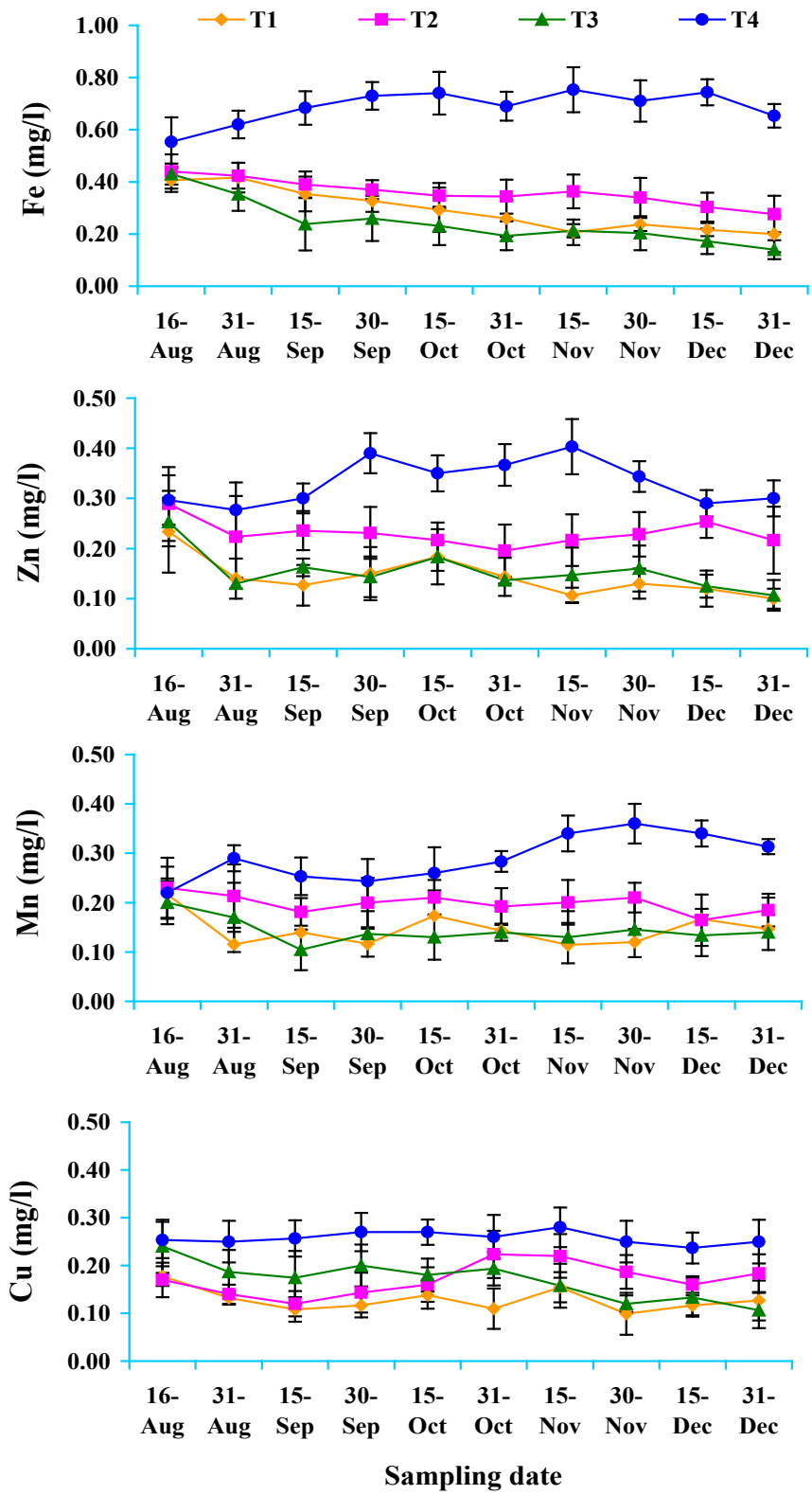


Figure 3.3: Fortnightly variations in Fe, Zn, Mn and Cu concentrations in T1, T2, T3 and T4

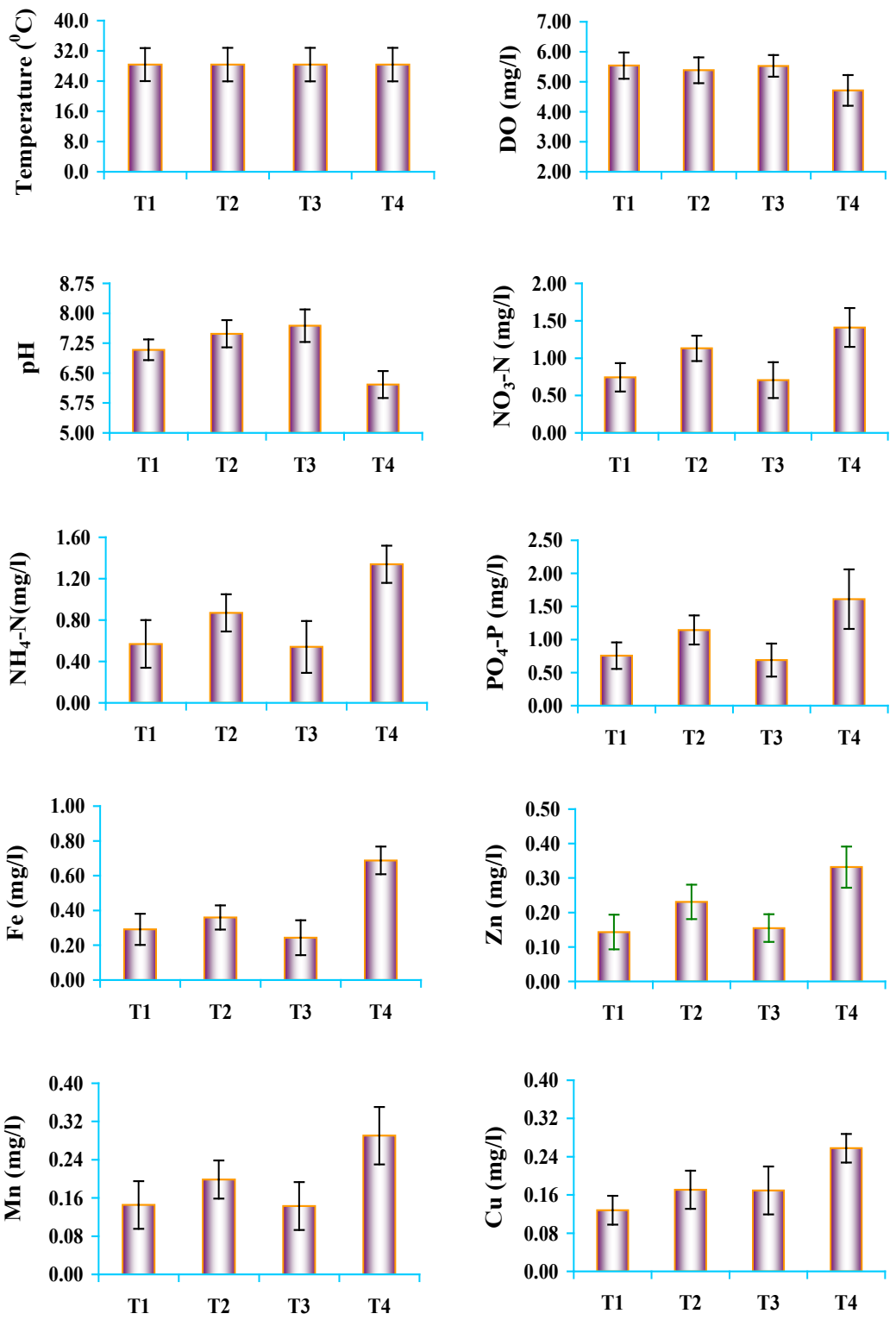


Figure 3.4: Variations in mean values of water quality parameters in T1, T2, T3 and T4

3.4.2. Soil organic matter

Fortnightly variations and mean values of soil organic matter in four treatments are shown in Figure 3.5 and 3.6. During this study, the values of soil organic matter were found to vary from 3.57 to 5.04, 3.05 to 4.71, 2.80 to 4.60 and 5.02 to 7.49% in T1, T2, T3 and T4, respectively. The mean value was significantly ($P<0.05$) high in T4 (6.66±0.82 %) followed by the T1 (4.19±0.59 %) but in T2 and T3, it was significantly low (3.40±0.57 and 3.29±0.60).

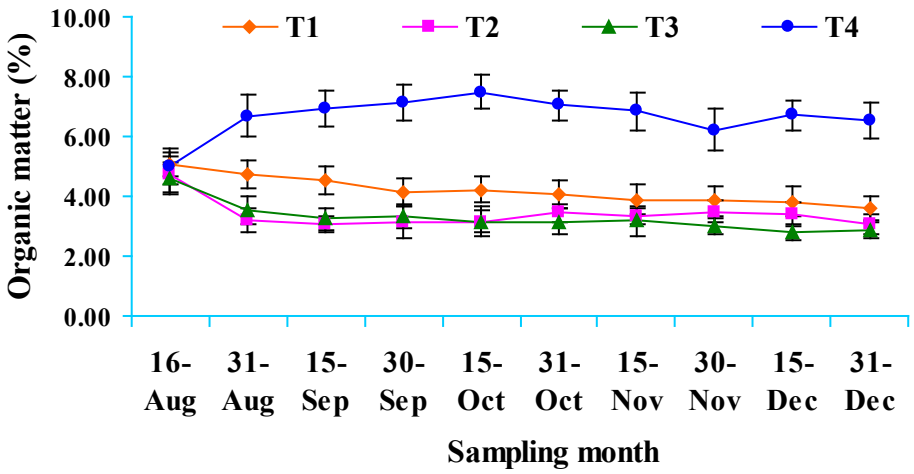


Figure 3.5: Fortnightly variations in soil organic matter in T1, T2, T3 and T4

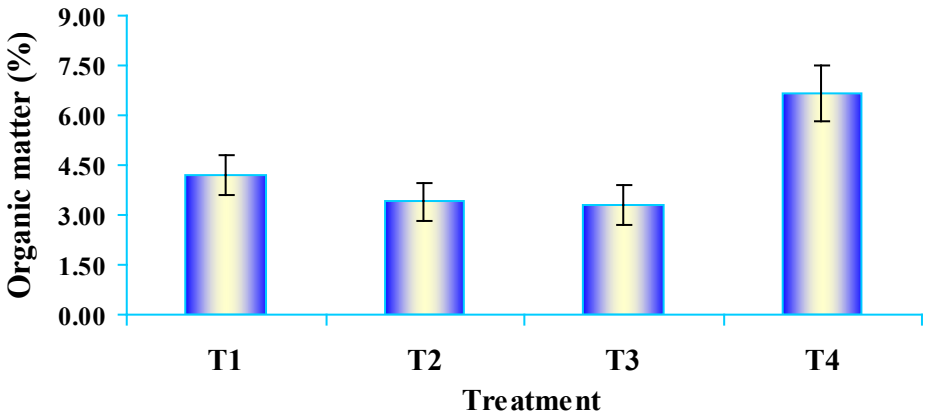


Figure 3.6: Variations in mean values of soil organic matter in T1, T2, T3 and T4

3.4.3. Planktonic algal community

During the study period, total 29 genera of planktonic algae belonging to chlorophytes, cyanophytes, bacillariophytes and euglenophytes were recorded from the study ponds (Table 3.3). There was no significant difference in number of planktonic algal genera in T1, T2 and T3 but relatively lower number was recorded in T4. The genera number varied from 23-29, 22-28, 23-28 and 14-21 in T1, T2, T3 and T4, respectively. The maximum number was recorded in T1 and the minimum in T4. Among these algal groups, chlorophytes had the maximum number of genera (11) followed by cyanophytes (8) and bacillariophytes (7) whereas euglenophytes had the minimum number of genera (3) in all treatments during the study periods.

Table 3.3: Planktonic algal genera found in the ponds under four treatments

Group of algae	Genera under each group
Chlorophytes	*Chlorella, Closterium, Coelastrum, Pediastrum, Scenedesmus, Spirogyra*, *Staurastrum*, *Teraedon, Ulothrix*, *Volvox* and *Zygnema*
Cyanophytes	*Anabaena, Anabaenopsis, Apanizomenon, Aphanocapsa, Chroococcus, Gomphospheria, Oscillatoria* and *Microcystis*
Bacillariophytes	*Asterionella, Cyclotella, Fragilaria, Navicula, Nitzschia*, *Synedra* and *Tabellaria*
Euglenophytes	*Euglena, Phacus* and *Trachelomonas*

3.4.4. Planktonic algal density

The mean density and density ranges of different groups of planktonic algae in the ponds under four treatments are shown in Table 3.4. Fortnightly variations

in density, variations in mean density and percent contributions of different algal groups in four treatments are shown in Figure 3.7, 3.8, 3.9, 3.10 and 3.11.

3.4.4.1. Density of total planktonic algae

The densities of total planktonic algae were found to vary from 16.68 to 21.91, 14.37 to 19.97, 11.56 to 19.47 and 20.03 to 34.81 x 104 cells/l in T1, T2, T3 and T4, respectively. The maximum density (34.81 x 104 cells/l) was recorded in T4 in mid of December and the minimum (11.56 x 104 cells/l) was recorded in T3 in end of December (Figure 3.7). Significantly higher mean density of total planktonic algae was recorded in T4 followed by T1 and T2 whereas in T3, it was quietly low (Table 3.4 and Figure 3.9).

3.4.4.2. Density of euglenophytes

During the study tenure, the densities of euglenophytes were found to be ranged from 8.31 to 9.49, 7.12 to 9.61, 4.02 to 9.49 and 11.14 to 29.22 x 10^4 cells/l in T1, T2, T3 and T4, respectively. The maximum density (29.22 x 10^4 cells/l) was recorded in T4 in mid of December and the minimum density (4.02 x 10^4 cells/l) was recorded in T3 in end of November (Figure 3.7). Significantly ($P<0.05$) higher mean density was recorded in T4 followed by T1 and T2 whereas lower mean density was recorded in T3 (Table 3.4 and Figure 3.9). Fortnightly percent contributions of euglenophytes in T3 were almost lower than T1 and T2 whereas in T4, it was quietly high (Figure 3.10). The average percent contributions of these algae were 49.64, 49.24, 37.25 and 69.54% in T1, T2, T3 and T4, respectively (Figure 3.11).

3.4.4.3. Density of cyanophytes

The density of cyanophytes showed no significant difference among the treatments ($P>0.05$) but the mean density was relatively high in T3 followed by T1 and T2 whereas in T4, the density was low (Table 3.4 and Figure 3.9). During

the study period, the densities of these algae were found to vary from 3.90 to 9.77, 3.69 to 7.89, 3.58 to 8.53 and 3.31 to 7.81 x 10^4 cells/l in T1, T2, T3 and T4, respectively. The maximum density (9.77 x 10^4 cells/l) was recorded in T1 in end of August and the minimum density (3.31 x 10^4 cells/l) was recorded in T4 in mid of December (Figure 3.8). Fortnightly percent contributions of these algae in T3 were almost higher than T1 and T2 whereas in T4, it was quietly low (Figure 3.10). The average percent contributions of these algae were 28.49, 29.95, 36.92 and 20.40 % in T1, T2, T3 and T4, respectively (Figure 3.11).

Table 3.4: Mean density (±SD) and ranges of different groups of algae in the ponds under four treatments

Algal group	Treatment			
	T-1	T-2	T-3	T-4
Total algae (x 10^4 cells/l)	17.99 ± 2.25^{b} (16.68-21.91)	16.66 ± 2.97^{bc} (14.37-19.97)	14.49 ± 2.89^{c} (11.56-19.47)	26.55 ± 5.65^{a} (20.03-34.81)
Euglenophytes (x 10^4 cells/l)	8.87 ± 1.58^{b} (8.31-9.49)	8.15 ± 2.23^{b} (7.12-9.61)	5.40 ± 2.13^{c} (4.02-9.49)	18.97 ± 6.78^{a} (11.14-29.22)
Cyanophytes (x 10^4 cells/l)	5.21 ± 1.92^{a} (3.90-9.77)	5.07 ± 1.54^{a} (3.69-7.89)	5.43 ± 1.83^{a} (3.58-8.53)	5.04 ± 1.57^{a} (3.31-7.81)
Chlorophytes (x 10^4 cells/l)	3.30 ± 0.61^{a} (2.56-4.21)	2.96 ± 0.36^{a} (2.44-3.25)	3.11 ± 0.64^{a} (2.01-3.97)	2.33 ± 0.37^{b} (2.09-2.82)
Bacillariophytes (x 10^4 cells/l)	0.60 ± 0.11^{a} (0.39-0.77)	0.48 ± 0.09^{b} (0.41-0.54)	0.55 ± 0.15^{ab} (0.23-0.69)	0.21 ± 0.08^{c} (0.17-0.25)

*__T1:__ The ponds treated with duckweed, **T2:** The ponds treated with lime, **T3:** The ponds treated with duckweed and lime, and **T4:** Control ponds (without duckweed and lime).

*Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different ($P<0.05$).

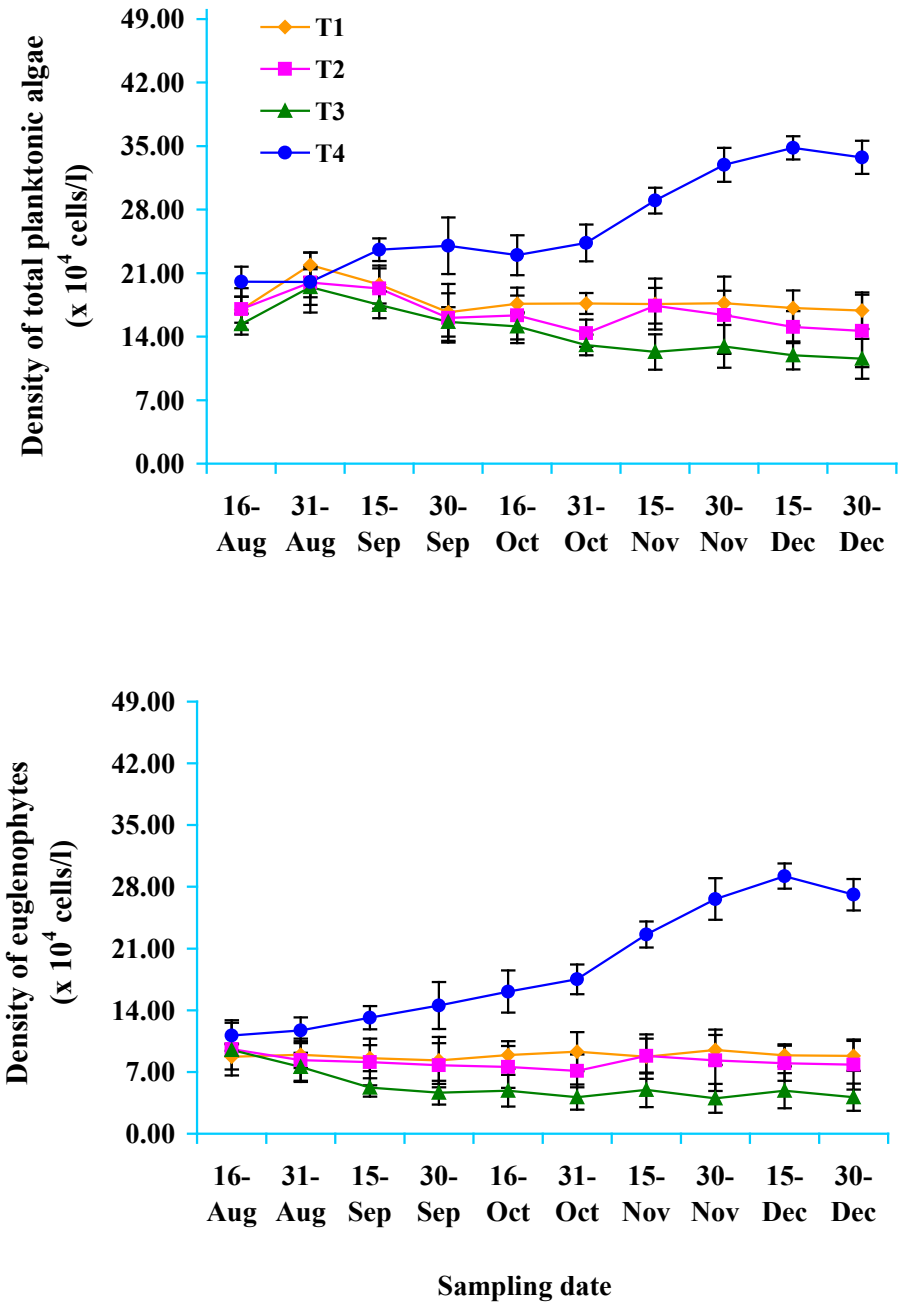


Figure 3.7: Fortnightly variations in density of total planktonic algae and euglenophytes in T1, T2, T3 and T4

3.4.4.4. Density of chlorophytes

Chlorophytes was the third abundant group of algae in the study ponds and its density varied from 2.56 to 4.21, 2.44 to 3.25, 2.01 to 3.97 and 2.09 to 2.82 x 10^4 cells/l in T1, T2, T3 and T4, respectively. The maximum density (4.21 x 10^4 cells/l) was found in T1 in mid of November and the minimum (2.01 x 10^4 cells/l) was found in T3 in mid of August (Figure 3.8). The mean density of this group of algae showed no significant difference in T1, T2 and T3 ($P>0.05$) but in T4, it was significantly low (Figure 3.9). The average percent contributions of these algae were 18.50, 17.90, 21.93 and 9.27 % in T1, T2, T3 and T4, respectively (Figure 3.11).

3.4.4.5. Density of bacillariophytes

Bacillariophytes was the least abundant group of algae in the study ponds and its density varied from 0.39 to 0.77, 0.41 to 0.54, 0.23 to 0.69 and 0.17 to 0.25 x 10^4 cells/l in T1, T2, T3 and T4, respectively. The maximum density (0.77 x 10^4 cells/l) was recorded in T1 in end of September and the minimum (0.17 x 10^4 cells/l) was recorded in T4 in end of December (Figure 3.8). Significantly higher mean density of this group of algae was recorded in T1 ($P<0.05$) followed by T3 and T2 whereas the lower mean density was recorded in T4 (Figure 3.9). The average percent contributions of algae were 3.38, 2.91, 3.91 and 0.83 % in T1, T2, T3 and T4, respectively (Figure 3.11).

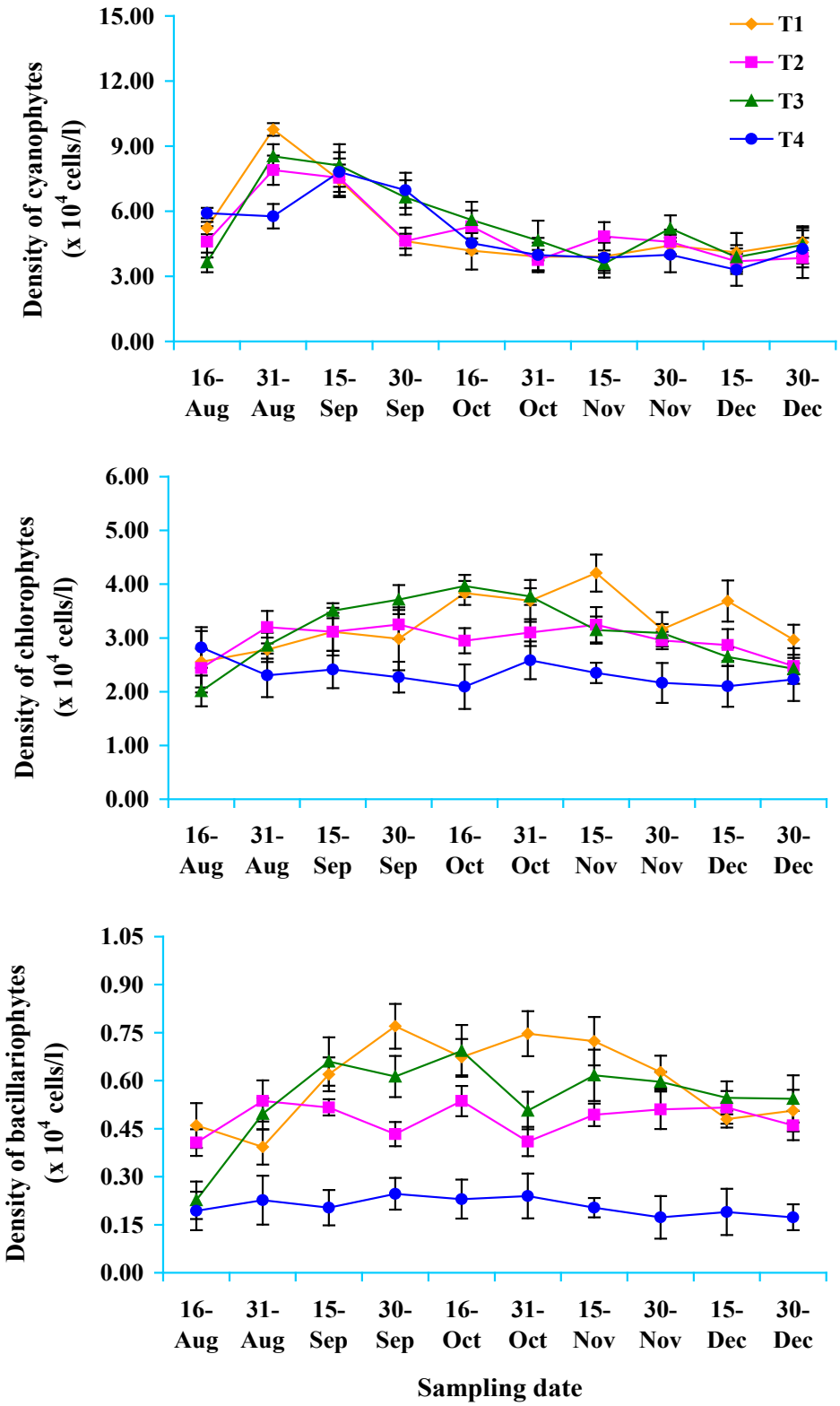


Figure 3.8: Fortnightly variations in density of cyanophytes, chlorophytes and bacillariophytes in T1, T2, T3 and T4

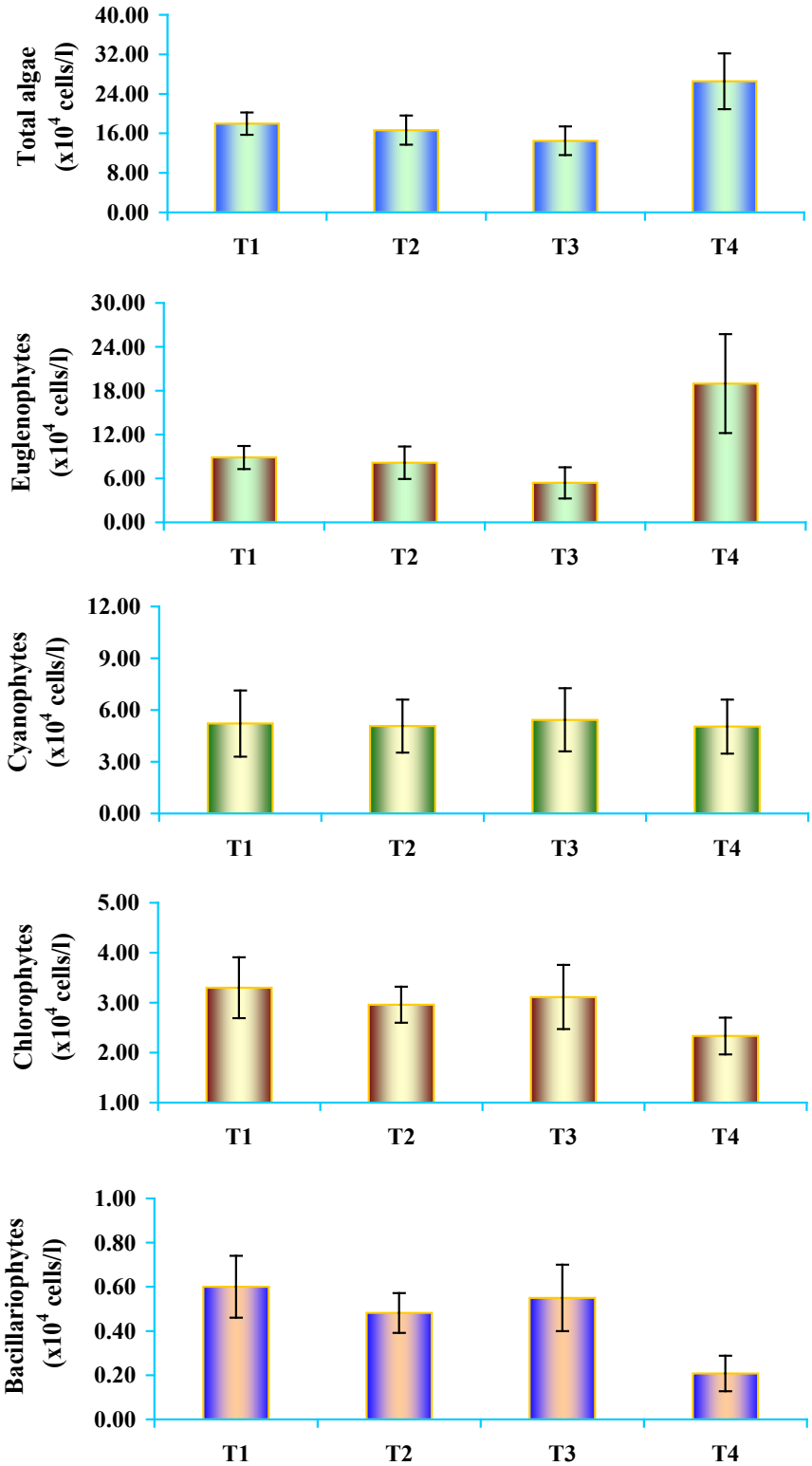


Figure 3.9: Variations in mean density of total algae, euglenophytes, cyanophytes, chlorophytes and bacillariophytes in T1, T2, T3 and T4

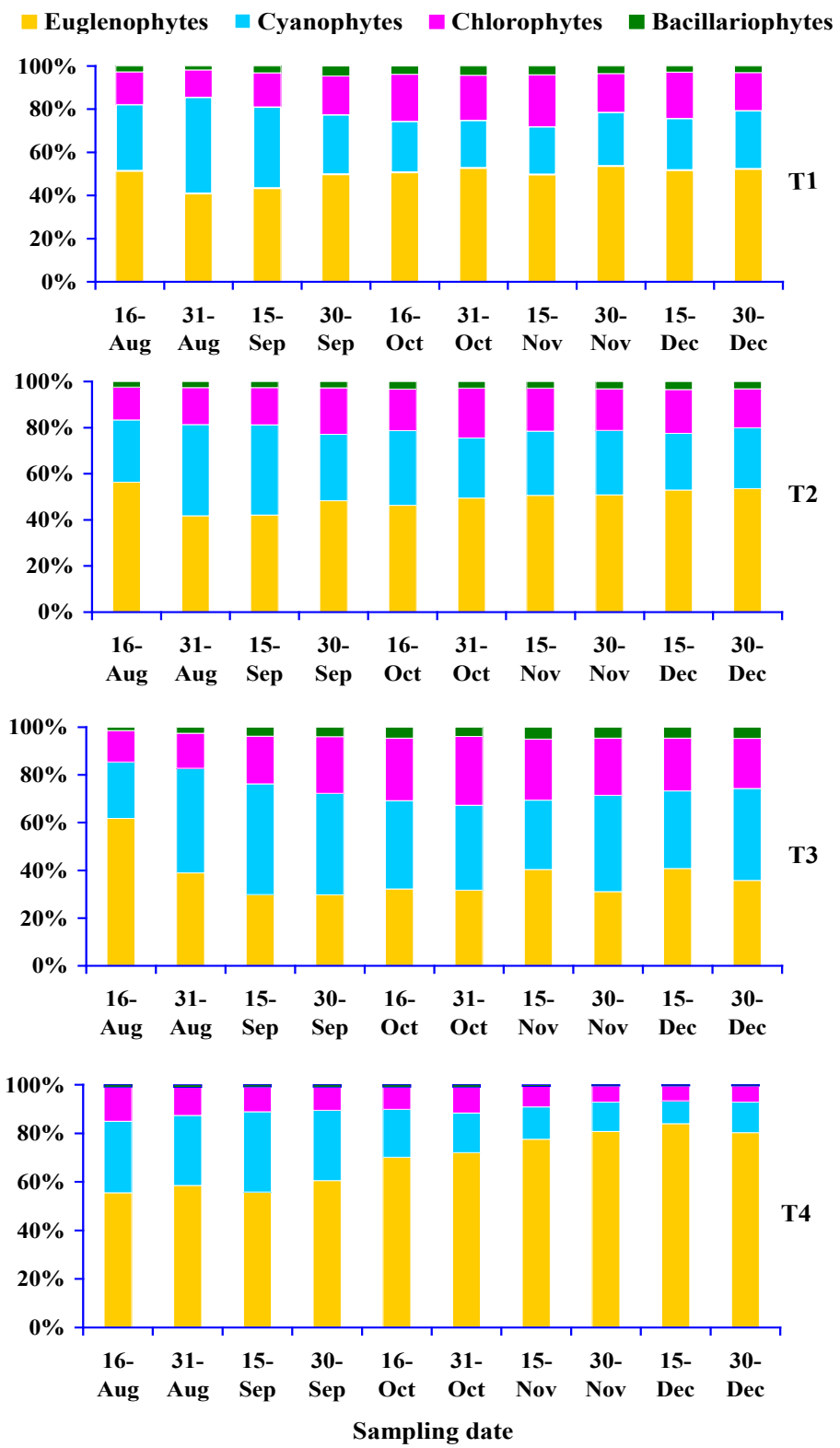


Figure 3.10: Fortnightly variations in percent contributions of different algal groups in T1, T2, T3 and T4

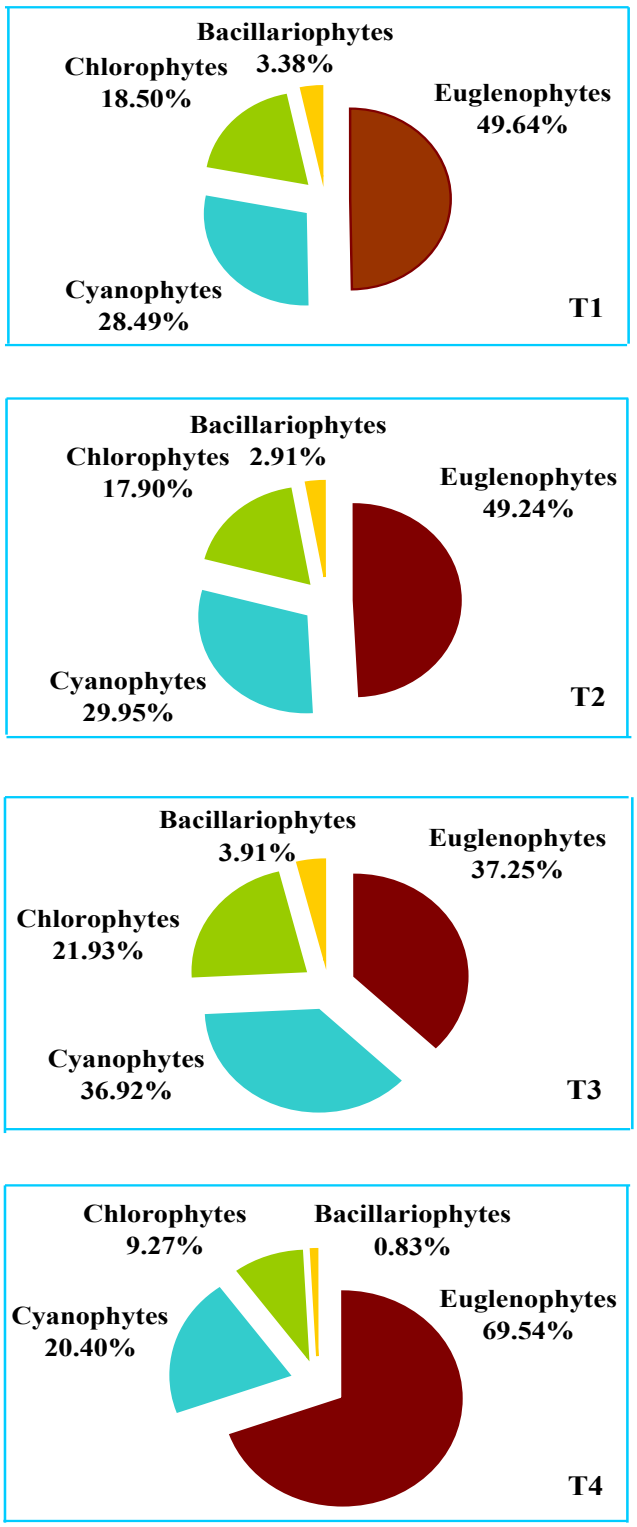


Figure 3.11: Average percent contributions of different algal groups in T1, T2, T3 and T4

3.4.5. Growth performance of fish

Growth performance of the fishes in terms of mean weight gain (MWG), average daily weight gain (ADWG) and specific growth rate (SGR) were analyzed and the results are shown in Table 3.5. Monthly weight increments of the experimental fishes in four treatments are shown in Figure 3.12.

3.4.5.1. Mean weight gain (g)

The variations in MWG of the fishes in four treatments are shown in Figure 3.13. Significantly higher MWG was recorded in T3 for all experimental fish species followed by T1 and T2 whereas in T4, it was quietly low ($P<0.05$).

3.4.5.2. Average daily weight gain (gbwd^{-1})

Average daily weight gain of the fishes in four treatments showed significant difference ($P<0.05$). Higher ADWG was recorded in T3 for all experimental fish species followed by T1 and T2 whereas in T4, it was quietly low. Among the fish species, the ADGW of silver carp was relatively high whereas it was relatively low for silver barb (Table 3.5). The comparison of ADWG of the fishes in four treatments is shown in Figure 3.14 (a).

3.4.5.3. Specific growth rate (% bwd^{-1})

Specific growth rate of the fishes in four treatments showed significant difference ($P<0.05$). In the present study, SGR of all experimental fish species was higher in T3 followed by T1 and T2 and the lower SGR was recorded in T4. Among the fish species, silver barb showed the maximum SGR in T1 and T3 whereas rohu showed the minimum SGR in T4 and T2 (Table 3.5). The comparison of SGR of the fishes in four treatments is shown in Figure 3.14 (b).

3.4.5.4. Survival rate (%)

There was no significant difference in survival rates of the fishes in T1, T2 and T3 but it was significantly low in T4 ($P<0.05$). The average values of survival rate were 90, 89, 93 and 82% in T1, T2, T3 and T4, respectively.

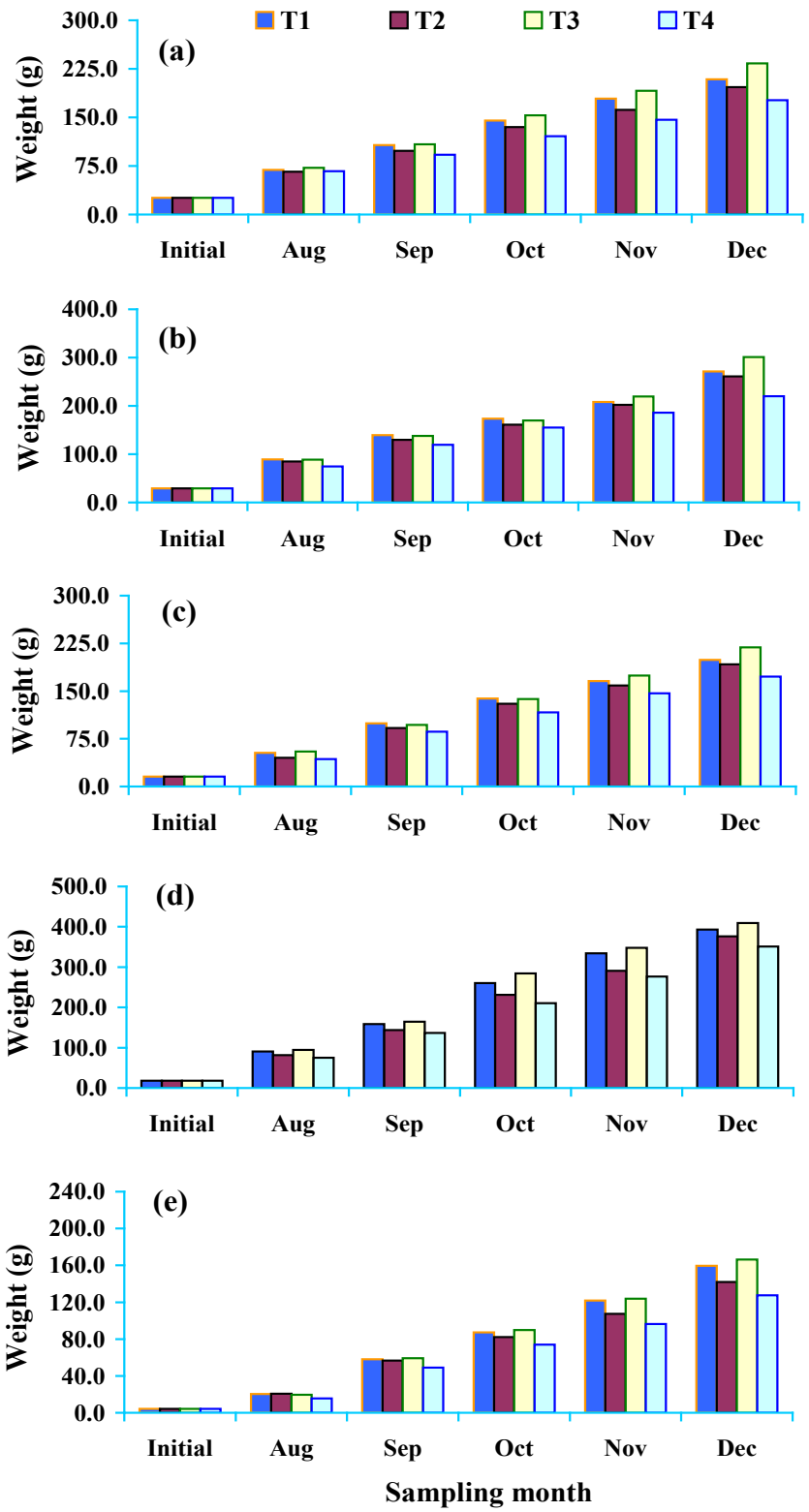


Figure 3.12: Monthly weight increments in (a) Rohu (b) Catla (c) Mrigel (d) Silver carp and (e) Silver barb in T1, T2, T3 and T4

Table 3.5: Growth parameters of the fish species in four treatments

Parameter	Fish species	Treatment			
		T1	T2	T3	T4
Initial weight (g)	Rohu	25.54±6.25	25.50±7.13	25.53±7.10	25.56±6.84
	Catla	29.25±7.14	29.27 ±6.28	29.31±7.11	29.29±7.16
	Mrigal	15.70±4.21	15.68 ±5.02	17.71±4.52	17.69 ±4.94
	Silver carp	17.85±4.03	17.80 ±3.44	17.82±4.12	17.83±3.17
	Silver barb	4.25±1.02	4.20 ±1.12	4.19±1.09	4.23±1.13
Final weight (g)	Rohu	209.02±15.84	196.96 ±17.25	233.71±18.95	176.67±18.69
	Catla	271.51±18.70	261.09 ±19.77	301.31±22.36	220.10±16.41
	Mrigal	199.06±12.43	192.03 ±13.61	218.77±15.73	172.60±18.55
	Silver carp	393.36±18.14	376.53 ±19.91	401.11±23.10	351.09±18.45
	Silver barb	159.51±15.46	141.93 ±18.49	166.32±15.16	127.40±17.54
Weight gain (g)	Rohu	183.38±19.24^{b}	171.32±18.25^{b}	208.07±21.67^{a}	151.03±19.47^{c}
	Catla	242.24±20.32^{b}	231.82±18.52^{b}	272.04±21.15^{a}	190.83±20.13^{c}
	Mrigal	183.36±20.21^{b}	176.33±19.78^{b}	203.07±17.25^{a}	156.90±21.25^{c}
	Silver carp	375.54±25.14^{b}	358.71±27.25^{b}	391.29±26.78^{a}	333.27±24.18^{c}
	Silver barb	157.31±16.25^{b}	139.73±22.10^{b}	164.12±19.25^{a}	125.20±18.39^{c}
Daily weight gain (gbw/d)	Rohu	1.22 ±0.04^{b}	1.14±0.05^{b}	1.39±0.06^{a}	1.01±0.06^{c}
	Catla	1.61±0.05^{b}	1.55±0.04^{b}	1.81±0.07^{a}	1.27±0.04^{c}
	Mrigal	1.22±0.06^{b}	1.18±0.02^{b}	1.35±0.04^{a}	1.05±0.06^{c}
	Silver carp	2.50±0.08^{b}	2.39±0.04^{b}	2.61±0.07^{a}	2.22±0.05^{c}
	Silver barb	1.04±0.04^{b}	0.92±0.06^{b}	1.08±0.03^{a}	0.82±0.05^{c}
SGR (%/day)	Rohu	1.40±0.02^{b}	1.36±0.02^{b}	1.47±0.03^{a}	1.29±0.03^{c}
	Catla	1.48±0.02^{b}	1.46±0.02^{b}	1.55±0.02^{a}	1.34±0.02^{c}
	Mrigal	1.69±0.03^{b}	1.67±0.01^{b}	1.76±0.02^{a}	1.60±0.03^{c}
	Silver carp	2.06 ±0.02^{ab}	2.03±0.01^{b}	2.09±0.02^{a}	1.99±0.01^{c}
	Silver barb	2.42±0.02^{a}	2.35±0.04^{b}	2.45±0.02^{a}	2.27±0.03^{c}

*Values are mean of triplicate determination. Values with different superscripts in the same row varied significantly ($P<0.05$).

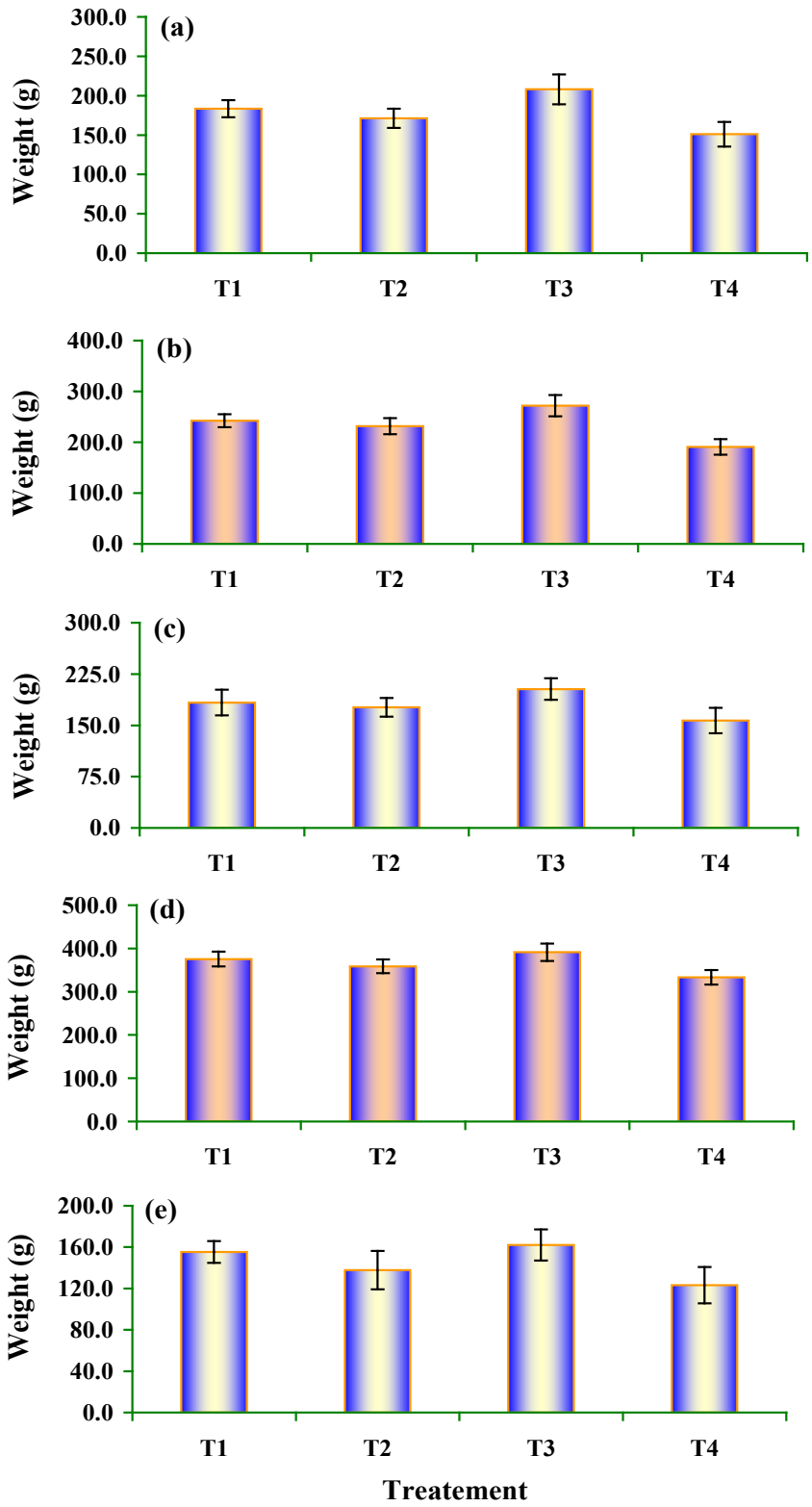


Figure 3.13: Variations in mean weight gain of (a) Rohu, (b) Catla, (c) Mrigel, (d) Silver carp and (e) Silver barb in T1, T2, T3 and T4

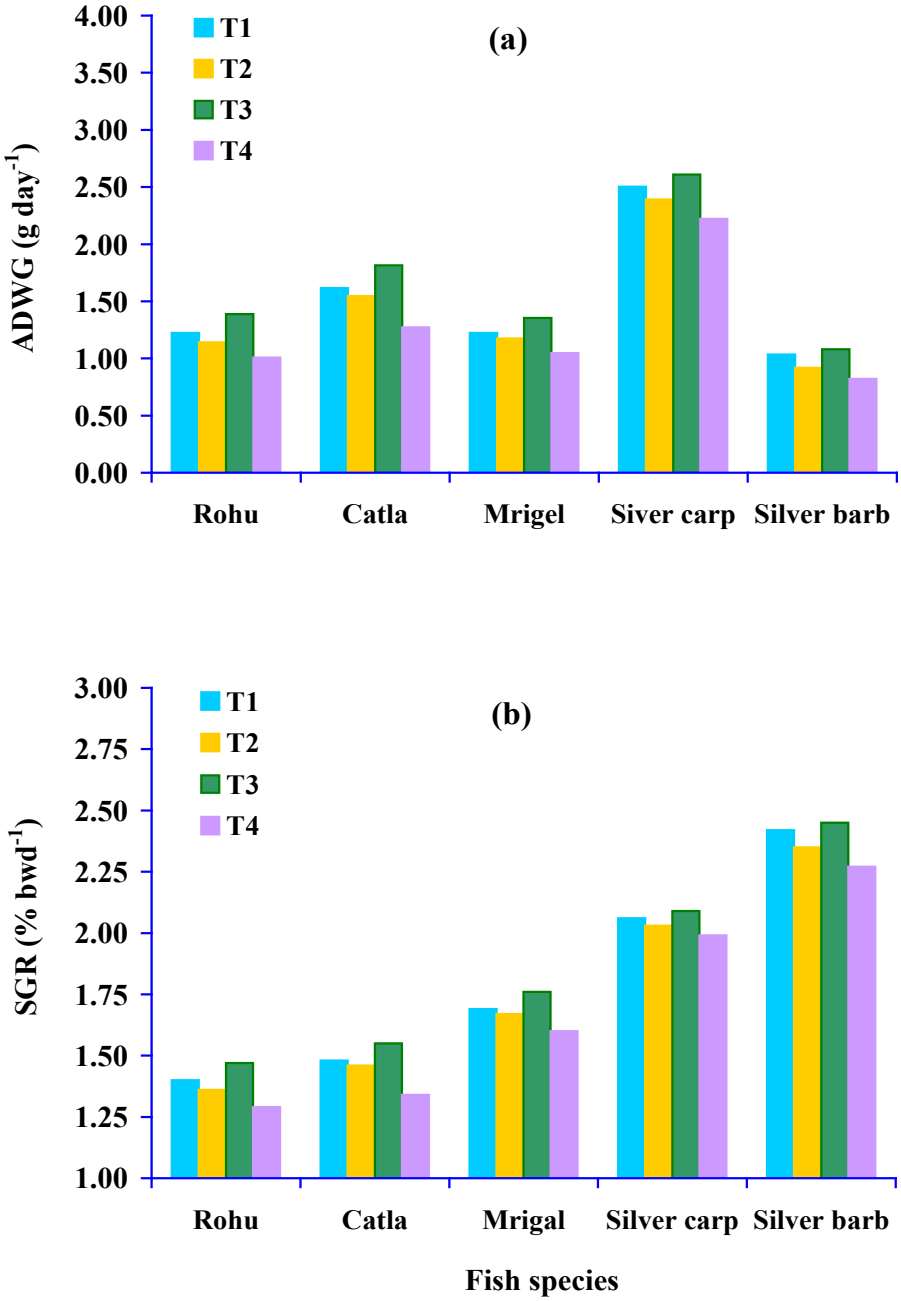


Figure 3.14: Comparison of (a) ADWG and (b) SGR of the fishes in T1, T2, T3 and T4

3.4.6. Gut contents of fish

The results of gut contents analysis showed that the experimental fishes ate various planktonic algae belonging to euglenophytes, cyanophytes, chlorophytes and bacillariophytes consisting 21 genera (Table 3.6). The average proportions (%) of planktonic algae in the gut contents (P_g) of the fish species in four treatments are shown in Table 3.7.

There was no significant difference in the proportions of euglenophytes algae found in the gut contents of the fish species in four treatments ($p>0.05$). But, relatively higher proportion of euglenophytes was found in the gut content of silver carp and silver barb while less proportion of these algae was found in the gut contents of rohu, catla and mrigel (Figure 3.15). Among the different groups of planktonic algae, chlorophytes were found to be the maximum proportion in the gut contents of all experimental fishes (except silver carp) followed by bacillariophytes and cyanophytes. In the gut content of silver carp, cyanophytes was found to be the maximum proportion followed by chlorophytes, euglenophytes and bacillariophytes.

Table 3.6: Planktonic algal genera found in the gut contents of the fish species

Algal group	Genera of each group
Chlorophytes	*Chlorella, Closterium, Pediastrum, Scenedesmus, Teraedon, Ulothrix*, *Volvox* and *Zygnema*
Cyanophytes	*Anabaena, Aphanocapsa, Apanizomenon, Gomphospheria, Oscillatoria* and *Microcystis*
Bacillariophytes	*Cyclotella, Fragilaria, Navicula, Nitzschia* and
Euglenophytes	*Euglena, Phacus* and *Trachelomonas*

Table 3.7: Proportions of planktonic algae found in the gut contents (P_g) of the fish species in four treatments

Group of algae	Fish species	P_g (%) of different fishes in four treatments			
		T1	T2	T3	T4
Euglenophytes	Rohu	4.30±2.48	5.03±2.71	3.51±1.52	4.41±1.26
	Catla	6.68±2.13	7.61±2.66	6.88±1.27	8.05±2.45
	Mrigal	3.27±1.49	4.22±1.84	3.91±1.43	2.37±1.89
	Silver carp	24.98±5.13	24.91±6.08	22.79±8.21	28.21±4.79
	Silver barb	23.18±7.11	23.04±5.29	22.98±3.61	24.58±6.57
Cyanophytes	Rohu	14.26±4.09	16.99±5.16	12.45±4.91	13.11±7.46
	Catla	13.74±3.82	10.16±6.94	12.01±3.28	11.60±5.64
	Mrigal	16.15±7.48	18.73±6.12	16.80±5.17	12.78±6.90
	Silver carp	31.72±14.86	33.56±12.26	45.08±16.24	31.51±13.89
	Silver barb	17.02±4.19	21.21±5.28	15.90±5.81	15.30±5.96
Chlorophytes	Rohu	49.20±9.57	47.31±12.14	55.27±13.27	55.95±8.47
	Catla	51.16±10.53	47.96±8.16	43.28±11.23	47.63±6.28
	Mrigal	47.42±7.19	48.86±9.54	42.41±6.41	45.22±5.69
	Silver carp	23.90±2.56	22.35±4.15	24.80±5.64	24.49±4.72
	Silver barb	35.73±5.42	28.41±6.51	30.89±7.68	32.23±6.24
Bacillariophytes	Rohu	32.24±10.49	30.67±8.46	30.77±9.14	26.53±8.14
	Catla	28.42±11.02	34.27±9.51	37.83±9.50	32.72±11.23
	Mrigal	33.16±11.45	28.19±13.25	36.88±9.53	39.63±14.78
	Silver carp	19.40±5.12	19.18±7.19	17.33±9.07	15.80±6.32
	Silver barb	24.08±8.51	27.35±10.42	30.23±9.05	27.89±8.40

*__T1:__ Ponds treated with duckweed, **T2:** Ponds treated with lime, **T3:** Ponds treated with duckweed and lime, and **T4:** Control ponds (without duckweed and lime).

*Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different ($P<0.05$).

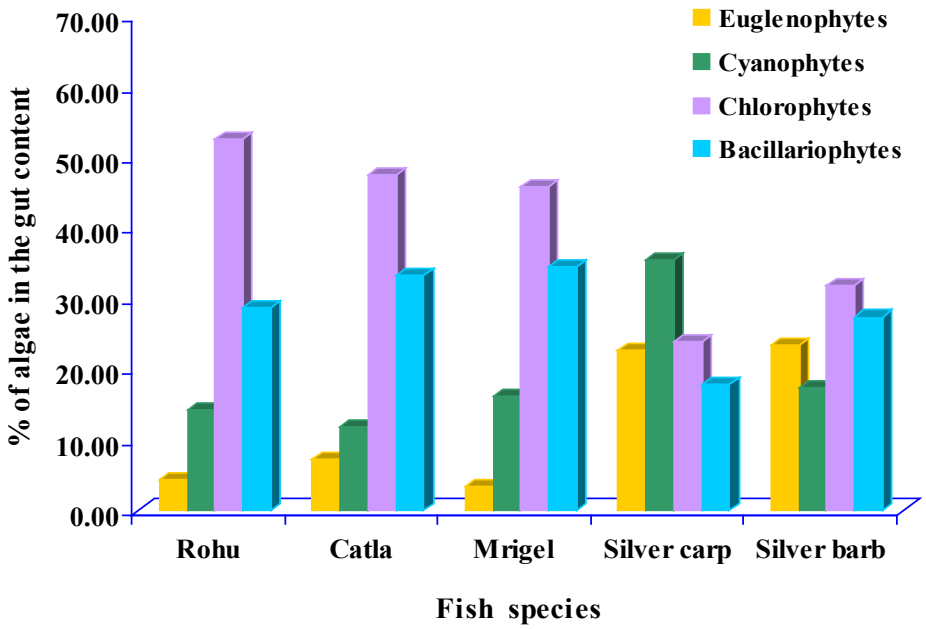


Figure 3.15: Average proportions of planktonic algae found in the gut contents of the fish species

3.4.7. Electivity index of fish

The results of electivity index of the experimental fishes are shown in Table 3.8. The values of electivity index of different experimental fishes ranged from -0.93 to + 0.96. In the present study, all types of experimental fishes showed negative electivity to euglenophytes. Conversely, they showed positive electivity to chlorophytes and bacillariophytes. Only silver carp showed positive electivity to cyanophytes but other fishes showed negative electivity to these algae. The results of electivity index also showed that rohu, catla and mrigel were actively avoided euglenophytes as food item than silver carp and silver barb.

Table 3.8: Electivity index of the fish species in four treatments

Group of algae	Fish species	Electivity index			
		T1	T2	T3	T4
Euglenophytes	Rohu	-0.84	-0.81	-0.83	-0.88
	Catla	-0.76	-0.73	-0.69	-0.79
	Mrigal	-0.88	-0.84	-0.81	-0.93
	Silver carp	-0.33	-0.33	-0.49	-0.42
	Silver barb	-0.36	-0.36	-0.24	-0.48
Cyanophytes	Rohu	-0.33	-0.28	-0.50	-0.22
	Catla	-0.35	-0.49	-0.51	-0.28
	Mrigal	-0.28	-0.23	-0.37	-0.23
	Silver carp	0.05	0.06	0.10	0.21
	Silver barb	-0.25	-0.17	-0.40	-0.14
Chlorophytes	Rohu	0.45	0.45	0.45	0.72
	Catla	0.47	0.46	0.33	0.68
	Mrigal	0.44	0.46	0.32	0.66
	Silver carp	0.13	0.11	0.06	0.45
	Silver barb	0.32	0.23	0.17	0.55
Bacillariophytes	Rohu	0.81	0.83	0.74	0.94
	Catla	0.79	0.84	0.81	0.95
	Mrigal	0.82	0.81	0.81	0.96
	Silver carp	0.70	0.74	0.63	0.90
	Silver barb	0.75	0.81	0.77	0.94

3.5. Discussion

In this section, the results of the present study on water quality parameters, algal community and density, growth of fish and grazing of euglenophytes algae are discussed and verified with the relevant research findings.

3.5.1. Water quality parameters

Water quality plays an important role in aquaculture and any undesirable changes in water quality cause stress, poor growth and mortality of culture species (Boyd and Tucker, 1998). Among different environmental causes, dense algal bloom is known to negatively affect water quality (Tucker *et al*., 1984; Armstrong *et al*., 1986; Rahman *et al*., 2007). Concurrently, duckweed and lime have potential usefulness to improve water quality (Sipauba-Tavares *et al*., 2003; Vanitha *et al*., 2013). However, in the present study, duckweed and lime were used separately or in combination to improve the water quality in the euglenophytes bloom ponds. By analyzing a series of water quality parameters, it was observed that there had been considerable variations (except water temperature) in response to use of duckweed and lime in the bloom ponds.

3.5.1.1. Water temperature

The temperature of water has extremely important ecological consequences. All organisms have preferred temperature in which they can survive and grow optimally. In the present study, there were no significant differences in water temperature in the ponds under four treatments which indicated that use of duckweed and lime had no significant effects on water temperature in the study ponds. But, the values of water temperatures were found to vary from 19.25 to 32.5 °C which might be due to the changes of weather condition from summer to winter season. The variations in water temperatures in four treatments were within the productive range according to the earlier reports of Jhingran (1991) and Rahman *et al*. (1982) who reported that water temperature as 18.5 to 37.5 °C and 20.0 to 30.0°C is favorable for ponds productivity.

3.5.1.2. Dissolved oxygen

Dissolved oxygen is considered to be the most important and critical one for all aquatic organisms. Dissolved oxygen, 5.0 to 7.0 mg/l is considered as fair or good in respect of productivity (Banerjee, 1967; Rahman, 1992). In the present study, significantly higher dissolved oxygen concentrations were recorded in duckweed and lime treated ponds (T1, T2 and T3) as compared to the control ponds (T4). This might be due to the addition of oxygen through duckweed and reduction of BOD and COD through duckweed and lime. This assumption is supported by pervious reports that duckweed supplied additional oxygen in water (Korner *et al*., 2003) and duckweed and lime reduced BOD and COD through declining organic matter and decomposition rate (Boyd and Tucker, 1998; Gurtekin and Şekerdag, 2008).

Though, algae are one of the major sources of dissolved oxygen in the pond water (Dupree and Huner, 1984) but in nutrients rich water bodies, depletion of dissolved oxygen occurs occasionally due to high organic decomposition as a result of dense algal bloom. During this study, significantly lower dissolved oxygen concentration was recorded in the control ponds (T4) which might be due to the dense bloom of euglenophytes. This result is in conformity with the findings of the previous reports (Boyd *et al*., 1975; Rahman *et al*., 2005) that algal bloom can lead to oxygen depletion.

3.5.1.3. pH

pH regulates the productivity of water body and it is considered as an important factor for aquaculture (Boyd, 1990). In the present study, significantly higher pH values recorded in T2 and T3, and remained within the acceptable range for pond productivity according to Swingle (1967) who repotted that pH range 7.0 to 8.5 is suitable for pond productivity. Increase pH values in T2 and T3 might be due to use of lime. This assumption is supported by the previous studies

(Ivahnenko *et al*., 1988; Boyd and Tucker, 1998) which stated that lime improves water quality by raising pH. The pH values in T1 were also within the acceptable range. This might be due to the use of duckweed which increased pH level through utilization of free carbon dioxide in water. Whereas, the pH values were almost below 6.5 in T4 which might be due to lower dissolved oxygen and higher carbon dioxide concentrations. Tucker (1984) reported that pH in water has a direct relation with dissolved oxygen and an inverse relation with free carbon dioxide concentration. This report is supportive to the present results.

3.5.1.4. Nutrients

The use of duckweed in eutrophic ponds may improve water quality through absorbing excessive nutrients loads (Alaerts *et al*., 1996; El-Kheir *et al*., 2007). In the present study, significantly low concentrations of nitrate, ammonium and phosphate were recorded in T1 and T3 (duckweed treated ponds) as compared to T2 and T4 (without duckweed). This might be due to absorption of these nutrients by duckweed. This assumption is strongly supported by the previous reports (Bergmann *et al*., 2000; Cheng *et al*., 2002; Janjit *et al*., 2007; Azeez and Sabbar, 2012) that duckweeds have shown their efficiency in absorbing nutrients from nutrients rich water systems. Ferdoushi *et al*. (2008) reported that aquatic macrophytes (*Lemna* sp. and *Azolla* sp.) appeared as a nutrient filter for absorption of nitrogen and phosphorus, and removed the excessive nutrients from the water body. Perniel *et al*. (1998) reported that *Lemna* consistently removed the largest amount of ammonia and phosphorus from eutrophic storm water. Again, Oron *et al*. (1988) reported that duckweed has a high rate of nutrient uptake and preferentially takes up ammonium ions. According to aforementioned previous reports and the result of the present study, it can be stated that use of duckweed in euglenophytes bloom ponds reduced excess nutrients and maintained its concentrations within more or less acceptable ranges for pond productivity.

3.5.1.5. Heavy metals

Aquatic macrophytes found to be the potential scavengers of heavy metals from aquatic environment and are being used in wastewater renovation systems (Abbasi and Ramasami, 1999; Kadlec *et al*., 2000). In the present study, the concentrations of Fe, Zn, Mn and Cu in T1 and T3 (duckweed treated ponds) were significantly low as compared to T2 and T4 (without duckweed) which might be due to absorption of these heavy metals by duckweeds. Supporting evidence to this assumption can be drawn from some previous studies (El-Kheir *et al*., 2007; Christian *et al*., 2012; Iram *et al*., 2012) which reported that duckweed can be used to effectively remove heavy metals from eutrophic water system. Azeez and Sabbar (2012) stated that duckweed can be used for pollutants removal and it has efficiency in improving the water quality by reducing heavy metals, nitrate and phosphate. This statement is strongly supportive to the present study. Moreover, use of lime might contributed to decrease exchangeable heavy metals ions from water as confirmed by relatively lower concentrations of heavy metals in lime treated ponds (T2) than the control ponds (T4). Sipauba-Tavares *et al*. (2003) reported that liming treatment reduced heavy metals in the pond sediment and water. This report is also supportive to the present results.

3.5.2. Planktonic algal community and density

3.5.2.1. Algal community

Planktonic algal community structure is regulated by environmental factors, growth rate of algal species and specific rate of loss attributed to grazing, sedimentation and dilution (Fogg, 1975). The result of the present study showed that the number of algal genera in T4 were relatively low as compared to T1, T2 and T3. This might be due to the variation in ambient environmental factors as confirmed by the results of water quality parameters of the study ponds (Table 3.2). This assumption is consistent with the findings of Reynolds *et al*. (2000)

who reported that aquatic environment is subject to high temporal variation with frequent reorganization of algal communities, as a result of interaction among physical, chemical and biological factors. The variation in number of algal genera in the study ponds indicated that the use of duckweed and lime had positive bearing on algal communities whereas the bloom of euglenophytes had negative effects. On the basis of genera number, chlorophytes was the most dominant group of algae and euglenophytes was the least dominant group in all the ponds under four treatments. This result is consistent with the findings of Wahab *et al*. (1995) and Dewan *et al*. (1991) who recorded chlorophytes as the dominant group and euglenophytes as the least dominant group of planktonic algae in the aquaculture pond.

3.5.2.2. Density of total planktonic algae

Algal densities differ in water bodies depending on environmental factors, availability of nutrients and grazing (Rhee and Gotham, 1981a; Riegman *et al*., 1993). In the present investigation, significantly higher density of total planktonic algae was recorded in T4 (Control ponds) and the lower density was in T3 (Duckweed and lime treated ponds). Higher density of total planktonic algae in T4 might be due to higher organic mater in bottom soil and dissolved inorganic nutrients in water. This assumption is supported by the findings of Quader (1997) and Rosy *et al*. (1998) who reported that higher soil organic matter in ponds provides higher nutrients in water especially nitrogen and phosphorus that enhanced the growth of planktonic algae. On the other hand, relatively lower organic matter and dissolved inorganic nutrients in T1, T2 and T3 provide relatively lower density total planktonic algae. Therefore, the results of the present study indicated that use of duckweed and lime in separate or in combination had considerable effects on the density of total planktonic algae.

3.5.2.3. Density of euglenophytes

The growth and proliferation of euglenophytes algae in fish ponds depend on the combination of a set of environmental factors (Rahman *et al*., 2007). The results of the present study showed that density and percent contribution of euglenophytes algae were significantly high in T4 (control ponds) followed by T1 and T2 whereas the density and percent contribution of these algae were significantly low in T3 (Table 3.4. and Figure 3.10). According to previous reports, the density of euglenophytes increased in acidic pH (Zakrys and Walne, 1994; Olaveson and Nalewajko, 2000) with higher nutrients (Nwanknwo, 1995; Kim and Boo, 2001; Rahman *et al*., 2007) and heavy metal concentrations (Hutchinson and Nakatsu, 1984; Duttagupta *et al*., 2004). Previous reports are agreed the results of the present study in case of control ponds (T4).

Lower density of euglenophytes in T3 might be due to lower concentrations of nutrients and heavy metals, and alkaline pH (>7.0) which might reduce the proliferation of euglenophytes algae. This speculation is supported by the previous studies (Duttagupta *et al*., 2004; Rahman *et al*., 2007) which stated that decreasing concentration of nutrients and heavy metals, and alkaline pH collapsed the luxurious growth of euglenophytes. The use of duckweed and lime in T3 contributed to reduce nutrients and heavy metals concentrations and to increase pH value as confirmed by the results of water quality parameters (Table 3.2 and Figure 3.4). Moreover, duckweeds might be disturbed the ratio of nitrogen to phosphorous through ammonium reduction which also contributed to reduce euglenophytes density.

In the present study, it was observed that nutrients and heavy metals concentrations in T1 were relatively low due to use of duckweed, but relatively lower pH values in that treatment might increase density of euglenophytes than that of T3. Whereas in T2, pH values were relatively high (>7.0) due to use of lime, but relatively higher nutrients and heavy metal concentrations in that

treatment might also increase density of these algae than that of T3. These results revealed that use of duckweed and lime (in combination) was better to reduce euglenophytes density as compared to use of duckweed and lime separately.

However, the result of the present study indicated that use of both duckweed and lime in euglenophytes bloom ponds improved water quality particularly by reducing nutrients and heavy metals, and by increasing pH value which are responsible for reduction of euglenophytes density.

2.5.2.4. Density of other algae

In the present study, the mean density of cyanophytes did not show any significant difference among the treatments but the mean density and percent contributions of these algae were relatively high in T1, T2 and T3 as compared to T4 (Figure 3.9 and 3.10). The results of this study also showed that mean density and percent contribution of chlorophytes and bacillariophytes were significantly high in T1, T2 and T3 as compared to T4 (Figure 3.9 and 3.10). Higher density of cyanophytes, chlorophytes and bacillariophytes in T1, T2 and T3 might be due to suitable environmental factors for their growth. Whereas, lower density of these algae occurred in T4 might due to euglenophytes bloom and unfavourable environmental conditions. Rahman *et al*. (2007) reported that bloom of euglenophytes reduced the density of chlorophytes and bacillariophytes. Again, Hosmani (1988) stated that the blooms of *Euglena elastica, E. gracilis* and *Trachelomonas charkoweinis* have a significant effect in reducing the number of other algal species in fish ponds. These reports are supportive to the present study. Therefore, the results of the present study indicated that use duckweed and lime had positive bearing on the density of cyanophytes, chlorophytes and bacillariophytes whereas euglenophytes bloom had negative effects.

3.5.3. Growth performance

The growth rate of fish is controlled by a variety of factors of which water quality, nutrients, algal bloom, culture technique and genetic condition are the most important. In the present study, growth performance of the fishes in terms of weight gain, daily weight gain and SGR were relatively higher in T1, T2 and T3 as compared to T4. But, the highest growth was recorded in T3 than other treatments. This might be due to the use of both duckweed and lime which provide better water quality as confirmed by the results of water quality parameters in that treatment (Table 3.2). Several references indicated that duckweeds improve water quality by reducing nutrients (Cheng *et al*., 2002; Janjit *et al*., 2007) and heavy metals (Obek, 2009; Christian *et al*. 2012;) whereas lime improves water quality by raising pH in acidic water (Boyd, 1990; Ivahnenko *et al*., 1988). In addition, duckweed enhanced degradation of organic matter through additional oxygen supply in water (Korner *et al*., 2003) and lime reduced organic matters in sediment (Sipauba-Tavares *et al*., 2003) which might be helpful for fish growth.

More to the point, duckweed and lime reduced the density of euglenophytes by absorbing nutrients and heavy metals, and by increasing pH values which might contribute to better growth performances of fishes as dense bloom of euglenophytes hampered growth (Xaver *et al*., 1991; Rahman *et al*., 2007). In addition, duckweed might also be enhanced the growth of fish through use as fish food. There is strong supporting evidence that duckweed enhanced the growth of fish in polyculture system (Wahab *et al*., 1995; Skillicorn *et al*., 1993).

Significantly lower growth performances and survival rate of the experimental fishes were recorded in T4 (without duckweed or lime) which might be due to the dense bloom of euglenophytes algae, lower dissolved oxygen and acidic pH. Rahman *el al*. (2007) stated that euglenophytes form dense bloom in acidic

pH and nutrients rich environment caused dissolved oxygen depletion which hampered the growth of beneficial algae and fish. This statement is supportive to the present results. Water having dissolved oxygen below 5.0 mg/l is to be unproductive (Banerjee, 1967; Swingle, 1969) and neutral or almost alkaline waters are the most important for fish growth when pH 7.0 to 8.00 (Huet, 1973). Blooms make a problem with dissolved oxygen deficiency which greatly hampered the normal growth of fish. Furthering, acidic pH is conducive to the bloom of euglenophytes whereas acidic pH is unfavourable for the growth of fish.

3.5.4. Grazing of euglenophytes

Filter-feeding fish are selective phytoplankton grazers that can suppress phytoplankton directly through ingestion (Drenner *et al*., 1987). In the present study, grazing of euglenophytes was investigated through analysis of gut contents and electivity index. According to the results of gut contents, it was observed that the proportion of euglenophytes algae in the gut contents of the fishes in four treatments did not show any significant difference ($P<0.5$). But, relatively higher proportions of euglenophytes were recorded in the gut contents of silver carp and silver barb whereas relatively less proportions of these algae were recorded in the gut content of rohu, catla and mrigel. Similar results have been reported by Rahman *et al*. (2005, 2007).

In the present study, the experimental fish species (rohu, catla, mrigel, silver carp and silver barb) showed negative electivity to euglenophytes. Rahman *et al*. (2005) stated that rohu, catla and mrigel showed negative electivity to euglenophytes while silver carp and silver barb showed positive electivity to these algae. This report is partially supportive to the present results. Sarker (1992) reported that rohu showed a little or neutral electivity to euglenophytes. Ahmed (1993) reported that catla showed positive selection for these algae. These reports are not agreed with the present results.

Previous reports (Haider, 1996; Mondol, 2000) indicated that euglenophytes are less preferred food for silver barb which agreed the present study. In a different study, Rahman *et al*. (2005) reported that silver carp and silver barb preferred euglenophytes as their food item. Quadir (1997) also stated that silver barb showed positive electivity to this group of algae. These reports are controversial to the present results. The controversial results for feeding preference of fish species reported by different studies might be due to changes in the feeding activity with change in the seasons (Mirza, 1984) and also to shift in the electivity index in different species combinations considering the extent of intra and inter specific competitions of fish (Wahab *et al*., 1991 and 1992).

The results of electivity index proved that euglenophytes algae were not favourable food item and feeding preferences of the fishes were not influenced by the density of these algae in the study ponds. The results of the gut contents and electivity index of the fishes and the higher density of euglenophytes in T4 revealed that introduction of filter-feeding fish species in the euglenophytes bloom ponds had no significant effects in controlling their bloom. Nonetheless, relatively higher proportion of euglenophytes algae in the gut contents of silver carp and silver barb indicated that grazing by silver carp and silver barb had some contribution in controlling the bloom of these algae. Datta and Jana (1998), Jana and Datta (2000) and Ke *et al*. (2009) reported that silver carp is suitable for cleaning cyanobacterial bloom. In a different study, Zhou *et al*. (2011) reported contrasting findings. In fact, the use of filter-feeding fish species to reduce algal biomass in lakes and reservoirs is still controversial (Domaizon and Devaux 1999b; Radke and Kahl 2002).

3.6. Conclusion

The results of the presents study revealed that the use of duckweed and lime had a positive bearing to improve water quality and to decrease density of euglenophytes algae by reducing nutrients, heavy metals and organic matter, and by increasing pH value in the euglenophytes bloom ponds. Duckweed might be acted as a biofilter by which nutrients and heavy metal concentrations reduced. On other hand, lime might be acted by increasing pH value through decreasing carbon dioxide from water and by reducing organic matters in the sediment of the pond. The overall study concluded that the use of both duckweed and lime are better for managing euglenophytes bloom as well as for increasing growth of fish and filter feeder fish species are not suitable for controlling euglenophytes bloom in eutrophic fish ponds. Further long-term studies are necessary to examine the ecological consequences of the management measures applied into euglenophytes bloom infested water bodies.

CHAPTER FOUR

Effects of Euglenophytes Algae Supplemented Feed on the Growth and Carcass Compositions of Common Carp (*Cyprinus carpio* L.)

Chapter Four

EFFECTS OF EUGLENOPHYTES ALGAE SUPPLEMENTED FEED ON THE GROWTH AND CARCASS COMPOSITIONS OF COMMON CARP (*Cyprinus carpio* L.)

4.1. Introduction

Fish has long been used as the cheapest source of protein for human nutrition worldwide – still with a gap in production and supply (Tidwell and Allan, 2001). Hence, accelerating the development of aquaculture industry is one of the important factors to fulfill the protein demand for increasing world's population. Alongside, aquaculture success depends on quality and quantity of water (Summerfelt, 2000). Bangladesh is a semiarid region; water quality is determines to success fish culture. Moreover, the presence of quality feed with low cost is one of the important factors for successful aquaculture (Cho and Slinger, 1979).

Traditionally protein sources used in fish feed include: fishmeal, mustard oil cake meal and soybean meal. Since many years ago, fishmeal is used as a protein sources in the aquaculture, poultry and pigs production (Hardy and Tacon, 2002). Recently, in many countries government constraints protecting against over-fishing because of overthrowing, thus, availability of fishmeal is decreasing and its price is increased. In terms of economy and availability, the first feed stuff to rather than fishmeal in aquaculture feed is mustard oil cake meal or soybean meal. Although they offer considerable nutritional potential, but they are also associated with negative qualities such as less than ideal amino acid balance and the presence of anti-nutritional factors (Tacon, 1997; Francis *et al*., 2001).

Because of uncertain availability and high cost of fishmeal, and the presence of antinational factors in mustard oil cake and soybean meal, the farmers are now compelled to search for cheaper alternative protein sources of either animal or plant origin (Higgs *et al*., 1995; Hardy and Tacon, 2002). Therefore, the potential use of unconventional feed stuffs such as algae, a fish feed input as a replacement of high cost feed stuffs such as fishmeal has been increasing.

Interestingly algae have been proved to be one of the important feed sources and feed additives in the commercial rearing of many fishes and penaeid prawn (Belay *et al*., 1996; Borowitzka, 1997; Khatoon *et al*., 2010a). A number of studies have assessed the nutritive value of microalgae as feed ingredient for fish and crustacean (Navarro and Sarasquete, 1998; Khatoon *et al*., 2009; Ungsethaphand *et al*., 2010). According to various references (Broun, 1980; Mustafa *et al*., 1994b; Ibrahim *et al*., 2007; Tartiel *et al*., 2008), growth performances have improved in fish fed diets containing algae cells. Zeinhom (2004) found that fish fed diet containing 15% algae increased the digestibility coefficient of dry matter (92.5%), crude protein (87.63%), lipid (88.45%) and energy (81.41%). Recently, attentions are giving to the nutritional value of algae as a potential substitute of protein and other ingredients in fish feed such as: alginates (Sorensen and Denstadli, 2008), fatty acids (Atalah *et al*., 2007) and feeding stimulants (Dworjanyn *et al*., 2007).

Because of the incredible impact of the algal meals on the growth of fish, a number of algae have been verified over time but less than forty genera have gained widespread use in aquaculture. Algae like *Skeletonema, Chaetoceros*, *Pavlova*, *Isochrysis*, *Tetraselmis* etc. are frequently used in aquaculture feeds due to high content of poly unsaturated fatty acids such as Docosahexanoic acid and Eicosapentanoic acid (Wang, 2003; Knuckey *et al*., 2005). Other algae like *Spirulina*, *Chlorella*, *Scenedesmus, Dunaliella* and *Nannochloropsis* are also widely used in aquaculture feeds for their high nutritional value (Avron and

Ben-Amotz, 1992; Lee, 1997; Yamaguchi, 1997). *Spirulina*, *Dunaliella*, *Haematococcus* are also used as good sources of anti-oxidant pigments like carotenoids, lutein, astaxanthin, zeaxanthin etc. in fish farming mainly for coloured fishes (Chiu *et al*., 2001; Hanaa *et al*., 2003).

But, most of the reports are available on use of single algal genus as fish feed ingredients, therefore availability of algal biomass may be the major constrain in using them (Appler and Jauncey, 1983; Guroy *et al*., 2007; Azaza *et al*., 2008). Moreover, the production costs of these micro-algae are quite expensive making them almost unaffordable in developing country like Bangladesh and others. Therefore, exploration of local available algae for aquaculture usage is much more relevant for useful cheap production of aquaculture feed.

Euglenophytes algae are common in eutrophic fish pond of Bangladesh. They form thick red scum on the surface of the fish pond responsible for water quality problems and hampered growth of fish (Rahman *et al*., 2005, 2007). But, a number of studies reported that the cells of euglenophytes algae contained quality protein, polyunsaturated fatty acids and vitamins that can be used to improve dietary value of fish feed. In a study, Becker (1994) observed that *Euglena* cells contained 39-61% protein, 14-18% carbohydrate and 14-20% lipid. Nakano *et al*. (1987) reported that the outer membrane of *Euglena* cells contains about 60% protein and it is readily digested by ordinary digestive enzymes. Hayashi *et al*. (1993a) stated that 13.25% *Euglena* cells in a casein diet improved growth and feed conversion efficiency of prawn juvenile and it contained growth promoting factors in addition to essential amino acids. The dietary value of rotifers and Artemia to red-sea bream can be effectively improved by enrichment with *Euglena* (Hayashi *et al*., 1993b).

Continuously increasing demand for fish feed, pressures the consideration of every possible natural resource as potential ingredient in fish feed. Therefore,

the locally available algae, euglenophytes can be considered as an alternative, economically viable and nutritive feed ingredient. In Bangladesh, most of the fish farmers use the mixture of rice bran and mustard oil cake (conventional fish feed) for carp culture together with natural fauna of the pond. For utilization of euglenophytes algae as a fish feed ingredient, supplementation of euglenophytes algae in the conventional feed can be tested using common carp (*Cyprinus carpio*) as a model fish. It is an omnivorous fish and can utilize feed including plant materials effectively.

However, the nutritive value of different algae and the effects of various algal meals on the growth and carcass composition have been examined for several species of fish in different parts of the world (Mustafa *et al*., 1995; Olvera-Novoa *et al*., 1998; Nandeesha *et al*., 2001; Tongsiri *et al*., 2010; Ahmadzadenia *et al*., 2011). But, researches on the utilization of euglenophytes algae as a fish feed ingredient and its effects on the growth and carcass composition of fish are scare in Bangladesh. Therefore, the present study was planned to investigate the effects of euglenophytes algae supplemented feed on the growth and carcass compositions of common carp with a view to the following specific objectives:

1. To determine the nutritive value of euglenophytes algae and the euglenophytes algae supplemented feeds.
2. To study the effects of euglenophytes algae supplemented feeds on the growth of common carp.
3. To study the effects of euglenophytes algae supplemented feeds on the carcass compositions of common carp.
4. To recommend the optimum dietary inclusion levels of euglenophytes algae in the diet for better growth and carcass of common carp.

4.2. Review of literatures

There are many published reports on the nutritive value of different algae and the effects of algal meal on the growth and carcass composition of various fish species in different parts of the world. But, very little reports are available on the nutritive value of euglenophytes algae and their effects on the growth and carcass composition of fish. However, some of the research findings relevant to the present study are reviewed below.

Kim *et al.* (2013) conducted 8-week feeding trial to investigate the effects of fish meal (FM) replacement with *Spirulina* on growth performance and body composition of parrot fish. They formulated four diets to replace FM with 0 (as control), 5, 10 and 15% *Spirulina* (designated as Con, S5, S10 and S15, respectively). They recorded significantly ($P<0.05$) higher weight gain, protein efficiency ratio and feed intake in fish fed S5 diet compared to other diets. They also recorded significantly ($P<0.05$) higher muscle protein and lower whole-body lipid in fish fed with S15 diet than control diet. The results of their study concluded that *Spirulina* can replace up to 15% FM protein diets for parrot fish.

Wassef *et al.* (2013) carried out a study to evaluate the suitability of two marine macroalgae, *Ulva lactuca* (Chlorophyta) and *Pterocladia capillacea* (Rhodophyta), meals as a supplement to enhance the nutritional value of formulated feeds for European sea bass (*Dicentrarchus labrax*) fry. Seven diets containing four levels (0 or control, 5, 10, and 15 %) of either *Ulva* meal (UM) or *Pterocladia* meal (PM) were tested in this study. The results of their study indicated that feeding seabass at 5 % UM or PM level (U_5 and P_5 diets) produced the best growth, feed utilization, nutrient retention and survival rates among all the dietary groups. Their findings suggested that both *Ulva* and *Pterocladia* meals could be potentially used as an additional feed component (at 5 %) for enhancement of sea bass fry performance and nutrients composition.

Saroch *et al.* (2012) carried out an experiment to evaluate the influence of *Spirulina* impregnated feeds on the growth of catla (*Catla catla*) for 60-days feeding trial. They formulated diets in which fish meal protein replaced with *Spirulina* at 5, 10 and 15% levels. They recorded significant difference in the final weight attained by catla at all levels of *Spirulina* incorporation as compared to the control diet and the replacement of fish meal with 5% *Spirulina* resulted in significantly superior growth. Specific growth rate and protein efficiency ratio improved with higher levels of *Spirulina* inclusion in comparison to control feed. They concluded that the usefulness of *Spirulina* for partial or complete replacement of fish meal in the diets of culturable fishes proves cost effective.

Sirakov *et al.* (2012) investigated the effect of algae meal (*Spirulina* sp.) on the growth performance and carcass parameters of rainbow trout (*Oncorhynchus mykiss*) for 35 days feeding trail. For this trial, they formulated an experimental diet – consisting of 10% *Spirulina* meal + basal diets (10% SD) and a control diet – a basal diet (BD) with 0% algae. They recorded higher weight gain, condition factor and average daily growth of the fish group fed with 10% SD than the fish group fed with control diet. They also recorded better weight of eviscerated fish, consumable yield and carcass weight of fish fed with experimental feed compared with the carcass parameters of fish fed with BD.

Ahmadzadenia *et al.* (2011) undertook an experiment to study the replacing of soybean meal with *Spirulina* on growth and chemical composition of carcass in rainbow trout for 50 days feeding trail. They formulated experimental diet by replacing soybean meal with *Spirulina* at 0% (T1, control meal), 20% (T2), 40% (T3), 60% (T4): and 80% (T5). They reported that replacing different levels of *Spirulina* significantly increased body length, mean weight, crude protein and ash, and decreased crude fiber in fish compare to control meal ($P<0.01$).

Mukherjee *et al.* (2011) performed a 12-week laboratory feeding trial to evaluate the efficacy of two different algae based feeds (one containing *Spirulina platensis* and *Enteromorpha intestinalis* and other containing *Phormidium valderianum* and *Catenella repens*) against conventional feed (rice bran and mustard oil cake) for fingerlings of Indian major carp, Rohu (*Labeo rohita*). They evaluated the diets on the basis of feed intake rate, body weight gain, feed conversion ratio, specific growth rate, metabolic growth rate, protein productive value, protein efficiency ratio, muscle protein, lipid, carotenoid, ash and accumulated muscle glycogen. They reported that the algal feed 2 (*Phormidium valderianum* and *Catenella repens*) was more suitable diet than the other two feeds for *L. rohita* fingerling.

Roy *et al.* (2011) conducted a study on the use of composite algal mix as feed supplement in nutrition of *Oreochromis mossambicus* and its effect on growth performance, feed efficiency, nutrient utilization and body composition. During this study, three diets containing 0 % (conventional feed, CF), 35% (value added feed, VAF) and 100% (algal feed, AF) algal supplementation were used in combination with other conventional fish feed ingredients. They recorded increase growth performance ($P<0.05$), feed efficiency and nutrient utilization and decrease carcass lipid in fish fed with VAF (35% supplementation level) as compared to 0 % and 100% supplemented diets. The results of their study suggested that 35 % supplementation of conventional feed with composite algal mix can be used in tilapia diet.

El-Tawil (2010) studied the effects of green seaweeds (*Ulva* sp.) as feed supplements in red tilapia (*Oreochromis* sp.) diet on growth performance, feed utilization and body composition. Six diets with different levels of *Ulva* sp. (0, 5, 10, 15, 20 and 25% of fish diet) were used in this study. The results of this study showed that final weight, weight gain and specific growth rate increased significantly ($P<0.05$) with increasing *Ulva* level in fish diet up to 15%.

Significant (P<0.05) values of feed conversion ratio were recorded in fish fed diet with 20% *Ulva* (1.49) followed by diets with 15 and 10 % *Ulva* (1.52 and 1.53 respectively). Fish fed diet containing 10% *Ulva* got the highest lipid content. Carcass protein concentration increased significantly (P<0.05) with increasing *Ulva* level in the diet and the highest value was maintained at fish fed the diet containing 25% *Ulva*. Feed utilization parameters were affected significantly by different *Ulva* level in the diet. In conclusion, he stated that green seaweeds (*Ulva* sp.) could be supplemented to red tilapia diet at optimum level of 15% to improve growth performance.

Tongsiri *et al.* (2010) carried out a study on the effect of replacing fishmeal with *Spirulina* on growth, carcass composition and pigment of the Mekong Giant Catfish. In their study, four diets were formulated in which fishmeal replaced with *Spirulina* at 0, 15, 30 and 100% (contained 18.81 to 20.71% protein, 10.85 to 12.03% lipid, 40.59 to 46.01% carbohydrate, 8.42 to 11.41% ash and 9.38 to 12.93% moisture). The results of their study showed that average daily gain, specific growth rate and feed conversion rate were not significantly different. The total biomass increase in fish fed with 0% *Spirulina* was significantly lower than fish fed with 15, 30 and 100% *Spirulina* (p<0.05).

Ungsethaphand *et al.* (2010) studied the effect of feeding *Spirulina platensis* on growth and carcass composition of hybrid red tilapia (*Oreochromis mossambicus* × *O. niloticus*). They incorporated *S. platensis* into the fishmeal-based diet at 0, 5, 10 and 20%. After trail they observed that the final weight gain, specific growth rate, feed conversion ratio and survival rate of fish were not affected by *Spirulina* supplementation (P>0.05). They also observed no significant difference (p>0.05) in carcass composition of the fish fed on *Spirulina* diets as compared to those on control diet. They suggested that up to 20% of *Spirulina* can be substituted for fishmeal in a fishmeal-based diet for hybrid red tilapia without any adverse effect on fish growth.

Ergun *et al.* (2009) conducted a nutritional trial to investigate the effects of dietary lipid levels and supplemental *Ulva* meal on growth performance, feed efficiency, nutrient utilization and body composition of juvenile Nile tilapia, *Oreochromis niloticus*. During this study, four diets containing 0% and 5% *Ulva* meal, and 10% (low-lipid; LL) and 20% (high-lipid; HL) crude lipid were formulated. The results of their study showed that fish fed 5% *Ulva* meal increased growth performance ($P<0.05$) compared with fish fed control diets, irrespective of dietary lipid level. The incorporation of *Ulva* meal improved specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER). Significantly lower carcass lipid found in fish fed 5% *Ulva* meal. The results of their study indicated that 5% inclusion of *Ulva* meal improves growth performance, feed efficiency, nutrient utilization and body composition of Nile tilapia.

Soler-Vila *et al.* (2009) conducted a study on the effects of red alga *Porphyra dioica* as a fish-feed ingredient on the growth, feed efficiency and carcass composition of rainbow trout (*Oncorhynchus mykiss*). They added *P. dioica* meal at 5, 10 and 15% to a diet whereas the control diet was without seaweed meal. The results of their study showed that seaweed meal inclusion did not affect significantly weight gain, specific growth rate, feed efficiency and protein efficiency ratio for any of the diets. Voluntary feed intake increased for all seaweed diets compared to the control diet. Significantly higher carcass protein content increased for the diet with 10% seaweed inclusion. The results of their study suggested that up to 10% *P. dioi*ca can be included in diets for rainbow trout without negative effects on growth performance.

Abdel-Tawwab *et al.* (2008) studied the use of *Spirulina* (*Arthrospir platensis*) as a growth promoter for Nile tilapia fry, *Oreochromis niloticus* for 12 weeks trail. They formulated diets containing 0.0, 1.25, 2.5, 5.0, 7.5, or 10.0 g *Spirulina*/kg diet. They recorded optimum growth and feed utilization at 5.0 g

Spirulina/kg diet. They reported that *Spirulina* supplementation increased protein deposition in fish body and no significant effects on carcass lipid and ash contents.

Tartiel *et al.* (2008) studied the effect of algae diets on growth performance, feed efficiency and body composition of Nile tilapia (*Oreochromis niloticus*) for 90 days trail. They formulated diets which contain *Chlorella* and *Scenedesmus* as fish meal replacers at zero (control), 10, 25, 50 and 75% substitution. They found significantly higher growth performance, feed conversion ratio and protein productive value in fish fed diets containing 50% of both algae ($P<0.05$). whereas fish fed diets containing 75% algae had significance lower performance ($P<0.05$). They recorded significantly higher amount of dry matter and crude protein, but lower lipid in carcass composition of fish which fed algae at 50% compared to other treatments ($P<0.05$). They concluded that dried *Chlorella* spp. and *Scenedesmus* spp. could be replaced fish meal up to 50% substitution level in Nile tilapia diets.

Ibrahim *et al.* (2007) studied the effects of *Ulva rigida* on the growth, feed intake and body composition of common carp (*Cyprinus carpio*). They formulated five diets by supplementation of *Ulva* meal into wheat meal at 0, 5, 10, 15 and 20%. Growth performances of fish were evaluated in terms of survival, final weight, percent weight gain, SGR, FCR, PER and body composition. They recorded poorest growth performance of the fish fed the diet with 20% *Ulva* meal supplementation and the best growth performance of the fish group fed with 5% *Ulva* meal supplementation ($P<0.05$). Their results suggested that the dietary *Ulva* meal inclusion of 5 to 15% replacing wheat meal in carp diets could be acceptable.

Guroy *et al.* (2007) conducted a 12-week feeding trial to evaluate the effect of 2 algae meals (*Ulva rigida* or *Cystoseira barbata*) on feed intake, growth and nutrient utilization of Nile tilapia, *Oreochromis niloticus*. They formulated diets

by supplementation of *Ulva* meal (5%, 10%, or 15%) or *Cystoseira* meal (5%, 10%, or 15%) and a diet without algae meal served as a control diet. They recorded highest weight gain for fish fed 5% *Cystoseira* diet and 5% *Ulva* diet compared to other treatments, except for the fish fed on 15% *Ulva* diet ($P<0.05$) which exhibited the lowest weight gain, poorest feed conversion ratio and decreasing tended of protein and energy utilization. They also observed that carcass lipid levels decreased with increasing levels of *Ulva* meal, while an increase in carcass lipid level with increasing levels of *Cystoseira* meal ($P<0.05$). The results of their study suggested that *Ulva rigida* or *Cystoseira barbata* meals could be used in small percentages in Nile tilapia diets.

Nandeesha *et al.* (2001) investigated the growth performances of two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels of *Spirulina platensis* for 90 days culture trail. They formulated diets by replacing fishmeal with *Spirulina* at 25, 50, 75 and 100% levels. They recorded no significant difference in the final weight attained by catla at all levels of *Spirulina* incorporation as compared to the fish-meal-based control diets but the replacement of fishmeal with more than 25% *Spirulina* resulted in significant superior growth of rohu. They also recorded improved specific growth rate and protein efficiency ratio in rohu with higher levels of *Spirulina* inclusion; while in catla they did not observe significant difference from the control diet. They found an inverse relationship between protein and fat deposition. They concluded that usefulness of *Spirulina* for partial or complete replacement of fishmeal in the diets of catla and rohu proves cost effective.

Nandeesha *et al.* (1998) assessed the effect of feeding *Spirulina platensis* on the growth, proximate composition and organoleptic quality of common carp, *Cyprinus carpio* for 120 days culture trial. In this study, fishmeal in the diet replaced with *Spirulina* at 25, 50, 75 and 100%. They reported that final weight gain, specific growth rate, food conversion ratio and protein efficiency ratio of

common carp not affected by *Spirulina* supplementation. They also reported that no significant difference in carcass moisture and protein content in the fish fed *Spirulina* diets as compared to fish-meal-based control diet but carcass ash and fat contents positively and negatively correlated with dietary *Spirulina* level, respectively.

Olvera-Novoa *et al.* (1998) conducted 9-weeks feeding trail to evaluate the effects of microalga, *Spirulina maxima* in diets for tilapia fry. They replaced animal protein with algae protein at ratios of 20, 40, 60, 80 and 100%, and substitution effect was compared with a control diet in which fish meal was the sole protein. The results of their study showed that growth rate and protein utilization of fish fed the diet with 20% and 40% *Spirulina* ware not significantly different ($P<0.05$) from those fed the control diet but further increases in the algae protein significantly decreased the growth and feeding performance. They concluded that *Spirulina* diet did not provide any adverse effects on carcass composition and *Spirulina* can be replaced up to 40% of the fishmeal protein in tilapia diets.

Hayashi *et al.* (1993a) studied the supplemental effects of *Euglena* in a casein diet for *Penaeus japonicus*. They reported that supplementation with 13.25% *Euglena* cells in a casein diet improved growth and feed conversion efficiency of juveniles. They also reported that *Euglena* cells contain a growth promoting factor for the prawn in addition to essential amino acids.

Hayashi *et al.* (1993b) conducted a feeding experiment to evaluate the dietary value of living feed enriched with *Euglena*. The reported that Rotifers and Artemia enriched with *Euglena* contained much more DHA than those enriched with *Nannochloropsis* or methyl esters of n-3 HUFA. They concluded that the dietary value of rotifers and Artemia to red sea bream effectively improved by enrichment with *Euglena* and it is expected to be profitable feed for the DHA enrichment of rotifers and Artemia.

4.3. Methodology

The research tools and equipments, the methods for sample collection and analysis, and the methods for data collection and analysis used in the present study are described below.

4.3.1. Duration and location of the experiment

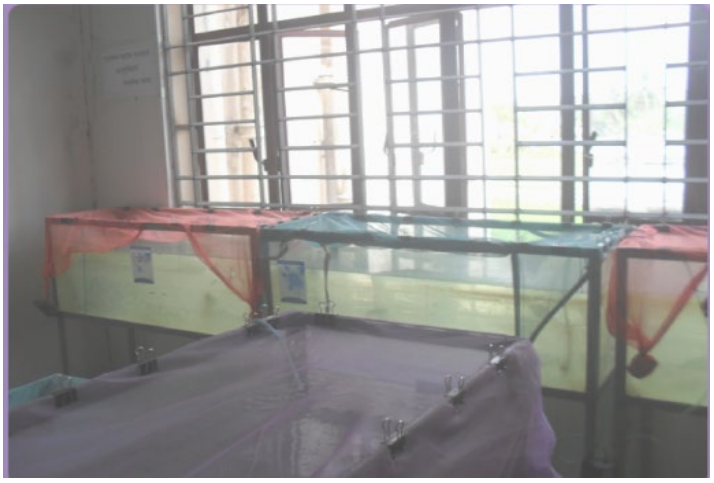
The experiment was conducted to investigate the supplementation of euglenophytes algae in the conventional fish feed and its effects on the growth and carcass compositions of common carp (*Cyprinus carpio*) for a period of 12 weeks feeding trail from August to October 2012 at the wet laboratory in the Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh.

4.3.2. Experimental unit

The experiment was carried out in twelve glass aquariums. The aquariums were rectangular in shape (90 × 30 × 30 cm) each of which contains 80 liters of water. The experimental unit provides continuous water supplying and water exchanging facilities. The aquariums were well aerated by the aerator (Model AC-980) and covered with fine net to avoid jumping of fish. The experimental unit is shown in Plate 4.1.

4.3.3. Collection of euglenophytes algae

In late autumn and early winter, euglenophytes algae as bloom are common event in fish culture ponds at Rajshahi district, Bangladesh. For collection of the bloom, some ponds were selected. To collect the bloom, at first it was gathered in a corner of the ponds by agitating water. The bloom was collected by using small mesh cloth and kept in a jar. Collected bloom was thoroughly cleaned with distilled water. After that it was dried in oven at 50.0 ^{o}C for 48-72 hours. After drying, it was grinded to powder and kept in a jar for nutritional analysis.



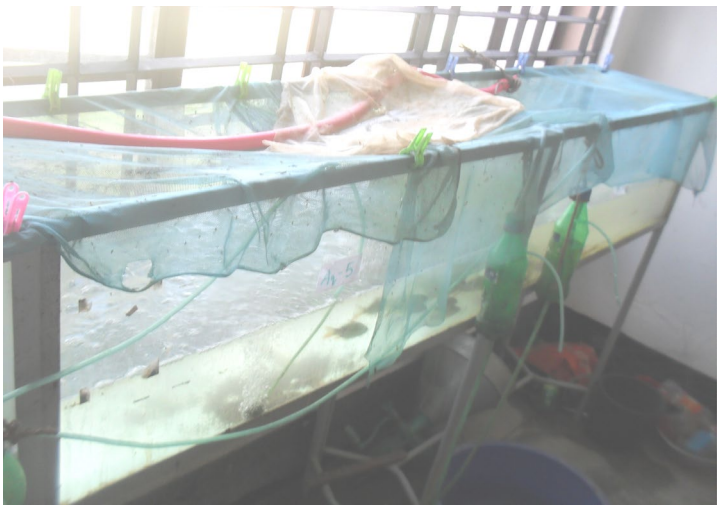


Plate 4.1: The experimental unit

4.3.4. Experimental feeds

Four types of feeds were prepared using conventional fish feed ingredients (Rice bran and mustard oil cake) and dried euglenophytes algae by following combinations. The conventional fish feed (mixture of rice bran and mustard oil cake in 1:1 ratio) was considered as control feed. Three euglenophytes supplemented feeds were prepared using dried euglenophytes algae at 20% 30% and 40% along with the control feed.

- **Feed-1:** The combination of feed containing 50 % rice bran and 50 % mustard oil cake (Control feed).
- **Feed-2:** The feed containing Feed-1 replaced with 20% dried euglenophytes algae.
- **Feed-3:** The feed containing Feed-1 replaced with 30% dried euglenophytes algae.
- **Feed-4:** The feed containing Feed-1 replaced with 40% dried euglenophytes algae.

The feeds were prepared on the basis of crude protein requirement of common carp (about 40%). The feed ingredients were mixed according to the combinations. The experimental feeds were prepared from the well mixed ingredients by using manually operated pellet machine. The photographs of feed ingredients and experimental feeds are shown in Plate 4.2 and 4.3.

4.3.5. Experimental design

The study was conducted in 12 glass aquariums under four treatments viz. Treatment-1(T1), Treatment-2 (T2), Treatment-3 (T3) Treatment-4 (T4) with three replicates in each treatment. T1 was assigned to the fish group fed with Feed-1 (Treated as control group), T2 was assigned to the fish group fed with Feed-2, T3 was assigned to the fish group fed with Feed-3 and T4 was assigned to the fish group fed with Feed-4. A lay out of the experimental design is shown in Table 4.1. The plan of works of this study is shown in Chart 4.1.

Rice bran	Mustard oil cake	Dried euglenophytes

Plate 4.2: The feed ingredients

Feed-1 (50% Rice bran and 50 % MOC)	Feed-2 (20% euglenophytes replacement)
Feed-3 (30% euglenophytes replacement)	Feed-4 (40% euglenophytes replacement)

Plate 4.3: The experimental feeds

Table 4.1: Layout of the experimental design

Treatment	Aquarium No.	Stocking density	Feeds
T1	A_1, A_2 and A_3	15 fishes/aquarium	Feed-1
T2	A_4, A_5 and A_6	Do	Feed-2
T3	A_7, A_8 and A_9	Do	Feed-3
T4	A_{10}, A_{11} and A_{12}	Do	Feed-4

4.3.6. Rearing of fish

Fingerlings of common carp (same aged group) were procured from local fish breeding farm in Rajshahi and were brought to the laboratory in the oxygen packed plastic container. The fishes were acclimatized in the laboratory condition for 7 days in glass aquaria. During acclimatization period, the fishes were fed with artificial feed. They were starved for 24 hrs prior to the onset of experiment. A total of 180 fish fingerlings were stocked at the rate of 15/aquarium. The fishes were fed daily (two times) at 5% of body weight throughout the study tenure. The water was replenished every day to avoid accumulation of unutilized feeds and metabolic wastes of the fish. The leftover feed materials were collected and dried in incubator at 60°C for 24 hrs. The dried materials were weighed to measure feed intake.

4.3.7. Monitoring of physico-chemical parameters

During the study period, some physico-chemical parameters viz., water temperature, dissolved oxygen (DO), pH, nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N) and phosphate-phosphorus (PO_4-P) were monitored weekly. Water temperature, DO and pH were measured by Celsius thermometer, DO meter (HANNA, model: HI-9142) and pH meter (HANNA, model: H I-9142), respectively. The NO_3-N, NH_4-N and PO_4-P were measured by using a HACH Kit (DR/2010 model).

4.3.8. Sampling and harvesting of fish

After successive intervals of three, six and nine weeks, body weights of experimental fish were measured using digital electric balance (Plate 4.4). After 12 weeks of feeding trail all fishes were harvested and the weights were measured. Five fishes were randomly selected from each experimental aquarium and decapitated to collect muscles for analyzing carcass composition. The fish carcass was dried at 60ºC and blended, kept in desiccators jar for subsequent study.

Plate 4.4: Measuring weight of fish

4.3.9. Analysis of growth and feed utilization

The growth performance and feed utilization in terms of mean weight gain (MWG), average daily weight gain (ADWG**),** specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR) and survival rate were analyzed by using standard methods.

4.3.9.1. Mean weight gain

Mean weight gain was computed by using the following equation

MWG (g) = Mean final weight (g) - Mean initial weight (g).

4.3.9.2. Average daily weight gain

Average daily weight gain was computed by subtracting the initial body weight from the final body weight and then divided by the number of rearing days. Average daily weight gain was computed by following formula.

ADWG (g day^{-1}) = {Mean final weight – Mean initial weight}/No. of days

4.3.9.3. Specific growth rate

Specific growth rate is the instantaneous change in weight calculated as the percentage increase in body weight per day over a given time interval. The SGR was computed by the following formula.

$$\textbf{SGR (\% bwd}^{-1}\textbf{)} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1} \times 100 \text{ (Brown, 1957)}$$

Where, W_1 = Initial body weight (g) at time T_1 (day)

W_2 = Initial body weight (g) at time T_2 (day)

3.3.9.4. Feed intake

Feed intake rate was computed by the following formula.

FI (g fish^{-1} day^{-1}) = ¼ DI x 100/ [(w_1+w_2)/2]/T

Where, DI is dry matter intake; w_1 and w_2 are start and final weights respectively and T is the feeding days.

3.3.9.5. Food conversion ratio

Food conversion ratio is the amount dry feed fed per unit of live weight gain. It was computed by the following formula.

$$\textbf{FCR} = \frac{\text{Feed fed (dry wt)}}{\text{Live weight gain}} \quad \text{(Castell and Tiews, 1980)}$$

3.3.9.6. Survival rate

Survival rate was calculated on the basis of total number of fishes during harvesting using the following formula.

Survival rate (%) = (Number of fishes harvested/Total number of fishes stocked) x 100

4.3.10. Chemical analysis

The chemical compositions in terms of protein, lipid, carbohydrate, ash and moisture content of dried euglenophytes algae, formulated feeds and carcass of fish were determined using standard methods at the Nutrition Laboratory, Department of Aquaculture, BAU, Mymensingh, Bangladesh.

4.3.10.1. Determination of protein

Protein content was determined by micro-Kjeldahl method. Percent crude protein content was calculated by the following formula.

$$\textbf{\% Nitrogen } (N_2) = \frac{\text{ml of titration} \times \text{strength of HCl (0.2N)} \times \text{mili equivalent of N}}{\text{Weight of the sample}} \times 100$$

Where, mili equivalent of Nitrogen (N_2) = 0.014

% Crude protein = % N_2 x 6.25 (animal source)

= % N_2 x 5.85 (plant source)

4.3.10.2. Determination of lipid

Lipid content was determined by ether extraction through Soxhlet method. The percent crude lipid was calculated by the following formula.

$$\textbf{\% Crude lipid} = \frac{\text{Weight of the lipid}}{\text{Weight of the sample}} \times 100$$

4.3.10.3. Determination of carbohydrate/glycogen

Carbohydrate content was determined according to the method of AOAC (1995). Percent carbohydrate was calculated by the following formula.

$$\textbf{\% Carbohydrate} = \frac{\text{Amount of Carbohydrates}}{\text{Weight of the feed}} \times 100$$

4.3.10.4. Determination of ash

Ash content was determined by burning the sample in muffle furnace at 550ºC for 12 hrs. Percent ash content was calculated by the following formula.

$$\textbf{\% Ash} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100$$

4.3.10.5. Determination of moisture

Moisture content was determined according to the method of AOAC (1995) by oven heated treatment at 105ºC for 24 hrs. Percent moisture content was calculated by the following formula:

$$\textbf{\% Moisture} = \frac{B - D}{C} \times 100$$

Where, B = Weight of crucible + Sample (g), D = Weight of crucible + Dry sample (g) and C = Weight of sample (g)

4.3.11. Statistical analysis

For statistical analysis of data, one way analysis of variance (ANOVA) was performed using computer software SPSS. Significance was assigned at the 0.05 level. The mean values were compared to see the significant difference from the DMRT (Duncan Multiple Range Test).

Plan of works for the Study on Effects of Euglenophytes Algae Supplemented Feed on the Growth and Carcass Compositions of Common Carp (*Cyprinus carpio* L.)

(3rd Experiment)

Activities	**Month (May to December 2012)**							
	May	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Review of literature collection								
Preparation of experimental unit								
Feed preparation								
Fish fingerling collection and stocking								
Regular feeding								
Monitoring of physico-chemical parameters								
Growth monitoring								
Harvesting of fish								
Analysis of carcass								
Data analysis								

Chart 4.1: Plan of works for the study on effects of euglenophytes algae supplemented feeds on the growth and carcass compositions of common carp (*Cyprinus carpio*)

4.4. Results

During the study period, nutritive values of euglenophytes algae and experimental feeds, physico-chemical parameters of water, growth performance and carcass compositions of common carp ware analyzed. The results of these parameters are presented below.

4.4.1. Nutritive value of euglenophytes algae

To determinate the nutritive value, the chemical compositions of dried euglenophytes algae (in terms of crude protein, crude lipid, total carbohydrate, moisture and ash) were analyzed and the results are summarized in Table 4.2. From the results of chemical analysis, it was observed that dried euglenophytes algae contained in average 49.64% crude protein, 14.40% crude lipid, 15.96% total carbohydrate, 9.29% moisture and 10.41% ash.

4.4.2. Nutritive value of experimental feeds

To determinate the nutritive value, chemical compositions of the experimental feeds were analyzed and the results are shown in Table 4.3. The variations in nutritive values of the experimental feeds are shown in Figure 4.1.

4.4.2.1. Crude protein

The crude protein contents in the experimental feeds were determined as 40.48, 42.60, 45.64 and 48.10% in Feed-1, Feed-2, Feed-3 and Feed-4, respectively. The maximum crude protein was found in Feed-4 (contained 40% euglenophytes algae) and the minimum in Feed-1(Control feed).

4.4.2.2. Crude lipid

The crude lipid contents in the experimental feeds were found to vary from 10.96 to 13.98 %. The maximum crude lipid was found in Feed-1 followed by Feed-2 and Feed-3 whereas in Feed-4, it was relatively low (Table 4.3).

Table 4.2: Chemical compositions of euglenophytes algae (% wet basis)

Component (%)				
Protein	Lipid	Carbohydrate	Moisture	Ash
49.64 ± 0.50 (49.25-50.21)	14.40 ± 0.20 (14.25-14.63)	15.96 ± 0.55 (15.32-16.30)	9.29 ± 0.24 (9.12-9.57)	10.41 ± 0.19 (10.29-10.62)

*Nutrient values are mean of triplicate determination.

Table 4.3: Chemical compositions of the experimental feeds (% wet basis)

Component (%)	Feed			
	Feed-1	Feed-2	Feed-3	Feed-4
Protein	40.48 ± 0.93^{d}	42.60 ± 1.63^{c}	45.64 ± 1.47^{b}	48.10 ± 1.75^{a}
Lipid	13.98 ± 0.61^{a}	12.65 ± 0.86^{b}	11.33 ± 0.54^{c}	10.96 ± 0.41^{c}
Carbohydrate	23.29 ± 1.13^{a}	21.12 ± 1.08^{b}	18.78 ± 1.24^{c}	16.57 ± 1.27^{d}
Ash	7.40 ± 0.49^{c}	8.15 ± 0.51^{b}	8.83 ± 0.58^{a}	9.03 ± 0.54^{a}
Moisture	11.65 ± 0.97^{a}	11.59 ± 0.53^{a}	11.36 ± 0.42^{a}	11.25 ± 0.76^{a}

- **Feed-1:** Containing 50 % rice bran and 50 % mustard oil cake (control feed), **Feed-2:** Containing Feed-1 replaced with 20% euglenophytes; **Feed-3:** Containing Feed-1 replaced with 30% euglenophytes; and **Feed-4:** Containing Feed-1 replaced with 40% euglenophytes.
- Values of nutrients are mean of triplicate determination. Values in the same row with different superscripts are significantly different ($P<0.05$).

4.4.2.3. Total carbohydrate

The total carbohydrate content was found higher in Feed-1 (23.29%) followed by Feed-2 (21.12%) and Feed-3 (18.78%) whereas in Feed-4, it was relatively low (16.57%).

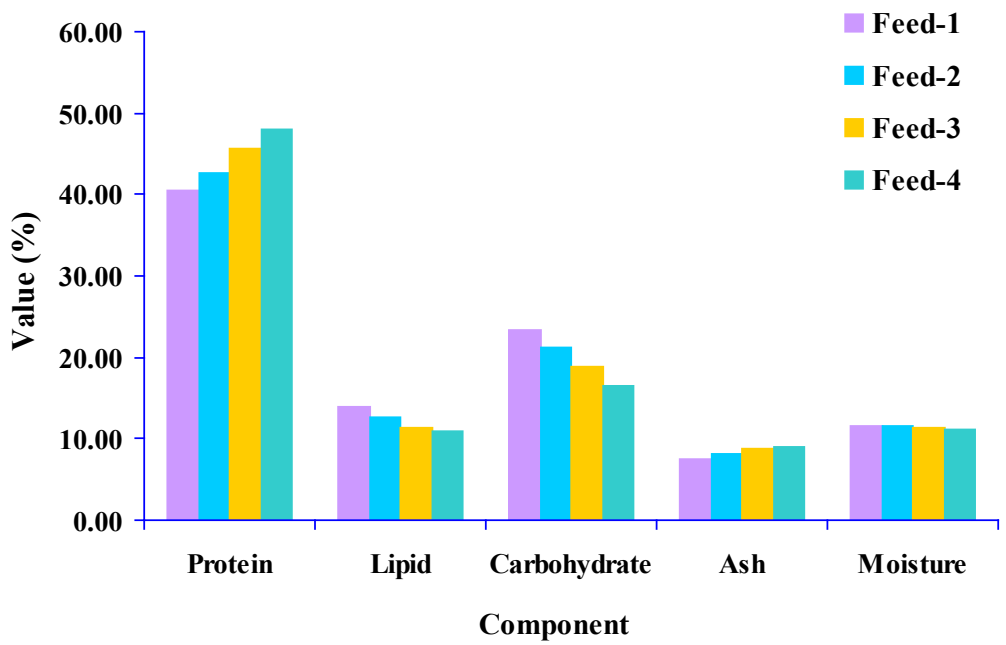


Figure 4.1: Variations in nutritive values of four experimental feeds

4.4.2.4. Ash

The ash contents in the experimental feeds were found to vary from 7.40 to 9.03%. Higher ash content was determined in Feed-4 and Feed-3 followed by Feed-2 whereas in Feed-1, it was relatively low (Table 4.3).

4.4.2.5. Moisture

There was no significant difference in moisture content of the experimental feeds but relatively higher moisture content was determined in Feed-1 and the lower in Feed-4 (Table 4.3).

4.4.3. Physico-chemical parameters of water

During the study period, water temperature, dissolved oxygen, pH and nutrients (nitrate-nitrogen, ammonium-nitrogen and phosphate-phosphorus) concentrations in the water of each experimental aquarium were analyzed weekly and the results are indicated in Table 4.4.

4.4.3.1. Water temperature

In all sampling time, the values of water temperatures were almost similar among the treatments. During the study period, the water temperature showed a decreasing trend and varied from 24.51-31.85 ºC.

4.4.3.2. Dissolved oxygen

During the study period, dissolved oxygen concentration was above 5.50 mg/l in all the treatments and showed no significant differences (P>0.05). The concentrations of DO among the treatments varied from 5.57 to 6.30 mg/l.

Table 4.4: The mean values (±SD) and ranges of physico-chemical parameters of water in the experimental aquariums under four treatments

Parameters	Treatments			
	T1	T2	T3	T4
Temperature (°C)	29.01 ± 2.44 (24.51-31.76)	29.03 ± 2.44 (24.57-31.81)	29.02 ± 2.48 (24.56-31.85)	29.02 ±2.45 (24.55-31.80)
DO (mg/l)	5.90 ± 0.20 (5.63-6.30)	5.83 ± 0.13 (5.70-6.07)	5.85 ± 0.12 (5.57-6.03)	5.88 ± 0.09 (5.70-6.07)
pH	7.15 ± 0.03 (7.12-7.21)	7.17 ± 0.04 (7.10-7.20)	7.16 ± 0.04 (7.12-7.20)	7.16 ± 0.03 (7.11-7.22)
NO_3-N (mg/l)	0.10 ± 0.04 (0.07-0.14)	0.11 ± 0.03 (0.08-0.18)	0.11 ± 0.03 (0.08-0.14)	0.11 ± 0.02 (0.08-0.15)
NH_4-N (mg/l)	0.08 ± 0.02 (0.06-0.10)	0.08 ± 0.04 (0.05-0.11)	0.09 ± 0.03 (0.07-0.10)	0.08 ± 0.03 (0.07-0.10)
PO_4-P (mg/l)	0.09 ± 0.03 (0.07-0.12)	0.09 ± 0.02 (0.07-0.13)	0.10 ± 0.03 (0.08-0.12)	0.10 ± 0.04 (0.07-0.13)

- **T1:** Fish group fed with Feed-1 (Control group); **T2:** Fish group fed with Feed-2; **T3:** Fish group fed with Feed-3; and **T4:** Fish group fed with Feed-4.
- Values of physico-chemical parameters are mean of triplicate determination.

4.4.3.3. pH

There was no significance difference in the pH values among the treatments (P>0.05). The values of pH were almost neutral and fluctuated between 7.10 and 7.22 among the treatments.

4.4.3.4. Nutrients

Relatively lower concentrations of nutrients were recorded in the water of each experimental aquarium under four treatments and did not show any significant differences (P>0.05). The concentrations of NO_3-N, NH_4-N and PO_4-P in four treatments varied from 0.07 to 0.18, 0.05-0.11 and 0.07-0.13 mg/l, respectively.

4.4.4. Growth performance and feed utilization

The growth performance and feed utilization in four treatments in terms of weight increment (WI), mean weight gain (MWG), average daily weight gain (ADWG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR) and survival rate were examined and the results are shown in Table 4.5 and 4.6.

4.4.4.1. Weight increment

Weight increments were observed in all feeding treatments till then harvesting but significantly higher WI was recorded in T3 after six to twelve weeks as compared to other treatments (P<0.05). The variation in WI in four treatments is shown in Figure 4.2a.

4.4.4.2. Mean weight gain

After 12 weeks study tenure, the values of MWG were recorded as 106.07±2.52, 108.81±5.16, 120.78±3.50 and 107.66±4.42 g in T1, T2, T3 and T4, respectively. The maximum MWG was recorded in T3 followed by T2 and T4 and the minimum was in T1. The MWG in T3 was significantly different from other treatments (P<0.05). The variation in MWG in four treatments is shown in Figure 4.2(b).

4.4.4.3. Average daily weight gain

The values of average daily weight gain were calculated as 1.26±0.10, 1.30±0.09, 1.44±0.10 and 1.28±0.11 gd^{-1} in T1, T2, T3 and T4, respectively. Significantly higher ADWG was recorded in T3 ($P<0.05$) and the lower ADWG was recorded in T1 but not significantly different from T2 and T4 ($P>0.05$). The variation in ADWG in four treatments is shown in Figure 4.2(c).

4.4.4.4. Specific growth rate

The values of specific growth rate were estimated as 2.52±0.11, 2.55±0.09, 2.66±0.09 and 2.54±0.10 % bwd^{-1} in T1, T2, T2 and T4, respectively. Significantly higher SGR was recorded in T3 ($P<0.05$) and the lower SGR was recorded in T1 but not significantly different from T2 and T4 ($P>0.05$). The variation in SGR in four treatments is shown in Figure 4.3(a).

4.4.4.5. Feed intake

The fish group in T1 and T4 showed lower feed intake and significantly ($P<0.05$) higher feed intake was observed in T3 followed by T2. The values of average feed intake were 1.87±0.09, 1.90±0.08, 1.99±0.08 and 1.86±0.09 g $fish^{-1}$ day^{-1} in T1, T2, T2 and T4, respectively. The variation in FI in four treatments is shown in Figure 4.3(b).

4.4.4.6. Feed conversion ratio

The values of FCR were calculated as 1.48±0.08, 1.47±0.07, 1.39±0.07 and 1.45±0.08 in T1, T2, T2 and T4, respectively. The results showed that replacement of euglenophytes algae in conventional feed improved FCR (1.48 to 1.39) of common carp. The FCR value in T3 was better than other treatments ($P<0.05$). The variation in FCR values in four treatments is shown in Figure 4.3(c).

4.4.4.7. Survival of fish

During the study tenure, there was no death of fish occurred in four treatments.

Table 4.5: Weight increments of common carp in four feeding treatments

Sampling time	Treatment			
	T1	T2	T3	T4
0 -Weeks	14.56±2.01[a]	14.51±1.77[a]	14.54±2.02[a]	14.53±1.98[a]
3 -Weeks	35.34±2.11[a]	36.26±2.74[a]	37.64±3.14[a]	35.19±3.50[a]
6 -Weeks	62.12±2.56[c]	64.27±2.65[bc]	67.22±4.30[a]	64.70±2.28[b]
9- Weeks	90.60±4.43[c]	92.89±3.17[bc]	99.40±3.62[a]	95.25±3.59[b]
12- Weeks	120.63±4.48 [b]	123.32±3.46 [b]	135.32±5.37[a]	122.19±5.59[b]

- **T1:** Fish group fed with Feed-1 (Control group); **T2:** Fish group fed with Feed-2; **T3:** Fish group fed with Feed-3; and **T4:** Fish group fed with Feed-4.

Table 4.6: Growth and feed utilization parameters of common carp in four feeding treatments

Parameters	Treatments			
	T1	T2	T3	T4
IW (g)	14.56 ± 2.01	14.51 ± 1.77	14.54 ± 2.02	14.53 ± 1.98
FI (g)	120.63±4.48	123.32±3.46	135.32±5.37	122.19±5.59
MWG (g)	106.07±2.52[b]	108.81±5.16[b]	120.78±3.50[a]	107.66±4.42[b]
ADWG (gd^{-1})	1.26±0.10[b]	1.30±0.09[b]	1.44±0.10[a]	1.28±0.11[b]
SGR (% bwd^{-1})	2.52±0.11[b]	2.55±0.0.09[b]	2.66±0.09[a]	2.54±0.10[b]
FI (g $fish^{-1}d^{-1}$)	1.87 ± 0.09[b]	1.90 ± 0.08[b]	1.99 ± 0.08[a]	1.86 ± 0.09[b]
FCR	1.48 ± 0.08[b]	1.47 ± 0.07[b]	1.39 ± 0.07[a]	1.45 ± 0.08[ab]

- **IW:** Initial weight; **FW:** Final weight; **MWG:** Mean weight gain, **ADWG:** Average daily weight gain; **SGR:** Specific growth rate; **FI:** Feed intake; and **FCR:** Feed conversion ratio.
- Values are mean of triplicate determination. Values in the same row with different superscripts are significantly different ($P<0.05$).

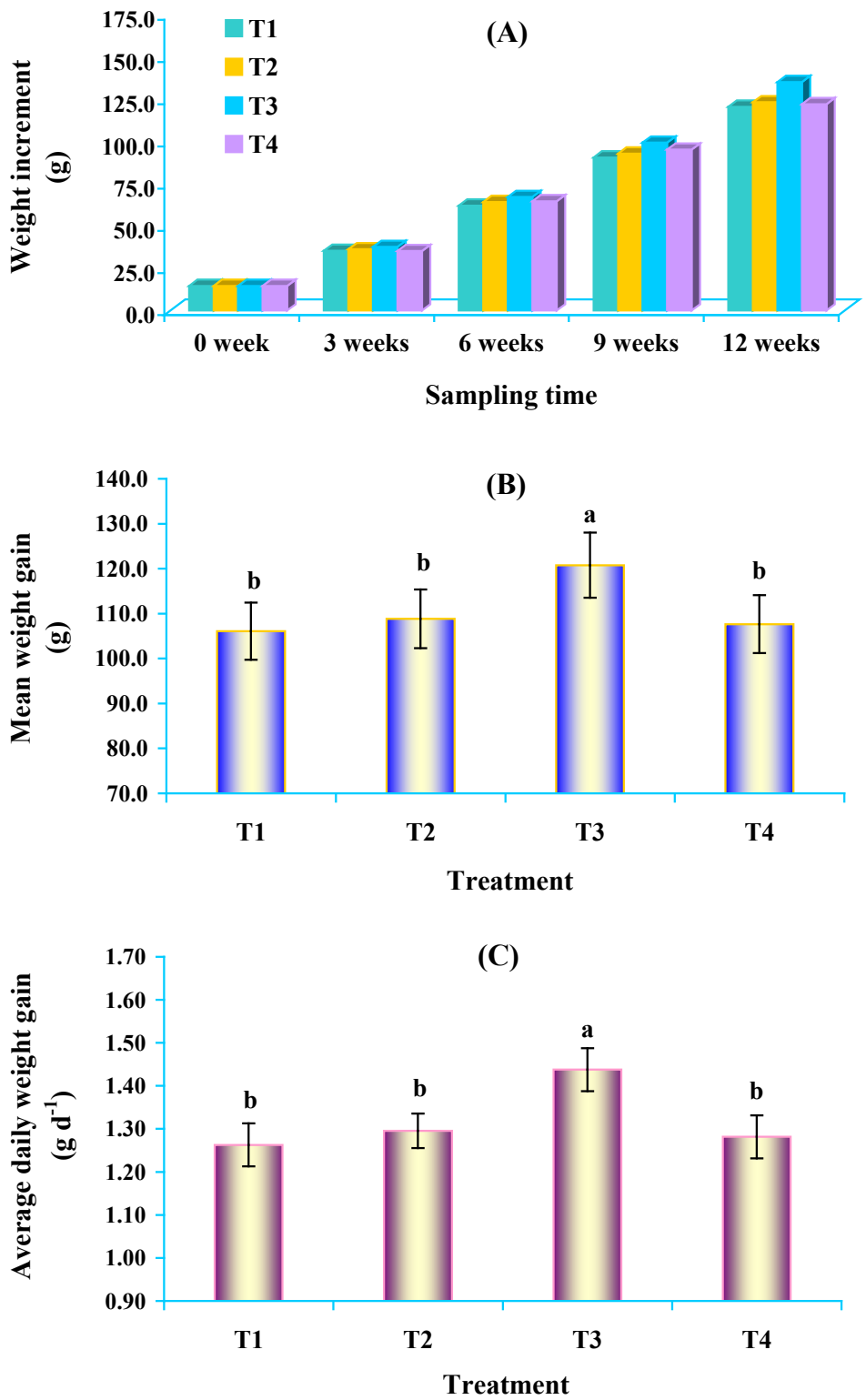


Figure 4.2: Variations in (A) Weight increment, (B) Mean weight gain and (C) Average daily weight gain in T1, T2, T3 and T4

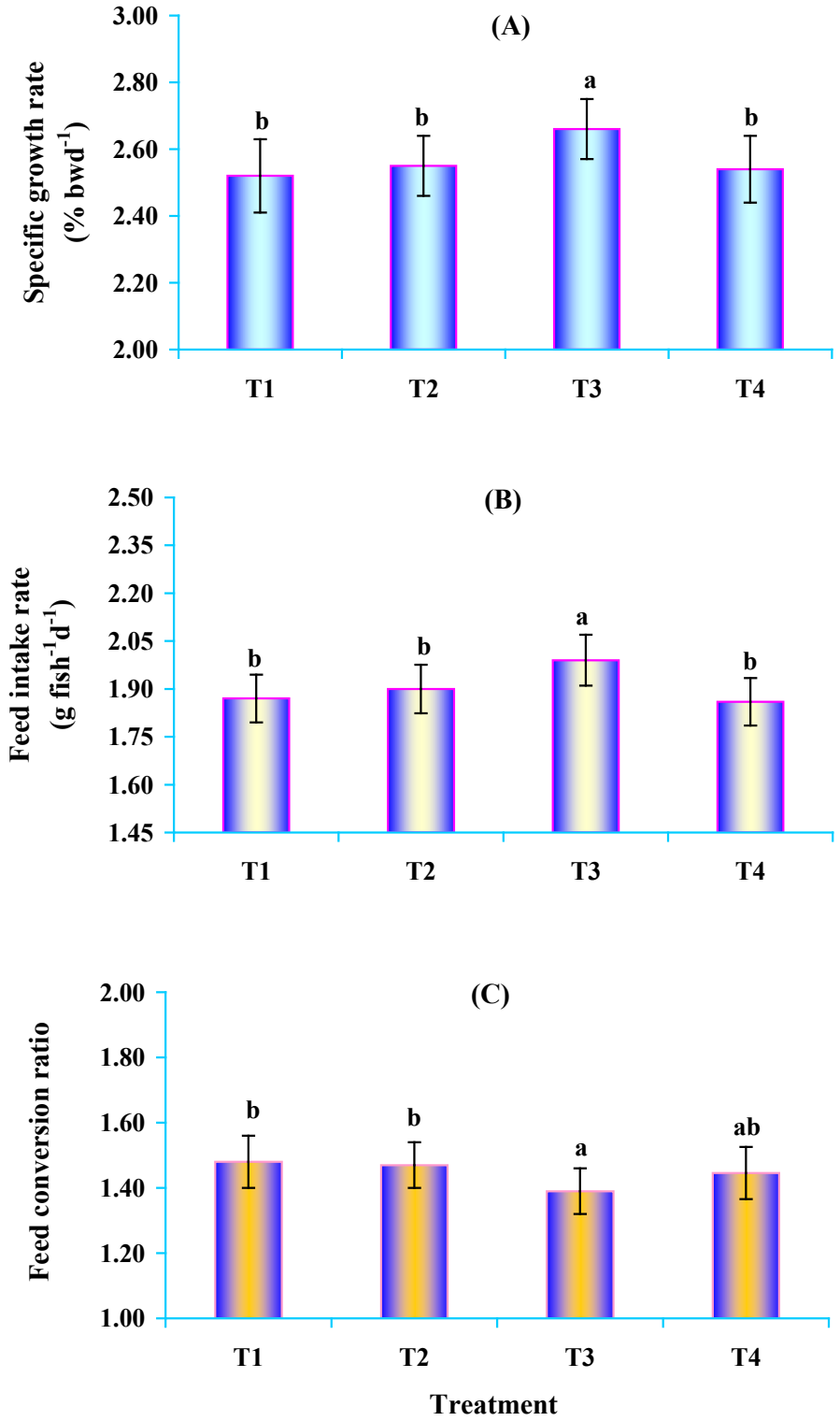


Figure 4.3: Variations in (A) Specific growth rate, (B) Feed intake rate and (C) Feed conversion ratio in T1, T2, T3 and T4

4.4.5. Carcass compositions

The carcass compositions of common carp as affected by four feeding treatments are summarized in Table 4.7. The variations in carcass compositions in four feeding treatments are shown in Figure 4.4.

4.4.5.1. Carcass protein

Carcass protein contents in four treatments were significantly different ($P<0.05$). Higher carcass protein was recorded in T3 (17.93%) followed by T4 (15.76%) and T2 (15.54%) whereas in T1 (Control group), it was quietly low (14.62%).

4.4.5.2. Carcass lipid

Carcass lipid contents were determined as 3.81, 3.69, 3.28 and 3.19% in T1, T2, T3 and T4, respectively. The maximum carcass lipid was found in T1 and the minimum was in T4. Carcass lipid contents in T1 and T2 were significantly different from T3 and T4 ($P<0.05$).

4.4.5.3. Carcass glycogen

Carcass glycogen in four treatments showed significant difference ($P<0.05$). Higher carcass glycogen was recorded in T1 (4.16%) followed by T2 (3.41%) and T3 (3.10%) whereas in T4, it was comparatively low (3.02%).

4.4.5.4. Carcass ash

Carcass ash contents were determined as 2.29, 2.41, 2.69 and 2.73% in T1, T2, T3 and T4, respectively. The maximum carcass ash was found in T4 and the minimum was in T1. Carcass ash contents in T3 and T4 were significantly different from T1 and T2 ($P<0.05$).

4.4.5.5. Carcass moisture

Higher carcass moisture was found in T1 (71.96%) followed by T2 (71.86%) and T3 (71.41%) whereas in T4, it was relatively low (71.34%).

Table 4.7: Mean values of carcass compositions (±SD) of common carp in four feeding treatments (% wet basis)

Component (%)	Treatments			
	T1	T2	T3	T4
Protein	14.62±0.66[c]	15.54±0.82[b]	17.93±0.97[a]	15.76±0.72[b]
Lipid	3.81±0.18[a]	3.69±0.15[a]	3.28±0.13[b]	3.19±0.15[b]
Glycogen	4.16±0.16[a]	3.41±0.12[b]	3.10±0.13[c]	3.02±0.11[c]
Ash	2.29±0.17[b]	2.41±0.13[b]	2.69±0.16[a]	2.73±0.14[a]
Moisture	71.96±0.24[a]	71.86±0.26[a]	71.41±0.23[b]	71.34±0.17[b]

- **T1:** Fish group fed with Feed-1 (Control group); **T2:** Fish group fed with Feed-2 **T3:** Fish group fed with Feed-3; and **T4:** Fish group fed with Feed-4.
- Carcass nutrient values are mean of triplicate determination. Values in the same row with different superscripts are significantly different ($P<0.05$).

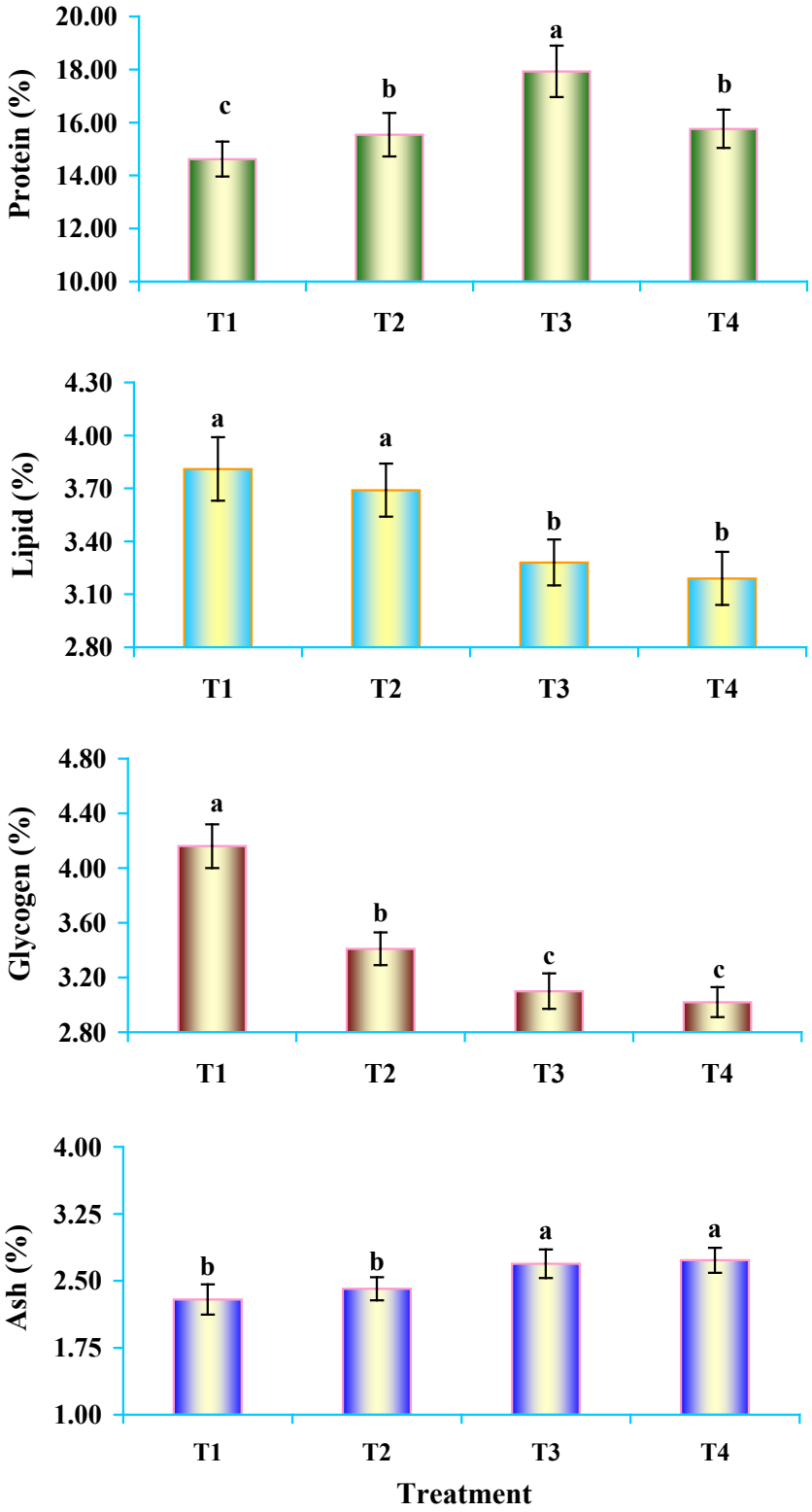


Figure 4.4: Variations in carcass compositions in T1, T2, T3 and T4

4.5. Discussion

In this section, the results of nutritive values of euglenophytes algae and formulated feeds, effects of euglenophytes algae supplemented feeds on the growth, feed utilization and carcass composition of common carp are discussed and corroborated with the previous relevant research findings.

4.5.1. Nutritive value of euglenophytes algae

Potential algae contained about 30-70% protein, 10-20% lipid, 5-15% carbohydrate and high amount of carotenoids with antioxidant property (Becker, 2004). Several references indicated that *Euglena* cell contains high quality proteins, polyunsaturated fatty acids, balanced carbohydrates and vitamins. In the present study, the analysis of chemical composition of euglenophytes algae showed that they contained average 49.64% crude protein, 14.40% crude lipid, 15.96% total carbohydrate, 9.29% moisture and 10.41% ash. These results are supported by Becker (1994) who reported that *Euglena gracilis* cells contained 39-61% crude protein, 14-20% crude lipid and 14-18% total carbohydrate.

The results of nutritive value of euglenophytes algae are comparable to the blue green algae, *Spirulina* sp. which contained 48-73% crude protein, 5-11% crude lipid, 8-19% total carbohydrate, 3-7% moisture and 5-8% ash (Ahmadzadenia *et al*., 2011). The results are also comparable to the green algae, *Chlorella* sp. which contained 46.7% crude protein, 14.8 % crude fat, 11.6 % total carbohydrate and 17.5 % ash; and to the *Scenedesmus* sp. which contained 52.3% crude protein, 12.20% crude fat, 10.06% total carbohydrate and 14.92 % ash (Tartiel, 2005).

4.5.2. Nutritive value of experimental feeds

Algae with high nutritional value have remarkable potential as fish feed ingredient (Navarro and Sarasquete, 1998; Atalah *et al*., 2007; Sorensen and

Denstadli, 2008; Khatoon *et al*., 2009; Wassef *et al*., 2013). The advantages of algal supplementation can be attributed to the balance of dietary protein, lipid, carbohydrates, fibers and minerals together with basic nutritional requirements in fish diets in comparison to commercial diets (Guroy *et al*., 2007; Azaza *et al*., 2008; Ergun *et al*., 2008; Tartiel *et al*., 2008).

In the present study, the results of chemical compositions of the formulated feeds showed that the feeds contained 40.48 to 48.10% crude protein, 10.96 to 13.98% crude lipid, 16.57 to 23.29% total carbohydrate, 7.40 to 9.03% ash and 11.25 to 11.65% moisture. From the results, it was observed that increasing level of euglenophytes algae in the feeds increased the protein and ash content whereas decreased the lipid and carbohydrate contents. These results are partially consistent to the report of Mukharjee *et al*. (2011) who found 48.61% crude protein, 4.93% crude lipid, 27.90% total carbohydrate, 18.20% ash and 15.40% moisture in *Spirulina* and *Enteromorpha* based formulated feeds. Tongsiri *et al*. (2010) found 18.81 to 20.71% crude protein, 10.85 to 12.03% crude lipid, 40.59 to 46.01% total carbohydrate, 8.42 to 11.41% ash and 9.38 to 12.93% moisture in *Spirulina* based diets. This report is also partially supportive to the results of present study. The differences in the nutritive values of the feeds used in this study from the previous reports might be due to differences in the nutritive values of algal species used and difference in inclusion levels in the feeds formulation.

4.5.3. Physico-chemical parameters of water

Physico-chemical parameters of water play an important role for the growth of fish (Rahman, 1992). Suitable physico-chemical parameters are prerequisites for healthy aquatic environment. So, suitable ranges of these parameters should be maintained in any culture system. In the present study, the physico-chemical parameters did not show any significant difference among the feeding treatments and remained within the acceptable ranges for fish growth.

The values of water temperature in four feeding treatments were found to vary from 24.51 to 31.85 ^{0}C which were within the acceptable range for fish growth according to Quddus and Banerjee (1989) who denoted that water temperature between 24.0°C and 32.0°C is suitable for fish growth. Again, Rahman *et al.* (1982) reported that water temperature range, 25.5°C to 30.0°C is favorable for fish culture. This report is accord with the present result.

The mean dissolved oxygen concentrations in four treatments were found to vary from 5.57 to 6.30 mg/l which were within the recommended range according to Rahman (1992) who stated that DO concentration of a productive water body should be 5.0 mg/l or more. The present result is also supported by the report of Bhuiyan (1970) who stated that water having DO 5.0 to 7.0 mg/l is fair for productivity.

The mean values of pH in four treatments varied from 7.10 to 7.22 which were also within the acceptable range according to Boyd (1990) who reported that pH range 6.5-9.0 is suitable for fish growth. Ahmed (1993) reported that the pH range, 6.70 to 7.20 is suitable for carp fingerlings rearing. This report is also consistent with the present study.

During the study tenure, relatively lower concentrations of nutrients (nitrate-nitrogen, ammonium-nitrogen and phosphate-phosphorus) were recorded in the water of all treatments. This might be due to use of tap water which contained poor nutrients and due to regular replenishing of water from the experimental aquariums which prevent loading of nutrients from decomposition of fecal metabolizes of fish and unutilized feeds.

4.5.4 Growth performance and feed utilization

4.5.4.1. Growth performance

Growth performances have improved in fish fed diets containing algae cells (Mustafa *et al*., 1994b; Ibrahim *et al*., 2007; Sirakov *et al*., 2012). In the present study, growth in terms of mean weight gain, daily weight gain and SGR was found to be increased in T2, T3 and T4 as compared to T1 (Table 4.6) which indicated that supplementation of euglenophytes algae in the conventional feed increased the growth of common carp. The present results are in agreement with those obtained by Mustafa and Nakagawa (1995), Zeinhom (2004), Tartiel *et al*. (2008) and Sirakov *et al*. (2012) who found that inclusion of algae in fish feed increased the mean weight gain, daily weight gain and SGR of different fish species. Supportive evidence to the present results can also be drawn by the findings of Saroch *et al*. (2012) who stated that the replacement of fish meal with *Spirulina* meal resulted in significantly superior growth of catla. Roy *et al*. (2011) reported that use of algal biomass as fish feed ingredient promoted body weight gain and SGR in tilapia. Again, El-Tawil (2010) stated that final weight, weight gain and specific growth rate increased significantly with inclusion of *Ulva* meal in red tilapia diet. Hayashi *et al*. (1993a) stated that *Euglena* cells in the casein diet improved growth of prawn. These reports are also supportive to the present results.

In course of the experimental tenure of 12 weeks, the fish group in T3 (fed with 30% euglenophytes algae supplementation) showed a significant improvement in growth parameters as compared to other fish groups ($P<0.05$). These results are in agreement with the findings of Nandeesha *et al*. (2001) who found that more than 25% *Spirulina* replacement in fish feed resulted in significantly superior weight gain and specific growth rate of rohu. Roy *et al*. (2011) reported that 35% composite algal mix can be supplemented in diet for better growth of tilapia. This report is supportive to the present results. Olvera–Novoa *et al*. (1998) stated that *Spirulina* can be replaced up to 40% of the fishmeal protein in tilapia diets.

This report is partially supportive to the present findings. Moreover, better growth performance in T3 might be due to the acceptability of the feed. This assumption is agreed with the report of Hasan and Macintosh (1992) who stated that the growth of common carp varied with the acceptability of feed.

From the present results, it was also observed that the fish group in T4 (fed with 40% euglenophytes algae supplementation) showed a considerable lower improvement in the growth parameters than the fish group in T3. This might be due to the dietary protein level in the feed above the optimum. This assumption is consistent with the findings of Rajbanshi and Mumtazuddin (1989) who reported that growth of Indian major carp showed a decreasing trend at dietary protein level above the optimum. Again, Singh *et al*. (2006) stated that SGR of carp fish is influenced by the dietary protein levels. Hence, the feed with 30% euglenophytes algae supplementation having optimum dietary protein level which might be more suitable to enhance growth than other experimental feeds.

The present results corroborated with the previous findings that optimum dietary lipid level resulted in improved growth rate, feed conversion ratio and utilization of other nutrients (Martins *et al*., 2007; Yigit *et al*., 2002). On the other hand, dietary lipid level above the optimum could have an adverse effect on growth and feed utilization as found in common carp (Bryant, 1980) and *Labeo rohita* (Hasan *et al*., 1993; Mukherjee *et al*., 2011). In the present study, the feed with 30% euglenophytes algae supplementation had lower lipid content which might be resulted into lower body lipid deposition and improve growth rate. Therefore, the study confirmed the fact that the high lipid containing feed, such as Feed-1 (conventional feed) used in the study might have a negative effect on fish growth as higher lipid content in feed could lead to reduce utilization of other nutrients, resulting in poor growth performance (Hemre and Sandnes, 1999).

4.5.4.2. Feed utilization

During the experimental tenure, higher feed intake rate was noticed in T3 (fed with 30% euglenophytes algae supplementation) compared to other treatments. Previously, Fournier *et al*. (2002) revealed that both dietary protein and amino acid levels in the feeds have effect on voluntary feed intake of fish. Tongsiri *et al*. (2010) reported that feed intake increased due to inclusion of *Spirulina* algae in the diet of Mekong Giant Catfish. According to the previous reports (aforementioned) and the present results, the feed with 30% euglenophytes algae supplementation might be contained optimum protein levels for which the fish seemed to prefer this feed.

The present results also showed that supplementation of euglenophytes algae in conventional fish feed improved FCR (1.48 to 1.39) of common carp and 30% supplementation resulted in the better FCR (1.39) as compared to other levels of supplementation. These results are partially agreement with those obtained by Zeinhom (2004) who found that inclusion of algae in fish diets insignificantly improved the FCR whereas feed intake was significantly increased. Dawah *et al*. (2002b) found that food conversion ratio was better when the fish were maintained on artificial diets with 10% and 20% dried algae. This report is also partially supportive to the present results.

4.5.5. Carcass compositions

The results of the present study showed that supplementation of euglenophytes algae in the conventional feed contributed to changes in carcass protein, lipid, glycogen, ash and moisture content of common carp.

4.5.5.1. Carcass protein

By carcass analysis, it was observed that carcass crude protein was increased in the all fish groups fed with euglenophytes algae supplemented feeds as compared to the control group. Abdel-Tawwab *et al*. (2008) and Kim *et al*. (2013)

reported that *Spirulina* algae supplementation in the diet increased protein deposition in fish body. In a different study Soler-Vila *et al*. (2009) found that *Porphyra dioica* algae inclusion in the diet increased carcass protein. These reports are supportive to the present study. The increased carcass protein in the fish might have been contributed by the higher dietary protein as confirmed by the results of nutritive value of the formulated feeds (Table 4.3).

In the present study, increase of carcass protein was found to be highest in the fish group fed with 30% euglenophytes algae supplementation (T3) and it showed a drop in the fish group fed with 40% euglenophytes algae supplementation (T4). Highest carcass protein found in the fish group in T3 might be due to the optimum ratio of euglenophytes algae supplementation in the feed. Supporting evidence to this assumption can be drawn from the previous report of Davies *et al*. (1997) who reported that carcass protein increased in grey mullet with an inclusion of 16 to 33% *Porphyra purpurea* algae. Tongsiri *et al*. (2010) stated that carcass protein tended to increase with the increase of *Spirulina* inclusion in the diets. Similar results have been reported by Ungsethaphand *et al*. (2010) and Ahmadzadenia *et al*. (2011). Their findings are partially supportive to the present results.

The feed with 40% euglenophytes algae supplementation had maximum dietary crude protein, but it neither could convert into higher carcass protein nor to the increased growth of the fish group in T4 as compared to the fish group in T3. This might be due to the imbalance of dietary protein in the feed. Earlier studies of Jauncey (1982) confirmed that dietary crude protein level above the optimum decreased the protein utilization and carcass protein in tilapia. In a different study, Singh *et al*. (2006) revealed that protein utilization and carcass protein in rohu fingerlings were influenced by the dietary crude protein. Hence, the feed with 30% euglenophytes supplementation having proper level of dietary protein which might be more suitable than other feeds to increase carcass protein in common carp.

4.5.5.2. Carcass lipid

The results of the present study showed that the carcass lipid contents decreased with the increasing level of euglenophytes supplementation in the conventional feed. These results are strongly agreement with those obtained by Guroy *et al*. (2007) and Azaza *et al*. (2008) who found that algal meals decreased carcass lipids of fish. The present results are also comparable to the earlier findings (Nandeesha *et al*, 1998; Puwastein *et al*., 1999; Justi *et al*., 2003; Ergun *et al*., 2009) that carcass crude fat decreased concomitant with an increased *Spirulina* algae supplementation in the diets.

4.5.5.3. Carcass glycogen

The similar trend, as observed in carcass lipid, was followed when the analysis was done on the basis of accumulating carcass glycogen i.e., carcass glycogen content was decreased with the increasing level of euglenophytes supplementation in the feed. Lower glycogen content in the fish groups in T4 and T3 might be due to lower dietary carbohydrate as confirmed by the nutritive values of the feeds (Table 4.2). Tongsiri *et al*. (2010) reported that 30% *Spirulina* supplementation in diet gave a low amount of glycogen deposition in the flesh of Mekong Giant Catfish. In another study, Roy *et al*. (2011) observed that carcass lipid levels decreased at 35% supplementation of algal mix in diet of *Oreochromis mossambicus*. These reports are supportive to the present results.

4.5.5.4. Carcass ash

It was revealed from the analysis of nutritive values of the formulated feeds that the euglenophytes algae supplemented feeds contained higher ash content than the conventional feed indicating more minerals in the euglenophytes algae supplemented feeds, which resulted into high deposition of nutrients in the carcass of fish, as confirmed by ash content (Table 4.7). The present results also showed that carcass ash content was increased with the increasing level of euglenophytes algae supplementation in the feeds. The present results are in

contrast to the previous finding by Khatoon *et al*. (2010a) showing less utilization of the minerals in optimum level by more nutrient containing algae based feed than the control feed. Contrasting result has also been reported by Ungsethaphand *et al*. (2010) that carcass ash content decreased with the increasing levels of *Spirulina* inclusion in the diets. The contrasting results might be due to the fact that the ability of fish to utilize dietary nutrients may differ among the species.

4.5.5.5. Carcass moisture

The results of carcass analysis showed that carcass moisture content decreased with increasing levels of euglenophytes algae in the feeds. This result is agreement with that obtained by Appler and Jauncy (1983) who found that the carcass moisture content decreased with increasing levels of algae in the diets.

4.6. Conclusion

The overall study indicated that supplementation of 30% euglenophytes algae in the conventional feed contributed better growth and carcass compositions of common carp as evident from the growth, feed utilization and carcass parameters. On the other hand, conventional feed resulted into poor growth and carcass compositions. The present study thus suggested that the efficacy of euglenophytes algae supplemented feeds was higher than the conventional feed in terms of growth performances and carcass compositions. Therefore, the locally available euglenophytes algae can be used in combination with conventional fish feed to achieve a comparable or better growth in common carp culture which may have a high commercial value. A comprehensive investigation is required in long-term feeding trials to evaluate the full potential of these algae as fish feed ingredient and to determine the optimum dietary supplementation levels.

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CHAPTER FIVE

Summary and Conclusion

Chapter Five

SUMMARY AND CONCLUSION

As frequent occurrence and harmful effects of euglenophytes bloom on fish production, three experiments were conducted to find out the management systems to minimize the euglenophytes bloom in fish pond and to explore the utilization of these algae as a fish feed ingredient during 2010 to 2012 in Rajshahi district, North-west part of Bangladesh.

Understanding the environmental factors which influence the density of euglenophytes will help to manage the bloom of these algae. Therefore, the first experiment was conducted to investigate the relationships of euglenophytes bloom to environmental factors in nine fish ponds for twelve months from July 2010 to June 2011. Among the ponds, three bloom ponds (BP) were selected at Raighati in Mohanpur Upazila (BP-R), another three bloom ponds at Yusufpur in Charghat Upazila (BP-Y) and three non-bloom ponds (NBP) at Meherchandi in Motihar Thana. Environmental factors (water temperature, dissolved oxygen, pH, NO_3-N, NH_4-N and PO_4-P, Fe, Zn, Mn and Cu), soil organic matter, planktonic algal community and density were monitored monthly by using standard methods. There was no significant difference in water temperature among the study ponds (BP-R, BP-Y and NBP) but significantly lower dissolved oxygen and pH, and higher concentrations of NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu were recorded in BP-R and BP-Y as compared to NBP ($P<0.05$). The mean values of water temperature, dissolved oxygen, pH, NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu varied from 26.29±4.47 (BP-R) to 26.39±4.52 ºC (NBP), 4.96±0.47 (BP-R) to 5.72±0.23 mg/l (NBP), 6.30±0.39 (BP-R) to 7.84±0.39 (NBP), 0.48±0.11 (NBP) to 1.24±0.29 mg/l (BP-Y), 0.23±0.07 (NBP) to 1.08±0.27 mg/l (BP-Y), 0.41±0.10 (NBP) to 1.19±0.32 mg/l (BP-Y), 0.18±0.05 (NBP) to 0.53±0.13 mg/l (BP-Y), 0.09±0.03 (NBP) to 0.28±0.10 mg/l (BP-Y), 0.11±0.03 (NBP) to 0.26±0.07 mg/l (BP-R) and 0.10±0.03 (NBP) to 0.26±0.07 mg/l (BP-Y), respectively. Significantly ($P<0.05$) higher mean concentrations of soil organic matter were recorded in BP-Y and BP-R (6.20±0.97 and 6.04±1.11 %) as compared to NBP (3.17±0.59 %). During this study, total 28 genera of planktonic algae were recorded and euglenophytes algae comprised three

genera, *Euglena, Phacus* and *Trachelomonas* among which *Euglena* was the most dominant genus. Significantly higher density of euglenophytes and lower density of other planktonic algae were recorded in BP-R and BP-Y as compared to NBP ($P<0.05$). The mean densities of euglenophytes varied from 2.95±1.12 (NBP) to 17.39±10.30 x 10^4 cells/l (BP-Y). The density of euglenophytes showed an increasing trend from autumn to winter, peaked in late autumn (November) and early winter (December) whereas in summer, monsoon and spring season, the density was relatively low. The density of euglenophytes was negatively correlated to water temperature, dissolved oxygen, pH and other planktonic algal density whereas positively correlated to NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu concentrations. This study indicates that higher concentrations of NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu under lower water temperature, DO and pH enhanced the density of euglenophytes.

Based on the findings of the first experiment, the second experiment was conducted to investigate the management of euglenophytes bloom by using duckweed and lime for five months from August to December 2011 in twelve euglenophytes bloom forming ponds at Raighati, Mohanpur Upazila under four treatments such as T1 (the ponds treated with duckweed), T2 (the ponds treated with lime), T3 (the ponds treated with both duckweed and lime) and T4 (the ponds without duckweed and lime) with three replications. The ponds were stocked with the fish species comprising *Labeo rohita*, *Catla catla*, *Hypophthalmichthys molitrix*, *Puntius gonionotus* and *Cirrhina mrigala* at 60/dec. Water quality parameters, soil organic matter, planktonic algal community and density, and growth performance and gut contents of fish were monitored regularly by using standard methods. The values of water quality parameters (other than water temperature), soil organic matter, density of total planktonic algae and euglenophytes, and growth of the fishes among the treatments were significantly different ($P<0.05$). The mean values of temperature, dissolved oxygen, pH, NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu varied from 28.36±4.43 (T4) to 28.38±4.43 ºC (T2), 4.72±0.51 (T4) to 5.54±0.44 mg/l (T1), 6.21±0.34 (T4) to 7.69±0.41 (T3), 0.71±0.24 (T3) to 1.41±0.26 mg/l (T4), 0.54±0.25 (T3) to 1.34 ±0.18 mg/l (T4), 0.69±0.25 (T3) to 1.61±0.39 mg/l (T4), 0.24±0.10 (T3) to 0.69±0.08 mg/l (T4), 0.14±0.05 (T1) to 0.33±0.06 mg/l (T4), 0.14±0.05 (T3) to 0.29±0.05 mg/l (T4) and

0.13±0.03 (T1) to 0.26±0.03 mg/l (T4), respectively. Significantly ($P<0.05$) higher concentration of soil organic matter was recorded in T4 (6.66±0.82%) and the lower concentration in T2 (3.29±0.60 %). Significantly higher density of total planktonic algae and euglenophytes were recorded in T4 and the lower density was in T3 ($P<0.05$). The mean density of total planktonic algae and euglenophytes varied from 14.49±2.89 (T3) to 26.55±5.65 x 10^4 cells/l (T4) and 5.40±2.13 (T3) to 18.97±6.78 x 10^4 cells/l (T4), respectively. Significantly higher growth of all experimental fish species (in terms of mean weight gain, average daily weight gain and SGR) were recorded in T3 ($P<0.05$) followed by T1 and T2 whereas the lower growth was recorded in T4. The proportions of euglenophytes in the gut contents of the fish species among the treatments did not show any significant difference ($P>0.05$) but relatively higher proportions was found in silver carp and silver barb. All experimental fish species showed negative electivity to eat euglenophytes as food. This study indicated that use of duckweed and lime had positive effects on water quality improvement and reduction of euglenophytes density. On the other hand, grazing of fish had no significant effect in controlling euglenophytes bloom.

For utilization of euglenophytes algae as a feed ingredient, the third experiment was conducted to study the effects of euglenophytes algae supplemented feed on the growth and carcass compositions of common carp (*Cyprinus carpio* L.) for 12 weeks feeding trail from August to October 2012. Four feeds containing 0 % (Feed-1, Control feed), 20% (Feed-2), 30% (Feed-3) and 40% (Feed-4) euglenophytes algae were used in combination with conventional fish feed ingredients (Rice bran and mustard oil cake). The experiment was carried out in 12 glass aquariums at the wet laboratory of the Department of Fisheries, University of Rajshahi, Rajshahi under four treatments such as T1 (the fish group fed with Feed-1), T2 (the fish group fed with Feed-2), T3 (the fish group fed with Feed-3) and T4 (the fish group fed with Feed-4) with three replications. The aquariums were stocked with same aged common carp fingerlings at 15/aquarium. Nutritive values of euglenophytes algae and experimental feeds, physico-chemical parameters of water, growth performances and feed utilization (in terms of mean weight gain, average daily weight gain, SGR, feed intake and FCR), and carcass compositions of fish were examined by using standard methods. The chemical analysis showed that euglenophytes algae contained

average 49.64% crude protein, 14.40% crude lipid, 15.96% total carbohydrate, 9.29% moisture and 10.41% ash. The experimental feeds contained 40.48 to 48.10% crude protein, 10.96 to 13.98% crude lipid, 16.57 to 23.29% total carbohydrate, 7.40 to 9.03% ash and 11.25 to 11.65% moisture. During the study period, physico-chemical parameters of water among the treatment did not show any significant difference ($P>0.05$) and remained within the suitable ranges. The values of mean weight gain, average daily weight gain, SGR, feed intake and FCR varied from 106.07±2.52 (T1) to 120.78±3.50 g (T3), 1.26±0.10 (T1) to 1.44±0.10 gd^{-1} (T3), 2.52±0.11 (T1) to 2.66±0.09 % bwd^{-1} (T3), 1.87±0.09 (T1) to 1.99±0.08 g $fish^{-1}d^{-1}$ (T3) and 1.39±0.07 (T3) to 1.48±0.08 (T1), respectively. The values of carcass protein, lipid, glycogen, ash and moisture varied from 14.62±0.66 (T1) to 17.93±0.97 % (T3), 3.19±0.15 (T4) to 3.81±0.18 % (T1), 3.02±0.11 (T4) to 4.16±0.16 % (T1), 2.29±0.17 (T1) to 2.73±0.14 % (T4) and 71.34±0.17 (T4) to 71.96±0.24 % (T1), respectively. The results showed that euglenophytes algae supplemented feeds had positive effects on the growth and carcass compositions as compared to control feed. Significantly higher mean weight gain, average daily weight gain, SGR and feed intake, improved FCR, higher carcass protein and ash, lower lipid and glycogen were recorded in T3 as compared to other treatments ($P<0.05$). This study revealed that euglenophytes algae could be used as a fish feed ingredient as evident from the growth and carcass compositions.

Overall study indicates that higher concentrations of NO_3-N, NH_4-N, PO_4-P, Fe, Zn, Mn and Cu under lower water temperature, dissolved oxygen and pH are responsible for the bloom of euglenophytes in fish ponds; use of both duckweed and lime is better for management of euglenophytes bloom in fish ponds; and 30% euglenophytes algae supplementation in the conventional feed is better for growth and carcass nutrients of common carp.

Based on the present findings, further long-term studies are necessary to examine the ecological consequences of management measures used in the bloom ponds and to evaluate the full potential of these algae as fish feed ingredient for various fish species. Furthermore, future study design including more ponds/aquariums would increase the statistical power in order to base conclusions on the effect of different treatments.

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Annexure

Annexure-1: Unprocessed data of environmental factors in the bloom ponds (BP-R and BP-Y) and non-bloom ponds (NBP) (Expt. 1)

Factors	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Water temperature (^{0}C)	BP-R	P1	32.10	31.47	30.05	28.60	24.90	21.63	17.21	23.00	24.10	25.39	26.41	31.42
		P2	32.28	31.50	30.00	28.30	24.45	21.85	17.33	22.36	24.11	25.55	26.36	31.58
		P3	32.10	31.42	29.98	28.40	24.02	21.50	17.10	22.41	24.20	25.42	26.35	31.50
	BP-Y	P4	32.09	31.49	30.03	28.45	24.68	21.74	17.37	23.14	24.21	25.47	26.39	31.50
		P5	32.12	31.40	29.94	28.28	24.45	21.70	17.32	23.15	24.29	25.45	26.44	31.54
		P6	32.07	31.84	30.43	28.43	24.63	21.57	17.20	23.06	24.17	25.40	26.45	31.50
	NBP	P7	32.18	31.53	30.10	28.40	24.33	21.60	17.43	23.30	24.20	25.39	26.60	31.59
		P8	32.12	31.80	30.60	28.25	24.70	21.05	17.03	23.50	24.45	25.29	26.27	31.74
		P9	32.58	31.70	30.65	28.12	24.55	21.00	17.36	23.39	24.37	25.12	26.22	31.62
Dissolved oxygen (mg/l)	BP-R	P1	5.00	5.01	4.55	4.30	3.94	4.60	4.59	5.14	5.19	5.49	5.34	5.62
		P2	5.29	5.30	5.00	4.12	3.88	4.86	5.14	5.36	5.42	5.78	5.06	5.26
		P3	5.18	4.80	4.70	4.40	4.40	4.48	5.04	5.11	5.09	5.29	5.10	5.61
	BP-Y	P4	5.30	5.06	4.93	4.32	4.01	4.84	4.84	5.35	5.29	5.32	5.15	5.10
		P5	5.16	5.29	4.55	4.66	3.96	4.32	5.01	5.52	5.51	5.56	5.13	5.16
		P6	5.21	4.95	4.81	4.39	4.22	5.04	4.66	5.19	5.45	5.63	5.44	5.30
	NBP	P7	5.72	5.34	5.38	5.86	5.47	5.34	5.23	5.42	5.89	5.95	5.62	5.94
		P8	5.80	5.82	6.00	5.70	5.67	5.78	5.63	5.65	5.92	6.12	5.91	5.91
		P9	5.46	5.62	5.65	5.37	6.05	5.81	5.70	5.86	6.10	5.87	5.80	5.64

Annexure -1: Continued

Factors	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
pH	BP-R	P1	6.29	6.38	6.24	6.32	5.95	5.88	6.45	6.52	6.38	6.41	6.36	6.29
		P2	6.59	6.14	6.39	5.99	6.23	6.19	5.97	6.41	6.24	6.15	6.12	6.70
		P3	6.52	6.13	5.91	6.04	5.84	5.75	6.51	6.87	6.88	6.50	6.60	6.81
	BP-Y	P4	6.34	6.16	6.22	6.30	5.84	6.00	6.60	6.22	6.71	6.30	6.69	6.50
		P5	6.69	6.32	5.70	6.13	6.30	6.32	6.70	6.74	6.82	6.32	6.36	6.71
		P6	6.45	6.53	6.04	5.85	5.84	5.85	6.14	6.27	6.32	6.68	6.64	6.73
	NBP	P7	7.60	7.58	7.44	7.44	7.58	7.81	7.85	8.05	7.91	7.80	7.73	7.48
		P8	7.83	8.06	8.30	7.80	8.09	8.27	7.41	7.35	7.31	7.54	7.72	7.90
		P9	8.33	8.16	8.34	8.20	8.15	7.67	7.74	8.13	7.89	8.01	7.95	7.99
Nitrate-nitrogen (mg/l)	BP-R	P1	0.95	1.12	1.30	1.52	1.69	1.68	1.09	1.29	1.06	1.00	1.03	0.93
		P2	1.02	1.09	1.19	1.34	1.81	1.76	1.17	1.13	0.89	0.88	1.11	0.97
		P3	0.90	0.89	1.48	1.49	1.54	1.83	1.26	0.97	1.11	1.07	0.96	1.22
	BP-Y	P4	1.06	1.21	1.36	1.37	1.67	1.81	1.29	1.21	0.87	0.93	1.07	0.92
		P5	0.96	1.12	1.29	1.42	1.83	1.59	1.37	1.05	1.08	0.88	1.09	0.98
		P6	1.04	1.01	1.41	1.59	1.93	1.66	1.10	1.19	1.04	1.03	1.26	1.06
	NBP	P7	0.20	0.47	0.53	0.40	0.33	0.39	0.36	0.39	0.58	0.53	0.62	0.55
		P8	0.40	0.30	0.49	0.69	0.60	0.67	0.57	0.56	0.43	0.38	0.46	0.40
		P9	0.28	0.51	0.40	0.53	0.54	0.57	0.50	0.53	0.56	0.50	0.50	0.38

Annexure -1: Continued

Factors	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Ammonium-nitrogen (mg/l)	BP-R	P1	0.78	0.89	1.18	1.40	1.38	1.22	0.97	1.01	0.84	0.80	0.92	0.94
		P2	0.52	0.83	1.08	1.44	1.50	1.53	1.14	1.03	1.12	0.88	0.80	0.82
		P3	0.74	0.62	1.10	1.33	1.42	1.44	1.21	0.95	0.94	1.01	0.89	1.01
	BP-Y	P4	0.91	0.86	1.23	1.34	1.32	1.38	1.16	1.02	0.98	0.78	0.98	0.83
		P5	0.63	0.74	0.99	1.36	1.51	1.32	1.26	0.89	0.90	1.02	1.12	1.04
		P6	0.57	0.89	1.19	1.52	1.64	1.59	1.15	1.12	0.85	0.82	1.03	0.80
	NBP	P7	0.13	0.30	0.36	0.24	0.16	0.14	0.33	0.32	0.33	0.26	0.16	0.31
		P8	0.29	0.17	0.21	0.31	0.39	0.16	0.17	0.16	0.14	0.19	0.28	0.24
		P9	0.20	0.25	0.26	0.20	0.20	0.32	0.23	0.17	0.25	0.29	0.13	0.15
Phosphate-phosphorus (mg/l)	BP-R	P1	1.02	0.91	1.11	1.40	1.79	1.92	1.30	0.80	1.01	0.80	0.92	0.85
		P2	0.89	0.71	1.23	1.35	1.50	1.71	1.08	1.13	0.86	0.91	1.17	1.12
		P3	0.98	1.03	1.30	1.56	1.72	1.95	1.53	0.80	0.79	0.71	1.23	1.09
	BP-Y	P4	0.91	0.81	1.10	1.28	1.55	1.88	0.93	1.13	0.89	0.83	0.89	1.01
		P5	1.02	1.09	1.27	1.52	1.83	1.83	1.19	0.78	0.92	0.96	1.04	0.86
		P6	1.13	1.19	1.25	1.47	1.88	1.69	1.26	1.20	1.06	1.01	1.13	0.98
	NBP	P7	0.34	0.24	0.50	0.48	0.58	0.50	0.56	0.41	0.52	0.39	0.50	0.49
		P8	0.27	0.37	0.36	0.30	0.48	0.54	0.49	0.37	0.34	0.36	0.33	0.30
		P9	0.19	0.34	0.39	0.37	0.35	0.36	0.39	0.56	0.47	0.62	0.39	0.37

Annexure -1: Continued

Factors	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Iron (mg/l)	BP-R	P1	0.32	0.48	0.43	0.68	0.79	0.62	0.41	0.38	0.33	0.37	0.45	0.39
		P2	0.35	0.39	0.52	0.70	0.69	0.73	0.53	0.50	0.42	0.28	0.53	0.44
		P3	0.40	0.51	0.53	0.63	0.87	0.72	0.42	0.49	0.33	0.41	0.57	0.38
	BP-Y	P4	0.34	0.46	0.48	0.69	0.59	0.76	0.49	0.47	0.48	0.45	0.52	0.42
		P5	0.48	0.55	0.40	0.67	0.82	0.82	0.63	0.53	0.56	0.48	0.61	0.57
		P6	0.36	0.46	0.45	0.48	0.81	0.68	0.57	0.47	0.40	0.36	0.53	0.40
	NBP	P7	0.10	0.09	0.12	0.29	0.21	0.27	0.13	0.28	0.24	0.22	0.23	0.18
		P8	0.15	0.15	0.19	0.21	0.25	0.19	0.24	0.20	0.18	0.16	0.19	0.13
		P9	0.09	0.11	0.17	0.21	0.15	0.14	0.19	0.17	0.12	0.18	0.16	0.10
Zinc (mg/l)	BP-R	P1	0.12	0.13	0.21	0.30	0.32	0.33	0.30	0.24	0.19	0.23	0.23	0.12
		P2	0.23	0.21	0.30	0.38	0.42	0.40	0.37	0.18	0.26	0.18	0.13	0.21
		P3	0.18	0.22	0.19	0.34	0.39	0.36	0.30	0.29	0.15	0.25	0.21	0.17
	BP-Y	P4	0.18	0.17	0.30	0.39	0.45	0.40	0.37	0.29	0.20	0.21	0.18	0.14
		P5	0.13	0.28	0.31	0.36	0.47	0.46	0.42	0.34	0.25	0.22	0.27	0.26
		P6	0.26	0.21	0.20	0.24	0.34	0.35	0.30	0.24	0.26	0.14	0.23	0.15
	NBP	P7	0.10	0.01	0.08	0.14	0.10	0.12	0.09	0.13	0.16	0.11	0.10	0.08
		P8	0.05	0.04	0.03	0.07	0.13	0.07	0.12	0.10	0.08	0.10	0.03	0.04
		P9	0.07	0.07	0.10	0.08	0.09	0.15	0.09	0.09	0.10	0.06	0.09	0.07

Annexure -1: Continued

Factors	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Manganese (mg/l)	BP-R	P1	0.12	0.16	0.23	0.33	0.32	0.29	0.30	0.27	0.22	0.20	0.23	0.18
		P2	0.14	0.13	0.27	0.31	0.36	0.35	0.31	0.34	0.20	0.19	0.22	0.22
		P3	0.21	0.28	0.24	0.38	0.40	0.38	0.33	0.31	0.27	0.26	0.23	0.20
	BP-Y	P4	0.13	0.15	0.25	0.32	0.34	0.32	0.31	0.31	0.21	0.20	0.23	0.20
		P5	0.18	0.21	0.26	0.35	0.38	0.37	0.32	0.33	0.24	0.23	0.23	0.21
		P6	0.15	0.20	0.17	0.26	0.26	0.23	0.20	0.20	0.21	0.20	0.17	0.17
	NBP	P7	0.09	0.12	0.10	0.14	0.11	0.08	0.06	0.09	0.15	0.13	0.11	0.13
		P8	0.06	0.10	0.07	0.10	0.18	0.15	0.13	0.12	0.13	0.09	0.14	0.10
		P9	0.04	0.05	0.11	0.09	0.13	0.16	0.14	0.14	0.09	0.11	0.08	0.08
Copper (mg/l)	BP-R	P1	0.25	0.19	0.20	0.24	0.29	0.27	0.22	0.18	0.16	0.21	0.25	0.20
		P2	0.20	0.30	0.29	0.27	0.31	0.33	0.20	0.24	0.21	0.18	0.22	0.17
		P3	0.27	0.28	0.33	0.26	0.34	0.31	0.28	0.32	0.26	0.25	0.18	0.24
	BP-Y	P4	0.21	0.28	0.25	0.36	0.39	0.40	0.21	0.21	0.19	0.20	0.24	0.19
		P5	0.28	0.27	0.34	0.27	0.40	0.39	0.24	0.24	0.24	0.22	0.20	0.21
		P6	0.29	0.34	0.25	0.35	0.32	0.29	0.31	0.24	0.16	0.14	0.22	0.13
	NBP	P7	0.10	0.06	0.13	0.15	0.11	0.08	0.09	0.12	0.16	0.13	0.14	0.10
		P8	0.06	0.13	0.07	0.13	0.09	0.09	0.12	0.09	0.11	0.07	0.09	0.08
		P9	0.04	0.08	0.12	0.10	0.12	0.15	0.13	0.14	0.10	0.11	0.08	0.07

Annexure -2: Unprocessed data of soil organic matter in the bloom ponds (BP-R and BP-Y) and non-bloom ponds (NBP) (Expt. 1)

Factors	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Soil organic matter (%)	BP-R	P1	5.55	4.26	4.83	5.88	6.10	7.07	7.38	6.17	5.52	4.40	5.12	5.17
		P2	5.00	5.79	6.21	6.76	7.65	7.29	7.57	5.69	4.93	5.41	6.03	5.76
		P3	5.38	5.14	5.90	5.69	7.24	8.36	8.98	7.24	6.24	5.64	5.17	4.91
	BP-Y	P4	5.28	5.12	5.52	6.76	6.36	7.18	7.09	5.93	5.22	6.19	5.58	6.15
		P5	5.90	5.78	6.33	6.57	7.71	8.29	7.90	6.91	6.91	5.27	6.15	5.34
		P6	5.35	4.89	5.57	6.03	7.60	7.45	7.84	6.45	5.64	4.60	5.02	5.22
	NBP	P7	3.43	3.62	3.67	3.26	3.00	3.86	3.41	3.50	3.03	3.28	3.93	2.76
		P8	2.33	2.90	4.38	2.84	2.31	2.53	4.50	3.00	3.97	2.84	2.28	3.33
		P9	3.00	3.14	2.59	3.72	3.50	2.88	2.43	2.69	2.59	2.52	3.00	4.15

Annexure -3: Unprocessed data of algal density in the bloom ponds (BP-R and BP-Y) and non-bloom ponds (NBP) (Expt. 1)

Group of algae	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Total planktonic algae (x 10^4 cells/l)	BP-R	P1	14.18	21.28	28.25	29.44	35.72	38.93	22.61	15.37	15.99	18.00	23.05	14.33
		P2	17.06	21.95	28.69	33.36	34.83	38.98	24.54	22.01	12.42	24.03	25.56	21.15
		P3	14.84	20.25	24.18	29.99	28.86	31.66	25.36	13.09	21.36	13.58	16.05	12.88
	BP-Y	P4	15.62	21.61	29.69	32.07	41.92	40.97	22.64	19.79	20.44	24.62	16.86	14.65
		P5	20.26	19.60	25.85	26.20	36.28	39.80	31.99	16.76	18.04	16.87	24.80	13.92
		P6	15.93	26.54	31.56	36.26	39.33	44.92	23.41	16.84	16.09	17.07	20.61	17.05
	NBP	P7	16.24	20.09	18.84	17.67	15.33	17.70	15.66	13.52	17.68	16.80	17.18	15.37
		P8	15.98	16.02	23.17	20.25	19.69	16.39	16.04	16.08	12.36	14.55	15.89	15.87
		P9	14.27	23.05	20.52	20.66	19.08	12.72	16.93	13.15	15.11	12.44	13.85	13.96

Annexure -3: Continued

Group of algae	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Euglenophytes (x 10^4 cells/l)	BP-R	P1	5.62	9.57	20.36	23.39	31.48	34.85	16.78	9.85	10.25	11.23	17.27	8.64
		P2	10.51	12.43	17.92	24.81	28.86	34.99	16.49	11.81	6.84	14.47	16.54	11.61
		P3	8.94	7.72	13.47	19.44	22.48	25.81	18.78	6.85	12.25	7.69	10.25	6.98
	BP-Y	P4	8.07	10.00	20.54	24.22	37.86	38.04	16.64	13.43	14.28	14.60	10.48	9.13
		P5	12.95	8.08	13.70	17.30	31.07	35.21	23.12	7.26	9.55	10.65	16.40	4.20
		P6	6.85	12.63	20.49	28.69	35.19	43.11	17.28	10.49	7.92	10.67	14.84	11.04
	NBP	P7	1.63	2.48	2.34	3.04	2.36	4.28	2.30	2.72	4.86	3.82	3.91	2.25
		P8	3.12	1.23	3.77	4.52	5.93	3.85	4.51	3.36	1.95	2.05	4.25	3.85
		P9	1.98	3.88	1.54	1.96	2.72	2.05	1.89	1.43	2.14	3.24	2.26	2.67
Cyanophytes (x 10^4 cells/l)	BP-R	P1	5.23	9.77	6.10	3.62	2.85	2.90	3.36	2.75	3.11	2.91	1.48	2.15
		P2	4.26	5.89	7.54	5.19	3.97	1.75	4.84	6.58	2.69	5.47	5.16	5.41
		P3	3.64	9.46	8.11	7.64	4.60	4.32	3.58	3.21	4.88	3.13	2.97	2.47
	BP-Y	P4	4.75	8.33	5.82	4.91	2.41	1.17	3.10	3.17	3.40	5.69	2.32	2.28
		P5	3.95	8.18	8.83	5.92	4.47	2.54	5.79	6.40	4.82	2.80	5.03	5.44
		P6	6.34	10.33	7.27	4.52	3.18	1.18	3.99	3.21	3.74	2.81	2.20	2.35
	NBP	P7	10.21	13.24	12.14	10.28	7.69	9.49	9.55	6.89	8.10	8.95	10.12	9.37
		P8	9.16	9.40	14.37	10.32	9.24	8.19	7.42	8.61	6.78	8.97	8.20	8.91
		P9	8.24	13.87	13.51	13.59	11.74	6.17	10.64	7.21	8.74	4.99	7.96	8.11

Annexure -3: Continued

Group of algae	Ponds		Sampling Month (2010-2011)											
			Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Chlorophytes (x 10^4 cells/l)	BP-R	P1	3.12	1.78	1.62	2.28	1.29	1.14	2.33	2.44	2.22	3.47	3.82	3.17
		P2	2.11	3.44	3.11	3.22	1.95	2.10	3.11	3.42	2.61	3.78	3.46	3.84
		P3	1.99	2.86	2.34	2.71	1.63	1.46	2.81	2.76	3.89	2.43	2.51	3.12
	BP-Y	P4	2.62	2.90	3.12	2.75	1.60	1.62	2.79	2.93	2.42	3.97	3.64	3.01
		P5	3.01	3.15	2.98	2.82	0.59	1.95	2.86	2.94	3.25	3.11	2.99	3.98
		P6	2.45	3.27	3.54	2.74	0.89	0.58	1.99	2.88	4.13	3.38	3.34	3.30
	NBP	P7	4.01	3.64	3.71	3.52	4.50	3.21	3.12	3.17	4.11	3.12	2.54	3.31
		P8	3.27	4.71	4.30	4.57	3.84	3.66	3.60	3.52	2.87	2.79	2.76	2.55
		P9	3.54	4.68	4.69	4.19	3.93	3.90	3.83	3.89	3.52	3.44	2.93	2.64
Bacillariophytes (x 10^4 cells/l)	BP-R	P1	0.21	0.16	0.17	0.15	0.10	0.04	0.14	0.33	0.41	0.39	0.48	0.37
		P2	0.18	0.19	0.12	0.14	0.05	0.14	0.10	0.20	0.28	0.31	0.40	0.29
		P3	0.27	0.21	0.26	0.20	0.15	0.07	0.19	0.27	0.34	0.33	0.32	0.31
	BP-Y	P4	0.20	0.38	0.22	0.20	0.05	0.14	0.11	0.27	0.35	0.36	0.42	0.24
		P5	0.35	0.20	0.34	0.17	0.15	0.10	0.22	0.16	0.42	0.32	0.39	0.30
		P6	0.29	0.31	0.26	0.31	0.07	0.05	0.15	0.25	0.30	0.21	0.24	0.36
	NBP	P7	0.39	0.73	0.65	0.83	0.78	0.72	0.69	0.74	0.61	0.91	0.61	0.44
		P8	0.43	0.68	0.73	0.84	0.68	0.69	0.51	0.59	0.76	0.74	0.68	0.56
		P9	0.51	0.62	0.78	0.92	0.69	0.60	0.57	0.62	0.71	0.77	0.70	0.54

Annexure-4: Unprocessed data of water quality parameters in the study ponds under four treatments (Expt. 3)

Parameter	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
Temperature (^{0}C)	T1	P1	32.43	32.28	31.72	31.61	30.65	29.39	28.00	25.76	22.40	19.22
		P2	32.58	32.39	31.57	31.49	30.90	29.42	27.80	25.53	22.60	19.34
		P3	32.53	32.48	31.80	31.68	30.84	29.50	27.75	25.70	22.48	19.28
	T2	P4	32.49	32.31	31.78	31.69	30.86	29.75	27.80	25.49	22.34	19.30
		P5	32.57	32.58	31.90	31.66	30.93	29.37	27.90	25.37	22.30	19.36
		P6	32.50	32.36	31.74	31.64	30.85	29.44	27.84	25.54	22.44	19.40
	T3	P7	32.49	32.39	31.84	31.72	30.93	29.47	27.70	25.44	22.39	19.19
		P8	32.52	32.40	31.78	31.67	30.94	29.46	27.70	25.43	22.44	19.39
		P9	32.48	32.42	31.93	31.81	31.00	29.50	27.60	25.33	22.33	19.51
	T4	P10	32.50	32.41	31.85	31.62	30.78	29.44	27.55	25.46	22.21	19.34
		P11	32.48	32.32	31.90	31.77	30.91	29.51	27.70	25.54	22.37	19.41
		P12	32.51	32.44	31.94	31.70	30.90	29.56	27.64	25.46	22.39	19.48
Dissolved oxygen (mg/l)	T1	P1	6.34	6.12	5.60	5.27	5.43	5.69	5.38	5.05	5.20	5.16
		P2	6.26	6.28	6.07	5.36	5.48	5.81	5.11	5.02	5.05	5.08
		P3	6.31	6.04	5.89	5.63	5.25	5.49	5.20	5.28	5.04	5.30
	T2	P4	6.32	6.10	5.61	5.51	5.03	5.09	5.01	5.10	5.02	5.10
		P5	6.21	6.04	5.40	5.49	5.10	5.01	5.04	5.01	5.11	5.06
		P6	6.21	6.17	5.35	5.27	5.29	5.30	5.24	5.23	5.19	5.20
	T3	P7	6.40	6.10	5.73	5.66	5.68	5.56	5.40	5.42	5.29	5.25
		P8	6.24	5.84	5.59	5.51	5.57	5.41	5.30	5.34	5.20	5.06
		P9	6.35	5.60	5.51	5.40	5.39	5.32	5.18	5.22	5.12	5.34
	T4	P10	5.62	5.54	5.28	5.23	4.97	4.46	4.67	4.21	4.29	4.33
		P11	5.24	5.13	5.02	4.92	4.56	4.23	4.41	3.92	4.01	4.09
		P12	5.50	5.39	5.16	5.00	4.72	4.38	4.42	4.13	4.33	4.29

Annexure-4: Continued

Parameter	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
pH	T1	P1	6.64	6.81	7.06	7.00	7.01	7.03	7.17	7.05	7.19	7.10
		P2	6.45	6.95	7.46	7.29	7.09	7.10	7.35	7.29	7.38	7.24
		P3	6.33	7.10	7.15	7.42	7.36	7.41	7.10	7.06	6.98	6.95
	T2	P4	6.75	7.03	7.12	8.12	7.40	7.53	7.61	7.60	7.86	7.70
		P5	6.86	7.33	7.43	8.02	7.39	7.64	7.83	7.57	7.40	7.49
		P6	6.98	6.96	7.46	7.74	7.77	7.35	7.39	7.85	7.50	7.90
	T3	P7	6.77	7.12	7.45	7.99	8.10	7.97	7.99	7.95	7.56	7.60
		P8	6.54	7.34	7.22	7.74	7.77	8.26	7.86	8.03	7.75	7.80
		P9	7.10	7.51	7.82	7.91	7.95	7.84	8.08	7.69	7.96	8.02
	T4	P10	7.12	6.42	6.53	6.42	6.34	6.38	6.23	6.14	6.07	6.18
		P11	7.04	6.39	6.25	6.19	5.83	5.94	5.80	6.01	6.15	5.90
		P12	6.76	6.67	5.95	6.03	6.04	5.98	6.02	5.88	5.89	5.86
Nitrate-nitrogen (mg/l)	T1	P1	1.23	0.94	0.95	0.55	0.57	0.66	0.59	0.64	0.60	0.75
		P2	1.05	0.76	0.81	0.83	0.65	0.77	0.86	0.52	0.42	0.49
		P3	1.20	0.80	0.70	0.79	0.85	0.59	0.68	0.75	0.70	0.63
	T2	P4	1.40	1.34	1.11	1.27	1.28	1.33	1.14	0.99	0.96	1.02
		P5	1.09	1.12	0.83	1.01	1.13	1.09	1.24	1.39	1.27	1.43
		P6	1.22	0.99	0.93	1.05	0.87	1.10	0.81	1.10	1.34	1.07
	T3	P7	1.24	0.78	0.83	0.69	0.74	0.79	0.82	0.64	0.63	0.59
		P8	0.98	0.86	0.71	0.62	0.54	0.51	0.36	0.49	0.46	0.42
		P9	1.29	1.12	0.97	0.78	0.66	0.60	0.64	0.50	0.36	0.57
	T4	P10	1.14	1.17	1.39	1.52	1.34	1.60	1.78	1.63	1.51	1.40
		P11	1.02	1.20	1.09	1.24	1.54	1.31	1.56	1.92	1.38	1.32
		P12	0.90	0.89	1.15	1.49	1.57	1.43	1.79	1.73	1.64	1.57

Annexure-4: Continued

Parameter	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
Ammonium-nitrogen (mg/l)	T1	P1	0.90	0.83	0.57	0.42	0.54	0.55	0.47	0.32	0.39	0.31
		P2	1.10	0.91	0.77	0.69	0.41	0.49	0.50	0.45	0.28	0.19
		P3	1.21	1.10	0.82	0.71	0.66	0.40	0.33	0.24	0.24	0.27
	T2	P4	1.08	0.86	0.89	0.83	0.80	0.72	0.83	0.64	0.66	0.51
		P5	1.23	1.01	0.97	0.94	0.95	0.64	0.84	0.75	0.79	0.68
		P6	1.16	1.12	1.09	1.01	0.98	0.86	0.97	0.85	0.64	0.70
	T3	P7	0.99	0.78	0.89	0.54	0.43	0.51	0.29	0.27	0.31	0.28
		P8	1.19	0.84	0.76	0.67	0.64	0.45	0.50	0.41	0.19	0.15
		P9	1.11	0.96	0.70	0.70	0.47	0.31	0.33	0.20	0.16	0.20
	T4	P10	1.15	1.20	1.14	1.02	1.29	1.31	1.40	1.55	1.53	1.51
		P11	0.98	1.24	1.30	1.23	1.28	1.49	1.67	1.56	1.39	1.34
		P12	1.24	1.13	1.19	1.21	1.50	1.42	1.37	1.72	1.45	1.37
Phosphate-phosphorus (mg/l)	T1	P1	1.24	0.76	0.65	0.89	0.57	0.45	0.56	0.49	0.50	0.67
		P2	0.96	0.89	0.84	0.65	0.88	0.65	0.68	0.66	0.54	0.64
		P3	1.16	1.02	0.94	0.97	0.72	0.92	0.84	0.73	0.76	0.43
	T2	P4	0.84	0.74	0.80	1.33	1.27	1.49	1.23	0.96	0.88	1.37
		P5	1.25	1.21	0.97	0.92	1.28	1.44	1.50	1.41	1.32	0.99
		P6	0.96	0.78	1.09	1.23	1.42	1.23	1.12	1.19	1.08	1.02
	T3	P7	1.34	1.20	0.83	0.81	0.73	0.52	0.70	0.63	0.59	0.41
		P8	1.26	0.81	0.71	0.57	0.49	0.54	0.50	0.43	0.60	0.60
		P9	1.03	0.88	0.63	0.56	0.62	0.71	0.61	0.61	0.37	0.39
	T4	P10	1.25	1.17	1.29	1.39	1.38	1.56	2.25	2.00	1.92	1.86
		P11	1.36	1.32	1.13	1.19	1.23	1.78	2.27	2.13	1.78	1.98
		P12	1.14	1.05	1.17	1.39	1.49	1.85	2.04	2.21	2.11	1.70

Annexure-4: Continued

Parameter	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
Iron (mg/l)	T1	P1	0.41	0.37	0.31	0.34	0.29	0.31	0.24	0.21	0.22	0.25
		P2	0.45	0.48	0.43	0.36	0.38	0.29	0.23	0.24	0.19	0.23
		P3	0.36	0.40	0.32	0.28	0.21	0.18	0.15	0.26	0.24	0.12
	T2	P4	0.51	0.48	0.39	0.40	0.37	0.41	0.43	0.35	0.24	0.21
		P5	0.38	0.39	0.34	0.38	0.29	0.34	0.30	0.26	0.34	0.27
		P6	0.43	0.40	0.44	0.33	0.38	0.28	0.36	0.41	0.33	0.35
	T3	P7	0.39	0.42	0.35	0.36	0.29	0.25	0.21	0.27	0.22	0.18
		P8	0.47	0.35	0.19	0.21	0.25	0.19	0.24	0.20	0.18	0.11
		P9	0.43	0.29	0.17	0.21	0.15	0.14	0.19	0.14	0.12	0.13
	T4	P10	0.66	0.56	0.71	0.75	0.83	0.74	0.66	0.62	0.75	0.65
		P11	0.48	0.64	0.73	0.77	0.72	0.63	0.83	0.74	0.69	0.70
		P12	0.52	0.66	0.61	0.67	0.67	0.70	0.77	0.77	0.79	0.61
Zinc (mg/l)	T1	P1	0.14	0.10	0.12	0.09	0.13	0.16	0.11	0.10	0.08	0.10
		P2	0.27	0.14	0.17	0.19	0.18	0.10	0.12	0.16	0.13	0.12
		P3	0.29	0.18	0.09	0.17	0.24	0.17	0.09	0.13	0.15	0.08
	T2	P4	0.28	0.13	0.22	0.21	0.18	0.14	0.16	0.19	0.24	0.16
		P5	0.37	0.28	0.28	0.29	0.25	0.24	0.26	0.27	0.29	0.29
		P6	0.22	0.26	0.21	0.19	0.22	0.21	0.23	0.23	0.23	0.20
	T3	P7	0.27	0.14	0.18	0.15	0.18	0.14	0.11	0.20	0.12	0.10
		P8	0.29	0.12	0.15	0.10	0.16	0.13	0.21	0.17	0.11	0.14
		P9	0.20	0.13	0.16	0.18	0.21	0.14	0.12	0.11	0.15	0.08
	T4	P10	0.24	0.22	0.27	0.39	0.39	0.32	0.44	0.31	0.30	0.31
		P11	0.33	0.28	0.30	0.43	0.32	0.40	0.43	0.35	0.26	0.33
		P12	0.32	0.33	0.33	0.35	0.34	0.38	0.34	0.37	0.31	0.26

Annexure-4: Continued

Parameter	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
Manganese (mg/l)	T1	P1	0.23	0.14	0.19	0.15	0.23	0.18	0.14	0.13	0.19	0.22
		P2	0.24	0.09	0.11	0.09	0.13	0.16	0.14	0.14	0.16	0.11
		P3	0.18	0.12	0.12	0.11	0.16	0.09	0.06	0.09	0.15	0.11
	T2	P4	0.27	0.26	0.15	0.25	0.17	0.19	0.19	0.21	0.11	0.18
		P5	0.16	0.14	0.20	0.20	0.24	0.23	0.25	0.24	0.21	0.22
		P6	0.26	0.24	0.19	0.15	0.22	0.16	0.16	0.18	0.17	0.15
	T3	P7	0.15	0.22	0.15	0.19	0.17	0.16	0.19	0.21	0.18	0.18
		P8	0.23	0.09	0.09	0.11	0.14	0.13	0.11	0.12	0.12	0.13
		P9	0.22	0.20	0.07	0.11	0.08	0.13	0.09	0.11	0.10	0.11
	T4	P10	0.16	0.26	0.21	0.20	0.23	0.29	0.33	0.32	0.31	0.30
		P11	0.26	0.30	0.28	0.29	0.32	0.26	0.31	0.36	0.35	0.31
		P12	0.24	0.31	0.27	0.24	0.23	0.30	0.38	0.40	0.36	0.33
Copper (mg/l)	T1	P1	0.17	0.14	0.11	0.12	0.17	0.06	0.13	0.06	0.10	0.14
		P2	0.16	0.12	0.08	0.09	0.11	0.12	0.14	0.15	0.14	0.08
		P3	0.20	0.14	0.13	0.14	0.13	0.15	0.19	0.09	0.11	0.16
	T2	P4	0.16	0.14	0.14	0.19	0.20	0.20	0.27	0.19	0.15	0.22
		P5	0.14	0.12	0.09	0.11	0.13	0.19	0.21	0.15	0.18	0.14
		P6	0.21	0.16	0.13	0.13	0.15	0.28	0.18	0.22	0.15	0.19
	T3	P7	0.29	0.24	0.11	0.25	0.16	0.19	0.21	0.11	0.18	0.15
		P8	0.18	0.16	0.21	0.17	0.16	0.16	0.14	0.14	0.11	0.08
		P9	0.25	0.16	0.20	0.18	0.22	0.23	0.12	0.11	0.11	0.09
	T4	P10	0.28	0.28	0.23	0.31	0.29	0.21	0.24	0.20	0.25	0.29
		P11	0.21	0.20	0.24	0.23	0.24	0.27	0.26	0.28	0.20	0.20
		P12	0.27	0.27	0.30	0.27	0.28	0.30	0.32	0.27	0.26	0.26

Annexure-5: Unprocessed data of soil organic matter in the study ponds under four treatments (Expt. 3)

Parameter	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
Soil organic matter (%)	T1	P1	5.05	4.29	4.22	4.15	4.00	3.52	3.31	3.29	3.26	3.10
		P2	4.64	5.21	5.05	4.62	4.71	4.28	4.26	4.00	4.09	3.91
		P3	5.43	4.71	4.31	3.64	3.95	4.41	4.07	4.28	4.15	3.69
	T2	P4	5.14	2.88	3.29	2.69	3.10	3.41	3.14	3.09	3.72	3.19
		P5	4.00	3.14	2.79	3.72	3.55	3.15	3.64	3.72	2.98	3.31
		P6	4.98	3.64	3.17	3.00	2.79	3.76	3.26	3.69	3.52	2.65
	T3	P7	4.03	3.34	3.05	2.84	2.65	2.88	3.00	2.97	2.81	3.00
		P8	4.97	3.17	2.97	3.62	3.69	3.65	3.72	3.29	3.09	3.10
		P9	4.81	4.09	3.69	3.52	3.14	2.97	2.81	2.74	2.52	2.53
	T4	P10	4.41	5.88	6.29	6.55	7.78	6.84	7.57	6.93	6.43	7.21
		P11	5.03	7.14	7.46	7.76	7.84	6.69	6.62	5.53	6.43	6.34
		P12	5.60	7.07	7.10	7.10	6.86	7.62	6.36	6.15	7.29	6.02

Annexure-6: Unprocessed data of algal density in the study ponds under four treatments (Expt. 3)

Group of algae	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
Total planktonic algae ($x10^4$ cells/l)	T1	P1	15.76	23.19	21.59	19.40	18.40	18.85	14.38	16.26	15.10	14.89
		P2	16.59	20.49	17.47	17.38	16.63	17.56	19.66	17.84	17.40	16.79
		P3	18.55	22.04	20.16	13.26	17.83	16.56	18.72	18.99	18.96	18.91
	T2	P4	17.24	22.78	21.86	17.80	19.84	14.99	16.34	16.32	13.10	11.15
		P5	14.65	16.32	18.11	12.95	14.15	12.66	16.18	12.13	15.53	18.99
		P6	19.24	20.81	17.94	17.46	15.07	15.47	19.66	20.64	16.54	13.74
	T3	P7	16.05	20.94	19.20	16.87	14.06	12.01	10.64	12.66	11.42	9.16
		P8	16.03	20.22	16.47	16.22	16.75	14.28	14.45	15.40	10.73	12.00
		P9	14.04	17.24	16.86	13.78	14.57	12.95	11.85	10.70	13.68	13.51
	T4	P10	21.49	20.30	23.32	20.48	24.27	26.27	27.76	34.44	34.27	32.77
		P11	20.45	18.22	22.50	25.19	20.43	24.46	30.49	33.54	36.27	32.66
		P12	18.26	21.56	24.93	26.40	24.22	22.22	28.41	30.83	33.90	35.86

Annexure-6: Continued

Group of algae	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
Euglenophytes (x10^4 cells/l)	T1	P1	7.13	10.64	10.10	11.06	10.24	11.70	6.21	7.54	7.49	6.91
		P2	9.14	7.66	7.15	8.16	7.18	8.94	11.24	10.32	9.94	9.33
		P3	9.90	8.58	8.46	5.71	9.37	7.31	8.78	10.62	9.21	10.21
	T2	P4	9.87	10.72	11.09	9.34	10.29	7.23	7.08	8.24	5.68	5.57
		P5	6.50	5.82	7.28	4.87	5.89	5.21	8.37	4.87	9.20	11.04
		P6	12.45	8.48	6.03	9.07	6.52	8.91	10.98	11.84	9.06	6.92
	T3	P7	9.48	8.12	6.15	4.76	4.25	3.69	3.11	3.97	4.08	2.39
		P8	10.20	8.84	4.10	5.94	6.89	5.75	7.01	5.68	3.41	4.74
		P9	8.79	5.79	5.46	3.27	3.47	3.02	4.79	2.41	7.13	5.29
	T4	P10	12.98	12.14	14.20	11.57	17.52	19.45	22.36	29.12	29.54	27.09
		P11	10.89	10.10	11.67	15.37	13.35	16.58	24.16	26.29	30.47	25.31
		P12	9.54	12.94	13.61	16.68	17.49	16.51	21.24	24.42	27.64	28.90
Cyanophytes (x10^4 cells/l)	T1	P1	5.55	9.52	8.13	4.08	3.59	3.23	3.29	4.99	3.70	4.76
		P2	5.10	9.71	6.69	5.31	5.18	3.97	3.88	4.07	3.49	3.84
		P3	5.03	10.09	7.48	4.46	3.77	4.51	4.57	4.19	5.13	5.12
	T2	P4	4.10	8.09	7.31	5.02	6.15	3.96	5.17	4.58	4.27	3.01
		P5	5.41	7.14	6.79	4.44	4.94	4.07	4.09	4.02	3.10	4.84
		P6	4.26	8.45	8.52	4.42	4.81	3.21	5.27	5.15	3.71	3.69
	T3	P7	4.07	9.18	8.94	7.55	5.22	4.10	3.89	5.17	4.30	3.80
		P8	3.69	8.20	8.36	6.11	5.01	4.14	3.76	5.83	4.09	4.12
		P9	3.17	8.21	7.02	6.26	6.56	5.71	3.09	4.63	3.24	5.42
	T4	P10	5.69	5.96	6.89	6.03	4.02	3.90	3.02	3.42	2.66	3.48
		P11	6.17	5.14	7.82	7.42	4.95	4.81	3.73	4.90	3.14	5.17
		P12	5.88	6.21	8.71	7.45	4.62	3.23	4.83	3.65	4.12	4.14

Annexure-6: Continued

Group of algae	Treatment/ Pond		Sampling time									
			16-Aug	31-Aug	15-Sep	30-Sep	16-Oct	31-Oct	15-Nov	30-Nov	15-Dec	30-Dec
Chlorophytes ($x10^4$ cells/l)	T1	P1	2.59	2.61	2.78	3.42	3.96	3.24	4.19	3.09	3.46	2.65
		P2	1.97	2.69	2.95	3.21	3.58	3.91	3.87	2.88	3.47	3.18
		P3	3.11	3.04	3.62	2.32	3.97	3.92	4.56	3.51	4.13	3.07
	T2	P4	2.85	3.46	2.92	2.98	2.81	3.38	3.60	3.02	2.69	2.10
		P5	2.30	2.87	3.52	3.25	2.82	3.02	3.19	2.77	2.70	2.61
		P6	2.17	3.27	2.90	3.52	3.22	2.90	2.95	3.07	3.21	2.72
	T3	P7	2.34	3.12	3.52	4.02	3.97	3.67	2.94	2.93	2.45	2.40
		P8	1.89	2.65	3.36	3.51	4.17	3.95	3.07	3.26	2.74	2.54
		P9	1.81	2.80	3.64	3.61	3.76	3.69	3.43	3.09	2.75	2.34
	T4	P10	2.62	2.04	2.09	2.60	2.57	2.63	2.48	1.77	1.80	1.99
		P11	3.26	2.77	2.78	2.13	1.86	2.91	2.43	2.21	2.53	2.00
		P12	2.59	2.10	2.37	2.08	1.85	2.21	2.13	2.51	1.97	2.69
Bacillariophytes ($x10^4$ cells/l)	T1	P1	0.49	0.42	0.58	0.84	0.61	0.68	0.69	0.64	0.45	0.57
		P2	0.38	0.43	0.68	0.70	0.69	0.74	0.67	0.57	0.50	0.44
		P3	0.51	0.33	0.60	0.77	0.72	0.82	0.81	0.67	0.49	0.51
	T2	P4	0.42	0.51	0.54	0.46	0.59	0.42	0.49	0.48	0.46	0.47
		P5	0.44	0.49	0.52	0.39	0.50	0.36	0.53	0.47	0.53	0.50
		P6	0.36	0.61	0.49	0.45	0.52	0.45	0.46	0.58	0.56	0.41
	T3	P7	0.16	0.52	0.59	0.54	0.62	0.55	0.70	0.59	0.59	0.57
		P8	0.25	0.53	0.65	0.66	0.68	0.44	0.61	0.63	0.49	0.60
		P9	0.27	0.44	0.74	0.64	0.78	0.53	0.54	0.57	0.56	0.46
	T4	P10	0.20	0.16	0.14	0.28	0.16	0.29	0.23	0.13	0.27	0.21
		P11	0.13	0.21	0.23	0.27	0.27	0.16	0.17	0.14	0.13	0.18
		P12	0.25	0.31	0.24	0.19	0.26	0.27	0.21	0.25	0.17	0.13

Annexure-7: Unprocessed data of weight increment (g) of the fish species in the study ponds under four treatments (Expt. 3)

Fish species	Treatments/Ponds		Sampling time					
			Initial	August	Sep	Oct	Nov	Dec
Rohu (*Labeo rohita*)	T1	P1	25.64	72.30	111.30	144.60	186.28	213.46
		P2	25.64	66.16	108.50	150.80	181.31	202.40
		P3	25.64	68.93	101.50	140.56	169.21	211.20
	T2	P4	25.64	70.14	102.30	142.50	167.45	202.36
		P5	25.64	67.70	97.80	128.26	157.87	188.72
		P6	25.64	60.10	95.35	135.00	159.54	199.80
	T3	P7	25.64	78.54	112.64	159.30	196.35	240.88
		P8	25.64	72.58	102.52	152.40	186.38	236.58
		P9	25.64	65.20	110.71	147.39	190.89	223.68
	T4	P10	25.64	65.19	89.36	123.47	139.36	186.54
		P11	25.64	63.76	92.60	120.30	147.99	170.18
		P12	25.64	71.50	94.62	118.55	152.32	173.28
Catla (*Catla catla*)	T1	P1	29.27	93.64	144.70	172.58	210.30	263.46
		P2	29.27	89.47	138.56	178.45	200.40	272.26
		P3	29.27	84.69	135.64	168.98	213.50	278.80
	T2	P4	29.27	89.48	136.22	165.29	205.84	254.80
		P5	29.27	81.27	129.54	156.32	199.26	266.14
		P6	29.27	84.12	124.16	162.40	201.30	262.34
	T3	P7	29.27	85.35	130.46	162.58	211.46	288.90
		P8	29.27	91.28	144.25	171.48	236.24	308.45
		P9	29.27	88.77	138.56	175.65	210.36	306.58
	T4	P10	29.27	77.98	122.34	145.68	195.24	214.58
		P11	29.27	73.58	115.89	155.26	180.20	220.31
		P12	29.27	72.89	119.36	164.10	182.48	225.40

Annexure-7: Continued

Fish species	Treatments/Ponds		Sampling time					
			Initial	August	Sep	Oct	Nov	Dec
Mrigel (*Cirrhina mrigala*)	T1	P1	15.70	51.28	99.60	143.27	170.25	189.76
		P2	15.70	48.48	96.13	131.25	161.16	201.21
		P3	15.70	58.46	102.58	140.54	166.22	206.20
	T2	P4	15.70	49.66	96.22	136.54	161.84	195.46
		P5	15.70	42.25	95.59	120.41	151.37	188.26
		P6	15.70	43.28	84.28	133.62	163.50	192.36
	T3	P7	15.70	61.25	89.94	139.36	170.48	225.28
		P8	15.70	50.37	98.87	131.70	183.24	214.50
		P9	15.70	53.19	102.23	141.00	169.33	216.54
	T4	P10	15.70	46.35	91.43	119.47	152.36	182.34
		P11	15.70	41.18	81.88	114.00	142.37	169.13
		P12	15.70	41.36	85.28	116.00	144.34	166.33
Silver carp (*Hypophthalmichthys molitrix*)	T1	P1	17.82	85.27	159.12	254.20	329.84	402.30
		P2	17.82	91.48	151.23	264.50	333.14	398.24
		P3	17.82	94.66	166.25	263.04	339.78	379.54
	T2	P4	17.82	77.46	141.80	226.80	280.30	370.60
		P5	17.82	82.25	137.57	230.23	295.75	382.42
		P6	17.82	84.44	152.30	237.55	297.11	376.58
	T3	P7	17.82	99.32	174.16	288.59	355.24	418.54
		P8	17.82	88.58	162.14	278.54	349.79	410.20
		P9	17.82	96.26	157.36	286.30	338.84	398.60
	T4	P10	17.82	79.84	132.64	199.58	270.46	352.30
		P11	17.82	70.21	130.20	211.24	277.44	357.40
		P12	17.82	75.68	147.82	221.30	281.38	343.56

Annexure-7: Continued

Fish species	Treatments/Ponds		Sampling time					
			Initial	**August**	**Sep**	**Oct**	**Nov**	**Dec**
Silver barb (*Puntius gonionotus*)	**T1**	**P1**	4.20	19.58	57.64	80.38	128.43	154.36
		P2	4.20	22.79	52.39	92.85	120.68	158.94
		P3	4.20	18.50	64.44	88.67	116.39	165.24
	T2	**P4**	4.20	24.20	60.25	86.35	108.42	145.28
		P5	4.20	19.90	53.98	78.13	110.25	148.24
		P6	4.20	17.76	55.46	82.35	103.54	132.28
	T3	**P7**	4.20	17.23	55.10	85.92	119.64	168.24
		P8	4.20	20.22	64.33	88.64	122.54	170.25
		P9	4.20	21.41	57.97	95.26	129.58	160.48
	T4	**P10**	4.20	13.24	42.58	70.25	91.39	126.34
		P11	4.20	16.24	53.16	74.40	102.17	120.45
		P12	4.20	17.11	51.28	76.80	95.26	135.42

Annexure-8: Unprocessed data of weight increment (g) of common carp in four feeding treatments (Expt. 4)

Fish species	Treatments/Aquarium		Sampling time				
			Initial	**After 3 Weeks**	**After 6 Weeks**	**After 9 Weeks**	**After 12Weeks**
Common carp (*Cyprinus carpio*)	**T1**	**A1**	14.54	35.52	62.31	93.48	119.84
		A2	12.56	33.15	59.47	85.44	116.59
		A3	16.57	37.36	64.58	92.89	125.45
	T2	**A4**	12.47	38.28	67.53	92.13	127.14
		A5	15.46	33.15	58.94	86.17	120.38
		A6	15.59	37.36	66.34	98.51	122.45
	T3	**A7**	16.55	40.15	62.30	96.84	139.45
		A8	12.52	38.64	69.13	93.15	129.25
		A9	14.56	34.12	70.24	98.21	137.25
	T4	**A10**	12.56	29.23	64.26	94.41	120.57
		A11	16.52	31.46	63.56	95.67	128.41
		A12	14.52	37.89	67.29	90.69	117.59