EVALUATION OF GROWTH, SURVIVAL AND HEMATO-BIOCHEMICAL INDICES OF COMMON CARP (*Cyprinus carpio*) THROUGH PARTIAL REPLACEMENT OF FISH MEAL BY MICROALGAE POWDER ISOLATED FROM SOUTH-EASTERN COAST OF BANGLADESH

Sumit Kanti Dey

Roll No.: 0122/03

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Aquaculture

Department of Aquaculture

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

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

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Supervisor

Department of Aquaculture

(Dr. Helena Khatoon)

Chairman of the Examination Committee

Department of Aquaculture

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Khulshi, Chattogram-4225, Bangladesh

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List of abbreviations

Acronym	Definition
sp.	Species
CD	Cell Density
OD	Optical Density
DO	Dissolved Oxygen
ppt	Parts Per Thousand
ppm	Parts Per Million
rpm	Rotation Per Minute
rcf/ ×g	Relative Centrifugal Field
pH	Power of Hydrogen
ºC	Degree Celcius
DB	Dried Biomass
FCR	Feed Conversion Ratio
SGR	Specific Growth Rate
hr	Hour
µ	Micro
ml	Mili Liter
L	Liter
%	Percentage
g	Gram
lbs	Pound
v/v	Volume/Volume
min	Minute
mg	Mili Gram
nm	Nanometer
cm	Centimeter
SAFA	Saturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
PUFA	Polyunsaturated Fatty Acid
HUFA	Highly Unsaturated Fatty Acid
ANOVA	Analysis of Variance
BUN	Blood Urea Nitrogen
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FAME	Fatty Acid Methyl Esters
GCMS	Gas Chromatography and Mass Spectrometry
Hb	Hemoglobin

Hct	Hematocrit
LYM	Lymphocytes
PLT	Platelet
RBC	Red Blood Cells
WBC	White Blood Cells
NO_2-N	Nitrite Nitrogen
SRP	Soluble reactive Phosphorus
TAN	Total Ammonium Nitrogen
SE	Standard Error

Abstract

A feeding trial of 60 days was conducted after the isolation of microalgae from marinewater to evaluate the effects of *Chlamydomonas* sp. on growth performance, survival, water quality, proximate composition, hemato-biochemical index of common carp (*Cyprinus carpio*) where the microalgae was incorporated with the fish diet as a partial replacement of fishmeal. Triplicate groups of twenty uniform sized common carp fries were kept in each rectangular glass tank (40 L). In diets, *Chlamydomonas* sp. was added at different levels-0% (control), 5% (C5), 10% (C10), 15% (C15), commercial feed (CMF) and fed to the fish twice a day at 5% of their body weight for 60 consecutive days. At the end of experiment, random sampling of fish was done for growth parameter assessment and further laboratory analysis. The protein content of fish fry gradually ranged higher to lower from C15(35.6%), C10(33.2%), CMF (29.8%), C5(25.2%) and control (24.8%). C15 group had the highest lipid value (15.7%) and control group had the lowest one (8.4%). However, there were significant differences ($p<0.05$) observed among the all five treatments in terms of Average Daily Gain (ADG) whereas C15(0.017 ± 0.00 g) and C10(0.015 ± 0.00 g) were found to be significantly higher ($p<0.05$) respectively. In case of SGR, significant differences ($p<0.05$) were recorded where C15 ($2.82 \pm 0.03\%$) showed the highest SGR among the five treatments. The result revealed the least FCR value ($p<0.05$) in C15 (2.4 ± 0.003) and highest FCR value in control group (3.7 ± 0.05). The highest survival rate was found in C15 followed by C10, CMF and C5 respectively (81.67%, 71.67%, 63.33%, 58.33%) compared to the control group (43.2%). The addition of *Chlamydomonas* sp. to the feed improved and maintained good water quality during the culture period. RBC, Hb, Hct, WBC were recorded higher in 15% inoculation of *Chlamydomonas* sp. Replacement of fish meal with 15% and 10% inclusion of microalgae reported higher amount of total protein (8.52 g/dl), (7.83 g/dl) respectively followed by CMF (7.46 g/dl), C5(6.82 g/dl) and control (6.27g/dl). This study indicates that partial replacement of fishmeal by microalgae powder of *Chlamydomonas* sp. can be a potential source of protein and lipid having cost effectiveness and beneficial effects on common carp fry.

Keywords: Common carp, microalgae, growth, water quality, proximate composition, hemato-biochemical parameters

Chapter-1: Introduction

The long-term viability of aquaculture operations significantly depends on the accessibility of high-quality and ecologically suitable feed supplies. Traditionally, fish meal has been an integral component in aquaculture diets owing to its great nutritional profile (Abdulrahman et al., 2014). However, the overexploitation of wild fish supplies and the growing costs associated with fish meal manufacturing have prompted the hunt for alternate protein sources (Deutsch et al., 2007). Microalgae, microscopic photosynthetic organisms, have emerged as an intriguing possibility for partial replacement of fish meal in aquaculture diets (Gao et. al., 2016). Microalgae have garnered attention in recent years in response to its countless benefits as a feed attribute. At their core, microalgae feature a rich nutritional content, making them an appealing source of protein, vital amino acids, vitamins, minerals, and omega-3 fatty acids (Khatoon et al., 2017). Some microalgal species, such as *Chlorella* sp., *Spirulina* sp., *Chlamydomonas* sp. and *Nannochloropsis* sp., have been uncovered to contain protein levels equivalent to or more substantial than fish meal (Safi et al., 2013). Additionally, microalgae comprise highly digestible carbohydrates and demonstrate distinctive bioactive substances, such as antioxidants, pigments, and immunostimulants, which could give rise to the general health and performance of aquaculture species (Granado-Lorencio et al., 2009). The equitable production of microalgae is another major trait. Microalgae may be tended in several sorts of systems, including open ponds, closed photobioreactors, and raceways, utilizing diverse water sources such as freshwater, saltwater, or wastewater. They have a rapid growth rate and may be harvested year-round, providing a steady supply of feed components. Furthermore, microalgae may be produced using non-arable soil, employing sunlight as the predominant source of energy and accumulating carbon dioxide during photosynthesis, thereby decreasing greenhouse gas emissions and escalating its commitment to sustainability (Lamers et al., 2012). Switching fish meal along with microalgae in aquaculture feeds could result in many perks. Firstly, it may ease the repercussions on wild fish demographics, since microalgae production does not need the capture of fish. The diminution in fish meal dependence favors to the protection of marine habitats and assists in preserving a healthy marine food web. On top of that, employing microalgae as a feed element may boost the nutritional content of aquaculture products, leading to better growth rates, feed conversion efficiency, and overall fish health. Microalgae-based diets have been

observed to spike fish coloring, taste, and omega-3 fatty acid levels, which are highly sought after by customers (Hosikian et al., 2010). Microalgae provide an avenue for possibility as a partial substitute for fish meal in aquaculture diets. Their remarkable nutritional profile, sustainable production capacity, and good influence on fish health and product quality make them a compelling option (Williams and Laurens, 2010). Further research and development efforts need to be conducted to enhance microalgae growing methods, augment cost-effectiveness, and facilitate their application across other aquaculture species. Embracing microalgae as a feed element marks a big step towards the continued existence and resilience of the aquaculture sector in addressing the world's ever growing demand for fish and fisheries products.

The Common Carp, scientifically designated as *Cyprinus carpio*, is a fish from the freshwater milieu that has been widely dispersed and comprehensively studied across the globe. Belonging to the family Cyprinidae, it is native to Eurasia and has been imported to other regions of the world because of its commercial and recreational utilitarianism. The Common Carp wields tremendous cultural and historical relevance, having been domesticated and meticulously bred for generations (Kiron et al., 2012) Microalgae might constitute an invaluable component in the diets of common carp attributed to its nutritional value and conceivable health advantages (Abdulrahman et al., 2019). It's worth mentioning that the precise kind and amount of microalgae used in common carp diets should be decided depending on criteria such as the fish's nutritional needs, availability of microalgae species, and the intended aims of fish farming. Consulting with aquaculture specialists or fish nutritionists may assist establish suitable and balanced feed compositions for common carp.

Chlamydomonas is a genus of single-celled green algae that could potentially be farmed and used as a feed element in aquaculture. While it may give some advantages with regard to nutrition, it is imperative to take into account that *Chlamydomonas* sp. alone cannot totally replace fish meal in aquaculture diets (Aldana-Aranda et al., 2017). Fish meal is a very nutritious and excellent source of protein, vital amino acids, fatty acids, vitamins, and minerals, which are required for the growth and development of many farmed fish species. However, it may be utilized as a partial substitute for fish meal in aquaculture feed regimens. It includes large quantities of protein, carbs, fats, vitamins, and minerals, making it a good alternative factor. It also offers the added incentive of being a sustainable and ecologically friendly alternative, since it can be produced using

renewable resources and has a lesser environmental effect juxtaposing them to fish meal manufacturing.

Incorporating *Chlamydomonas* sp. into aquaculture feeds could accelerate the nutritional profile and offer a source of critical nutrients (Garcia et al., 2008). However, it boils down to consider the special needs of the target fish species when developing diets. Different fish species have diverse nutritional demands, and the addition of *Chlamydomonas* sp. should be based on careful assessment of the fish's need for amino acids, fatty acids, and other vital elements. Furthermore, it is standard practice to integrate numerous alternative protein sources to enhance the nutritional content of aquaculture diets. This strategy helps to guarantee that the fish get a balanced diet that fits their nutritional needs for growth, health, and reproduction. Therefore, although the species may be used as a partial substitute for fish meal, it is often coupled with other protein sources such as plant proteins, insect meals, or microbial biomass to generate a well-rounded feed composition (Andreeva et al., 2021). The content of important nutrients in *Chlamydomonas* sp. was contrasted with *Chlorella* sp. and *Spirulina* sp., the species often recognized as a superfood. The results revealed that the protein content of *Chlamydomonas* sp. (56.9%) was greater than *Chlorella* sp. (45.3) and *Spirulina* sp. (50.4%) on a dry weight basis (Hu et al., 2015). *Chlamydomonas* sp. had all the indispensable amino acids with excellent scores based on FAO/WHO values (0.9–1.9) as in *Chlorella* sp. and *Spirulina* sp. (Hussain et al., 2017). Unsaturated fatty acids predominated the total fatty acids profile of *Chlamydomonas* sp. were ~74 of which ~48% are n-3 fatty acids. Alpha-linolenic acid (ALA) concentration in *Chlamydomonas* sp. (42.4%) was significantly greater than that of *Chlorella* sp. (23.4) and *Spirulina* sp. (0.12%) (Choong et al., 2020). For minerals, *Spirulina* sp. was rich in iron (3.73 mg/g DW) followed by *Chlorella* sp. (1.34 mg/g DW) and *Chlamydomonas* sp. (0.96 mg/g DW). *Chlamydomonas* sp., unlike the other two species, comprised of selenium (10 µ g/g DW), and exhibited a substantially reduced heavy metal load (Hyka et al., 2013). Moreover, it contains significantly high amounts of chlorophyll (a +b) and total carotenoids (28.6 mg/g DW and 6.9 mg/g DW, respectively) compared with *Chlorella* sp. (12.0 mg/g DW and 1.8 mg/g DW, respectively) and *Spirulina* sp. (8.6 mg/g DW and 0.8 mg/g DW, respectively) (Darwish et al., 2020). Based on its nutritional credentials, this species has considerable potential as a new superfood or component for a dietary supplement. No elaboarted research has been carried out till now

associated with the effects of *Chlamydomonas* sp. on the diets of common carp. Therefore, Common carp (*Cyprinus carpio*) was used in the experiment on the basis of availability and potentiality and the partial replacement of fish meal was accomplished by *Chlamydomonas* sp. powder after isolation, culture and assessment of its caliber for being used as a worthwhile feed ingredient.

1.1. Objectives

The present feeding trial was anticipated to comply with the following objectives-

i. To evaluate the effectiveness of *Chlamydomonas* sp. supplementation at different levels on the growth performance of common carp.

ii. To compare the proximate composition of fish fed with *Chlamydomonas* sp.

iii. To compare the hemato-biochemical indices of common carp fry, fed with microalgae powder of *Chlamydomonas* sp. and commercial feed.

Chapter-2: Review of Literature

2.1. Microalgae

Microalgae, a varied category of single-celled algae, are minuscule photosynthetic organisms. They may be discovered in a range of aquatic habitats, including freshwater, marine, and even moist soil (Hannon et al., 2010). These small creatures are remarkably adaptable and are essential to the health of the Earth's ecology. The renowned process of photosynthesis, in which microalgae use sunlight to change carbon dioxide and nutrients into organic molecules, chiefly carbohydrates, proteins, and lipids, is a skill that is unique to them. They are among the best solar energy converters in the world for their extraordinarily high photosynthetic efficiency (Barsanti et al., 2008). In fact, it's thought that microalgae provide close to 50% of the world's oxygen (Saha and Murray 2018; Khan et al., 2018). Microalgae are incredibly diverse, which is one of their most striking qualities. From tiny spheres to elongated rods and spirals, they come in a wide variety of sizes, colors, and forms. They can have different levels of pigmentation, which results in vivid shades like green, red, brown, and even golden. Chlorophyll, carotenoids, and phycobilins, among other pigments, are responsible for their unusual hues (Khan et al., 2018). Microalgae have drawn a lot of interest for its possible uses in a variety of sectors due to their quick growth rate and effective nutrient absorption. In addition to proteins, lipids, carbs, vitamins, and minerals, they are a great source of bioactive substances. Some microalgae species also generate secondary metabolites with potential for use in medicine and nutrition. In terms of sustainable food production, microalgae are immensely promising, especially as a nutrient-rich feedstock for the aquaculture and cattle sectors. They may be grown on non-arable soil with the help of sunshine, water, and carbon dioxide, relieving pressure on traditional agriculture and minimizing negative effects on the environment. Microalgae are also being investigated as a source of biofuels, including biodiesel and bioethanol, offering a sustainable and environmentally benign alternative to fossil fuels. Microalgae are essential in environmental cleanup in addition to their uses in nutrition and energy. They may absorb excess nutrients, heavy metals, and contaminants from aquatic bodies, reducing eutrophication and assisting in the treatment of wastewater. Furthermore, by trapping carbon dioxide from the atmosphere, microalgae might possibly aid in the fight against climate change.

2.2. Significance of microalgae

The food industry sector devoted to aquaculture is expanding the quickest. According to Marketwatch's forecast for 2020, the aquaculture industry will be valued US$34.94 billion in 2022. Between 2020 and 2027, it is anticipated to grow at a pace of over 7.6%. Fish that are good for a plant-based diet are being produced more often in aquaculture. Fine-tuning of fish nutrition has reduced feed waste, which has a positive impact on the industry's financial viability. The output, survival, and quality of farmed fish have all increased due to diets high in useful nutrients including omega-3 fatty acids, antioxidants, and prebiotic compounds. Due to the following characteristics, fish meal is a highly sought-after element in fish feed. The following factors contribute to increased growth in fish: (1) Excellent digestibility and palatability for fish; (2) Well-balanced composition and concentrations of protein, minerals, essential fatty acids, and essential amino acids; (3) Low convertibility of feed (i.e., a high percentage of feed is converted into fish biomass); (4) Increased immunity contributing to a higher survival rate. In the previous 10 years, consumer preference for fish meal, a vital component of feed, has surged by 300% (Indexmundi, 2021). In light of this, several sectors and researchers have taken steps to begin exploring for economically feasible and environmentally friendly options for supplanting fish meal. Microalgae have been illustrated to be a viable alternative source of balanced nutrients ideal for the generation of fingerling fish and fish in the burgeoning aquaculture industry after several research (Hodar et al., 2020). According to Rizwan et al. (2018), microalgae have the highest net biomass production of any terrestrial plant or animal. Microalgae do not require fertile ground to thrive, unlike land-based plants, and they may even be grown in seawater or waste water (Li et al., 2019). In the setting of a biorefinery, microalgae might potentially be utilized to provide fish feed (Arun et al., 2020; Nagappan and Nakkeeran, 2020). This idea, for instance, would enable the co-production of useful metabolites like colors with fish diet. The basic rationale for the microalgae promise is that it has the proper ratio of protein, lipids, and carbohydrates, which is necessary to safeguard the health of the fish. The well-balanced amino acid composition of microlagae negates the need for extravagant amino acid supplements in the diet.

For instance, methionine, which is frequently missing in substances derived from plants, is abundant in microalgae including *Chlorella* sp., *Chlamydomonas* sp., *Porphyridium* sp., *Isochrysis* sp., and *Nannochloropsis* sp. (Wan et al., 2019). Microalgal fiber, in contrast to plant fiber, lacks lignin and contains less hemicellulose, signifying improved digestion (Niccolai et al., 2019). Furthermore, certain microalgae create substantial quantities of vitamins and immunostimulants in their cells, and they have an array of pigments with antioxidant qualities that might benefit aquatic animals' health (Prabha et al., 2020; Zhou et al., 2019). The fish's marketability could elevate if microalgal pigments such as astaxanthin give them an enticing color (Posten and Schaub, 2009). In accordance with Ryckebosch et al. (2012), some microalgae are rich in omega-3 fatty acids like docosahexaenoic acid (DHA) and eicosa-pentaenoic acid (EPA), which are good for human health in alongside fish. Therefore, it can be concluded that a large demand for fish feed in the near future might be satiated by picking microalgal strains with desired cellular formulation and ameliorated production cost as feed (Nagappan et al., 2021).

2.3. Microalgae isolation

The initial and decisive phase in bioprospecting purposes of microalgae for any kind of economic use is species or strain selection (Barclay and Apt, 2013). The screening of microalgae species encompasses a number of protocols, which incorporates sample collection, isolation, purification, identification, maintenance, and characterization of prospective products (Gong and Jiang, 2011). Depending on the types of microalgae, 18S ribosomal RNA genes for eukaryotic and 16S ribosomal RNA for prokaryotic microalgae can be used to sequence microalgae taxonomy (Bellinger and Sigee, 2010). There are very few studies on the isolation and molecular identification of the algae in Bangladesh, despite the fact that this approach has become ubiquitous around the world. For the first time in Bangladesh, Tarin et al. (2016) identified and characterized *Chlorella vulgaris* and *Anabaena variabilis* from natural and manmade water bodies in Khulna and Dhaka University to assess their potential as feedstock for biofuel production. *Pithophora polymorpha* and *Spirogyra maxima* were successfully identified and newly reported microalgae from Bangladesh by examining partial 18S rDNA sequences (Alfasane et al., 2019). Four marine microalgae (*Chlorella* sp., *Nannochloropsis* sp., *Tetraselmis* sp., and *Chaetoceros* sp.), isolated from Bangladesh's Cox's Bazar coast, have recently been characterized by Islam et al. (2021) based on

growth performance, pigments, and nutritional content. The native microalgae of Bangladesh and its potential, particularly in the watery ecosystem, have received inadequate study.

2.4. Growth factors of microalgae

Growth of microalgae is affected by some factors such as length of photoperiod, temperature, pH and light intensity (Wahidin et al., 2013).

2.5. Microalgal growth

Microalgae show five phases during its growth (Lavens and Sorgeloos, 1996). These are well defined as i) lag phase, ii) exponential phase, iii) linear growth phase iv) stationary growth phase and v) decline or death phase. Under suitable condition microalgae show all that phases.

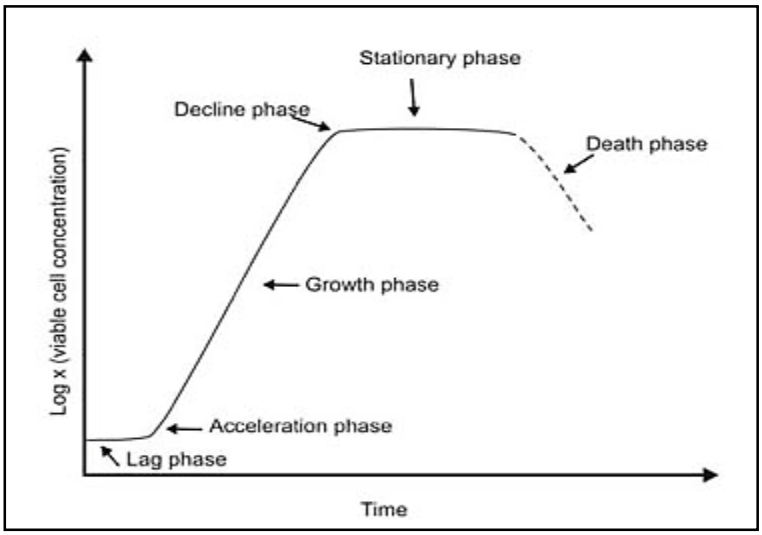


Figure 1: Microalgae growth curve (Teresa et al., 2010)

The cell is viable but not yet prepared to divide during this initial phase, known as the lag phase. The second phase saw the beginning of a rise in cell density with passing time. The biomass of microalgae often doubles during the exponential development phase (Chisti, 2007). Once at the stationary phase, when the growth rate is balanced, they enter. Microalgae are denser in this phase than in any other. The cell density began to fall during the last stage of the dying process. Growth is now starting to be constrained by nutrition availability, pH, CO2, and other physical variables (FAO, 1996).

For the use of microalgae in aquaculture, monitoring of cell development is deemed a core part (Santos-Ballardoa et al., 2015). When attempting to analyze the growth of microalgae, primarily two analytical techniques are used: spectrophotometric absorbance, which involves measuring the absorbance of the cell suspensions, and cell density determination, which involves counting the number of cells per milliliter (Godoy-Hernández and Vázquez-Flota, 2006). Optical density (OD) determination is an indirect approach for measuring the biomass of microalgae since it may be directly correlated with the number of cells present in the medium (Ribeiro-Rodrigues et al., 2011). The best way to maximize microalgal output is to have a thorough understanding of how a particular microalgal strain responds to different culture conditions, such as nutrition delivery (Hyka et al., 2013). The output of biomass and the accumulation of lipids are both known to be significantly influenced by a number of dietary variables, including nitrogen, phosphorus, carbon, and iron (White et al., 2013). Microalgae's production and growth vary from species to species. Due to their greater ecological suitability and high level of environmental adaptability, potentially significant indigenous species have a lower environmental impact where the system is operating and should therefore have their chemical composition and productivity analyzed to determine their nutritional properties.

2.6. Microalgal pigments

2.6.1. Chlorophyll

Chlorophylls, carotenoids, and phycobilins are the top three pigment groups found in microalgae, and they have been acknowledged as one of the most important products from microalgae (Granado-Lorencio et al., 2009). The two main categories of chlorophylls are chlorophyll-a and chlorophyll-b, and chlorophyll is one of the important bioactive substances that can be isolated from microalgae (Aris et al., 2010). Chlorophyll-b collaborates indirectly in photosynthesis by transforming the light it absorbs into Chlorophyll-a, which is the major pigment that is accountable for turning light energy into chemical energy (Nayek et al., 2014). Carrying antioxidant properties, chlorophyll and its derivatives are employed as natural food coloring agents (Hosikian et al., 2010) and are also commonly found in medicinal goods (Bhagavathy and Sumathi, 2012).

2.6.2. Carotenoid

Carotenoid is a naturally occurring, fat-soluble, yellow to red pigment that predominates in plants and is crucial for photosynthesis in algae and photosynthetic bacteria (Lamers et al., 2012). Salinity, light intensity, food deprivation, temperature, and other elements, however, have an impact on the formation of carotene. (Kleinegris and colleagues, 2009). Carotenoids are widely used in natural foods as coloring agents, including egg yolk, poultry, and fish. Only a few carotenoids, such as astaxanthin, canthaxanthin, lutein, lycopene, and -carotene, have been utilized commercially out of the more than 750 that have been discovered (Vlchez et al., 2011). The main advantage of the using microalgae as a carrier of carotenoids is their positive impact on human health due to the presence of many other antioxidant compounds. Astaxanthin is synthesized by Chlorophyceae family namely *Chlorella* sp., *Chlamydomonas, Dunaliella*, and *Haematococcus* sp., etc. (Pulz and Gross, 2004).

2.6.3. Phycobiliproteins

According to Ba'saca-Loya et al. (2009), phycobiliproteins are a set of proteins containing conjugated linear tetrapyrrole chromophoric groups. and the three predominant phycobiliproteins are phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) (Wiedenmann, 2008). Phycocyanin is a pigment used commercially in the pharmaceutical sector because it shields the photosystems from free radicals and has the potential to stop oxidative damage brought on by free radicals. In the words of Mathivanan et al. (2015), phycocyanin is a water-soluble, extremely fluorescent blue-green light-harvesting pigment that is unique to select algae species and is a member of the Rhodophyta and Cryptophyta families. It is specifically found in cyanobacteria. Phycobiliproteins are utilized extensively as nutraceuticals, as natural colors, and in various biotechnological applications in the food, cosmetics, pharmaceutical, and diagnostics domains (Becker, 2004).

2.7. Proximate composition of microalgae

Proximate composition of microalgae usually corresponds to the percentage composition that comprises multiple fundamental elements including water, protein, lipid, carbohydrate, and ash (primarily minerals) (Ganguly et al., 2018). In comparison to lipid and carbohydrate, protein generally makes up a larger fraction of microalgae (Lavens and Sorgeloos, 1996). According to Phukan et al. (2011), the total protein

content of microalgae, particularly *Chlorella* sp., ranges between 43-50%. Some algae, notably blue-green and green algae, have very high protein contents that can be employed as functional food components. These levels are generally between 40 and 60 percent (of dry matter). According to Reyes et al. (2012), algal proteins have a good nutritional value in terms of protein amount, amino acid purity, and nutritional acceptability. Lipid content of microalgae is usually in the range of 20–50% of the cell dry weight, and can be as high as 80% under certain conditions (Michael and Borowitzka, 2018). Microalgae can overproduce lipids or carotenoids under stress condition such as high salt, high light, or nutrient limitation. For instance, lipid accumulation in *Dunaliella* sp. and *Chlorella vulgaris* was significantly increased under high-salinity stress, reaching 70% and 21.1%, respectively (Singh et al., 2016). Microalgae strains with high oil or lipid content are of great interest in the search for a sustainable feedstock for the production of biodiesel. Compared to other microalgae compounds, carbohydrates have a lesser energy value but they are the preliminary raw component for the synthesis of biofuels via biotechnological conversion (Andreeva et al., 2021; Aytenfisu et al., 2018). The amount of lipids and their proximity or position of double bonds in the carbon chain (Villarruel-Lopez et al., 2017) and carbohydrate content (Park et al., 2014) can differ based on the species of microalgae in biomass depends on the type of microalgae and growth conditions.

2.8. General attributes of *Chlamydomonas* sp.

2.8.1. Taxonomic classification

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Chlamydomonadales

Family: Chlamydomonadaceae

Genus: *Chlamydomonas* (Ehrenberg, 1833)

2.8.1.1. Morphological characteristics

Motile unicellular algae. Generally oval and/or circular. Cell wall is made up of a glycoprotein and non-cellulosic polysaccharides instead of cellulose. Two anteriorly inserted whiplash flagella are found. Each flagellum originates from a basal granule in the anterior papillate or non-papillate region of the cytoplasm. Each flagellum shows a typical 9+2 arrangement of the component fibrils. Prominent cup or bowl-shaped chloroplast is present.

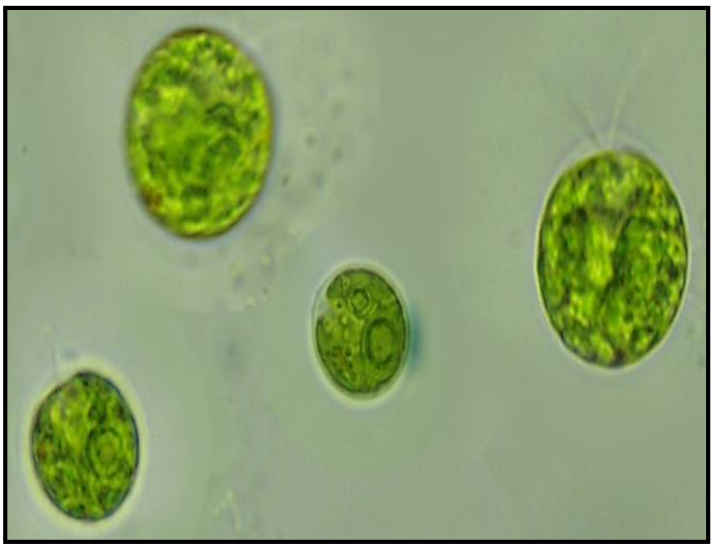


Figure 2: *Chlamydomonas* sp.

2.8.2. Source of nutritional components

Chlamydomonas sp. are among the most intriguing marine microalgae because of their tiny size, high protein content, acceptable amino acid profile, and particularly high lipid content (up to 40% of DM), which is often rich in EPA and DHA. (Becker 2007; Bellou et al. 2014; Molino et al. 2018; Ashour et al. 2019). The development of fish and the muscle amino acid profile in Atlantic salmon (*Salmo salar*) and turbot (*Scophthalmus maximus*) were not adversely affected by dietary supplementation with up to 10% *Chlamydomonas* sp. meal (Qiao et al. 2019). Moreover, *Chlamydomonas* sp. defatted meal could replace up to 15% fish meal in European seabass (Valente et al. 2019) and up to 10% crude protein from fishmeal and soy concentrate in red drum (*Sciaenops ocellatus*) diets (Patterson and Gatlin 2013) without affecting growth, feed utilization, protein, and energy retention of the fish. Vitamin B12, Eicosapentaenoic Acid (EPA), omega-3 HUFAs, protein, carbohydrate, fat, and vitamin C are all found in

Chlamydomonas species. *Chlamydomonas* (Eustigmatophyceae) genera have high protein (53%) and fat (12.6%) contents, according to Lupatsch and Gbadamosi (2018). *Chlamydomonas* sp. are abundant in methionine, a protein that is frequently deficient in components derived from plants (Wan et al., 2019). Additionally, proline, glutamate, and aspartate are the most prevalent amino acids, while tryptophan, cystine, histidine, and low levels of hydroxyproline are seen (7.02%). *Chlamydomonas* sp. varied in protein digestibility from 65-78% in aquatic species (Niccolai et al., 2019). Fatty acids are another characteristic principle of the *Chlamydomonas* sp. biomass; eicosapentaenoic acid (20:5ω3, EPA) is found in large amounts. Other fatty acids also found are 14: 0, 16:0, 16:1, and 20:4ω6 (13-15). The triacylglycerols are characterized by a high proportion of saturated and monounsaturated short-chain fatty acids 14:0, 14:1, 16: 0, and 16:1 (14). The high quality of *Chlamydomonas* sp. biomass for the aquaculture industry is attributed to its high EPA amount (16%) (Niccolai et al., 2019). Among sterol composition, cholesterol, fucosterol, and isofucosterol are the principal constituents in all *Chlamydomonas* species (17%) (Niccolai et al., 2019).

2.8.3. Source of valuable pigments

Chlamydomonas algae are different from other closely related microalgae. They contain chlorophyll a but are totally deficient in chlorophyll b and c. According to Macas-Sánchez et al. (2005), *Chlamydomonas* sp. is a significant source of pigments with high economic value. *Chlamydomonas* sp. has a very straightforward carotenoid composition, consisting of the main xanthophyll pigments carotene, violaxanthin, and a pigment that resembles vaucheraxanthin (9, 10). In cultures exposed to high light intensity, zeaxanthin and anteraxanthin can be found as minor components, both containing ketonic groups (Karlson et al., 1996).

2.8.4. Source of antioxidant

Today, both producers and consumers are very interested in the functional benefits of microalgae-based diets on stress response (Elabd et al., 2020). In a prior work, *Chlamydomonas* sp. shown significant antioxidant activity (Goh et al., 2010). Due to their capacity to accumulate high amounts of PUFAs (polyunsaturated fatty acids), they appear to be mostly rich in phytochemicals and ideal for use as a nutritional supplement and natural medicine for the management of a variety of diseases (Matos et al., 2017). *Chlamydomonas* sp. modulate the liver and intestine antioxidant enzyme response

without detrimental effects on overall lipid oxidative damage (Castro et al., 2020). According to Haoujar et al. (2019), *Chlamydomonas* sp. has higher polyphenol content associated with non-toxic antioxidant activity which could facilitate public health and safety (Yan et al., 2010). Moreover. *Chlamydomonas* sp. is rich in carotenoids which are effective substances in preventing oxidative damage to tissues, because they can absorb free radical energy (Guedes et al. 2011).

2.8.5. Growth factors and culture conditions

Chlamydomonas sp. culture is highly dependent upon culture media and environmental factors that influence it (Boroh et al 2019). Major factors supporting the microalgal life are light, water, and CO2 (Elystia et al 2019). Kitaya et al. (2008) reported direct effect of different parameters especially environmental parameters (light, salinity, nutrient types and composition, light period, and culture pattern). According to the review article of Parmar et al. (2011) the length of lighting period and cycle (light/dark) are the most validate factors influenced most in micro-algal cultivation. Most importantly the growth of microalgae affected by some illuminating factors such as length of photoperiod, temperature, pH and light intensity (Wahidin et al., 2013). In order to optimizing microalga growth in mass culture system the above-mentioned factors must have to maintain accurately.

2.8.5.1. Light

Intensity of light is an important factor for microalgae cultivation. Generally, for biomass growth, microalgae depend on enough carbon source (about 40-50% carbon) and light to carry out photosynthesis process (Moheimani, 2005). Requirement varies on basis of the conditions. For an Erlenmeyer flask; 1000 Lux is suitable where 5000-10000 Lux required for larger volume (FAO, 1996). The use of fluorescence light for indoor culture can promote a better growth and cell division of microalgae (Laing, 1991). However, maximum exposure of light can become limiting factor to microalgae density.

2.8.5.2. Temperature

Temperature, the 2nd most prior factor for culturing microalgae. Besides, above 27 ºC will make the algae die (Laing, 1991). Various microalgae are viable with very low temperature than its optimum (upto 15 ºC lower), but crossing limit above by 1-4 ºC

can create a great damage (Teresa et al., 2010). Changing of light intensity will influence temperature which indirectly affects growth of microalgae (Huang et al., 2013).

2.8.5.3. p^H

pH plays an important role for culturing microalgae. Many cellular activities disrupt in microalgae cell because of pH maintaining failure (Lavens and Sorgeloos, 1996). pH is directly related with CO2 accessibility and for that reason it is also essential for photosynthesis. In higher concentration pH may varies and reach at limiting values pH 9 (FAO, 1996).

2.8.5.4. Nutrient composition of media

Nitrate, phosphate and silicate are some examples of macronutrients essential for microalgae growth (Lavens and Sorgeloos, 1996). Nitrogen is considered as the most important type of nutrient and most common type of nutrient in the culture medium (Thompson et al., 1989). Zinc, cobalt, boron, iron and manganese are the most commonly used trace metals (Probert and Klaas, 1999). Others are thiamin (B1), cyanocobalamin (B12) (FAO, 1996). Lourenço (2006) reports direct interaction of nutrients on growth of microalgae. Commercial media contain all types of micronutrients and macronutrients to help the growth of microalgae.

2.8.5.5. Salinity

Salinity has direct effect on growth of microalgae. Salinity range varies on basis of species cultured. Every microalga has a different salinity range. Change in salinity might inhibit the growth of microalgae (Takagi et al., 2006). By adding fresh water or salt we can easily control the salinity of culture medium. Lipids were stored in salt-stressed cells and were degraded when stressed cells are crossed the optimal conditions (Shetty et al., 2019). A significant proportional increase in the lipid content of biomass occurred with an increase in salinity (up to 35 ppt), with a concomitant decrease in EPA percentage, in *Nannochloropsis oculata* (Renaud and Parry1994). Complete growth retardation also observed with the higher salinity levels in *Chlamydomonas* sp. (Hu and Gao, 2003); but higher EPA percentage has been noticed with lower brackish water salinities. (Hu and Gao, 2003).

2.8.5.6. Mixing and aeration

For homogenizing mixing and aeration is an important factor. Kaewpintong (2004) found fine development of cell growth in an aerated culture system (bioreactor) than non-aerated system. On the contrary, excess liquid pressure, velocity, over turbulence and excess bubbles can create stress to the cell which result damage of cell (Eriksen, 2008). To ensure better contacts with cell and nutrients it is also important to maintain homogenous conditions through balance aeration.

2.9. Common Carp (*Cyprinus carpio*)

Commonly referred to as the common carp, *Cyprinus carpio* is a freshwater fish species that is a member of the Cyprinidae family. It is a highly adaptable and widely dispersed fish that is well-known for its use in sport fishing, aquaculture, and its ecological effects in many water bodies across the world.

2.9.1. Taxonomic classification

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cypriniformes

Family: Cyprinidae

Subfamily: Cyprininae

Genus: *Cyprinus carpio* (Linnaeus, 1758)

Figure 3: *Cyprinus carpio*

2.9.2. Physical characteristics

The common carp is an aquatic fish with a prolonged, muscular body and a slightly arched back. Although some individuals can develop to lengths more than 1 meter (3.3 feet), it normally grows to a normative average length of 40–80 centimeters (16–31 inches). They may potentially weigh more than 40 kilograms (88 pounds) in certain circumstances. Large scales covering the body can be any hue from dark olive green to golden or bronze. The coloration can vary depending on the fish's environment, age, and genetic factors. One of the distinguishing features of the common carp is the presence of two pairs of barbels. These barbels, located near the mouth, are fleshy, whisker-like organs that aid in detecting food and sensory perception. The mouth is protractile, meaning it can extend outward to help the fish feed on bottom-dwelling organisms.

2.9.3. Historical background

In the middle and late Roman eras, carp was a luxury dish, and in the middle ages, it was eaten when one was fasting. The Romans stored the fish in ponds (referred to as 'piscinae'), and Christian monasteries eventually built fish ponds to house them. Carp were raised in monoculture in this European method. Broodfish were chosen from the biggest individuals. Unintentional artificial selection, the earliest steps towards domestication had occurred between the 12th and the middle of the 14th century A.D. In the nineteenth century, carp were first bred in controlled semi-natural ponds and raised as fry. More than 2000 years ago, cyprinids were maintained in impassable ponds for the purpose of breeding them. River fry were frequently added to the ponds for stocking. Natural food-based polycultural rearing technology was applied. Semi-domesticated carp races have developed in this system. Domesticated carps have been produced in most of the carp rearing areas recently. There are about 30-35 strains of domesticated common carps in Europe. Many strains are maintained in China. There are some Indonesian carp strains, which have not been scientifically examined and identified so far.

2.9.4. Habitat and distribution

The interior waterways of China and the adjacent areas are where the common carp is indigenous to Asia. But because it is so well-liked as a food fish and was made available for sport fishing, it has spread far over the world. Africa, Australia, North America,

South America, and Europe are currently among its current locations. Lakes, ponds, reservoirs, rivers, and even some saline estuaries have all seen the successful introduction of common carp. Common carp are extremely flexible and can survive in a variety of aquatic environments. Low oxygen levels and extreme turbidity are only two of the conditions that they can tolerate in water. They frequently inhabit slow-moving or still waterways that have dense vegetation and muddy bottoms. However, they can also inhabit faster-flowing rivers and streams, particularly during their reproductive migrations.

2.9.5. Behaviour and diet

Common carp are well recognized for being opportunistic eaters which take advantage of many food sources. They consume both plant debris and tiny invertebrates since they are omnivorous. They frequently eat aquatic vegetation, insects, crabs, mollusks, worms, and debris. They crush and grind food with their unique pharyngeal teeth, which are found in the back of their throats. Common carp are mostly bottom feeders during the day, using their delicate barbels to seek and ingest food from the substrate. However, they may also eat at the water's surface, particularly when there are lots of floating bugs or plant materials.

2.9.6. Reproduction

Depending on a number of variables, including water temperature and food availability, the common carp matures sexually between the ages of 2 and 5. When water temperatures climb over a particular threshold, spawning often takes place in the spring or early summer. During the reproductive season, common carp migrate and go to shallow, lush environments to breed. Males externally fertilize hundreds of sticky eggs released by females. Because they are adhesive, the eggs attach to any accessible substrate, including submerged plants. Depending on the water temperature, the eggs emerge within a few days after spawning with little to no parental care from the adults. The fry, or young fish, are originally translucent and go through several developmental phases before developing the adult coloring.

2.9.7. Global trade and economic value of Common Carp (*Cyprinus carpio*)

Statistical evidence suggest that common carp production may have reached the highest possible level. In contrast, common carp will continue to play a significant role in

regions where it is historically raised. Carp are primarily consumed domestically. The market requires live or freshly dressed fish, according to various studies on common carp processing that were conducted throughout Europe. It is impossible to predict a major growth in demand for processed carp products because processing raised the price of carp to less affordable levels. A typical year in Europe involves the commerce (import or export) of around 24 000 tonnes of live, fresh/chilled filleted, or frozen carp goods (all species) (FAO, 2009). Austria, the Czech Republic, Croatia, and Lithuania are the top exporters. Austria, Germany, Hungary, and Poland were the most prominent importers in 2002. International trade of all carp species is quite restricted in the rest of the globe (39 000 tonnes/yr in 2002), including the main production region (Asia) (FAO, 2009). There are various places where "bio carp" production has begun. Certain consumer groups may accept common carp more if quality labels are used and a focus is placed on the fact that the fish are grown in extensive or semi-intensive systems using eco-friendly technology. In Europe, there has been a shift in the primary objective of common carp farming. Fish was formerly primarily sought for human food. Recent years have seen a considerable increase in the amount of carp generated in aquaculture being placed into natural waterways and water reservoirs for fishing. Because cultivated carp are less aggressive on the hook than wild carp, anglers require wild carp or hybrids of domesticated and wild carp strains. Additionally, wild carp are needed to replenish natural bodies of water where the restoration of native wildlife is being done.

2.9.8. Ecological impact

The common carp's capacity to disrupt ecosystems has led to its classification as an invasive species in several areas. By uprooting aquatic plants and stirring up silt, their bottom-feeding habits can reduce water clarity and change the composition of the environment. They may, however, also function as a significant game fish in recreational fisheries and act as food sources for other creatures. As a strong freshwater fish, the common carp (*Cyprinus carpio*) is renowned for its flexibility, omnivorous food, and vast geographic range. Depending on the particular ecosystem it lives in, it can have both good and harmful ecological effects.

Chapter-3: Materials and Method

3.1. Microalgae sampling site

No.	Location	Type of Water	Coordinate
1	Naf Estuary, Teknaf, Cox's Bazar	Seawater	20° 47′ N, 92° 28′ E
2	Sonadia Island, Maheshkhali, Cox's Bazar	Seawater	22°29' N 91°25' E

3.2. Sample collection and concentration

For sample collection, 60 µm mash size plankton net was used where about 40-50L seawater was filtered through the plankton net and collected in a 300ml sample bottle and maintained at refrigerated condition while transferring to the laboratory. Then the samples were concentrated by centrifuging at 4000 rpm for 5 minutes. After centrifugation supernatant were discarded and the concentrate was used for isolation.

3.3. Determination of microalgal diversity

Filtered raw seawater samples from each of the sampling site were preserved by adding a few drop of lugol's iodine to determine the microalgal diversity. Then the samples were observed under microscope and microalgal diversity was determined by using phytoplankton identification books such as Belcher and Swale (1976); John et al. (2002) and Bellinger and Sigee (2010).

3.4. Isolation of microalgae

3.4.1. Agar plate preparation and streak plating method

For preparing the agar medium, 1.5 % agar was added to 1 L of Conway media and solution was sterilized in an autoclaved at 121˚C temperature for 15 min under 150 lbs pressure. Then the agar medium was cooled to about 50 ℃ and vitamin solution was added into it while mixing by gently rotating the flask to ensure mixing of the nutrients and avoid bubble formation. After that, the warm medium was aseptically poured into the sterile petri dish where the agar was at least ½ or ¾ the depth of the petri dish, and agar medium was left to cool and solidify and stored in plastic bags in a refrigerator until further use. After preparation of the agar plate 1-2 drops of the concentrated

natural collection was placed near the periphery of the agar. Following aseptic technique, wire loop or hockey stick was used to make parallel streaks of the suspension on the agar. The plates were covered, inverted in position and sealed with parafilm to prevent the petri dish from opening unnecessarily or accidentally while incubating. When growth was visible in the dish, the petri dish was taken out from the incubator and chosen colony was removed by using a sterile fine needle and place in 3-5 drops of liquid medium on a sterile dish and observed under microscope to select the desired colonies that are free from other organisms for further isolation. When the colony contained multiple microalgae, streaking procedure was repeated again to develop single colony.

3.4.2. Serial dilution

For serial dilution, test tubes were filled with 9 ml of culture medium (Conway media) prepared in the last lab. Tubes were labeled as 10^{-1} -10^{-10} to indicate dilution factor. After that, test tubes were put in the biological safety cabinet where test tube cap was removed and its neck was flamed and 1 ml of enrichment sample was added to the test tube (10^{-1}) and mixed gently. Then, 1 ml of this dilution was taken and added to the next tube (10^{-2}) and then mixed gently and this procedure was repeated for the remaining tubes (10^{-3} -10^{-10}). After completing the dilution procedure, test tubes were incubated under controlled temperature and light conditions. After 2-4 weeks, the cultures were examined microscopically by withdrawing a small sample aseptically from each dilution tube where unialgal culture found in one of the higher-dilution tubes, e.g., 10^{-6} -10^{-10}. When the tubes contain two or three different species, capillary pipetting method was applied for isolation of single algal species.

3.4.3. Picking up method (Capillary method)

For capillary picking up method, first a micropipette was prepared from a Pasteur pipette, where pasteur pipette was held in the hottest region of the flame, supported on the left by a hand and on the right by forceps and heated until it soft and pliable condition and when it became soft, the pipette was quickly removed from the flame with a gentle pull to produce a thin tube. The forceps were then relocated to the appropriate region of the thin tube to gently bend the thin area, so that it broke and formed a micropipette. A large drop of algal suspension growing by the dilution method was placed in the center of a glass slide. 4-6 drops of prepared Conway media were

placed in another slide. By using an inverted microscope to observe the cells, the desired single cell from the large drop of the algal suspension was transferred to one of the drops of liquid media. This process was repeated by pipetting the desired algal unit from liquid medium drop number one to two and continued until only one single algal unit was found in the last drop of liquid medium without any foreign substance. The single algal unit was then transferred into the Eppendorf containing liquid media and incubated at the desired environmental condition.

3.5. Morphological identification of microalgae

Morphological identification of isolated microalgae was done microscopically at 40X magnification and based on the morphological characteristics using phytoplankton identification books (Belcher and Swale, 1976; John et al., 2002 and Bellinger and Sigee, 2010).

3.6. Preparation of Conway media

Pure Conway medium was used for the culture of *Chlamydomonas* sp. Conway medium constitutes with different macronutrients, metal solutions and vitamins which are shown in Table 1. One ml of solution A, 0.5 ml of solution B and 0.1 ml of solution C were added with autoclaved and sterilized seawater to make 1 liter of Conway media.

Table 1: Constituents of Conway Media

Solution A-Macronutrients	
Compound name and molecular formula	**Proportions**
Sodium/Potassium nitrate ($NaNO_3$ /KNO_3)	10 0.0 0 g/116.0 0 g
EDTA Disodium salt ($C_{10}H_{16}N_2O_8$)	45.00 g
Boric acid (H_3BO_3)	33.60 g
Sodium di-hydrogen orthophosphate ($NaH_2PO_4.4H_2O$)	20.00 g
Ferric chloride hexahydrate ($FeCL_3.6H_2O$)	1.30 g
Manganese (II) chloride tetrahydrate ($MnCL_2.4H_2O$)	0.36 g
Deionized/distilled water	1 L

Solution B-Trace metal solution	
Compound name and molecular formula	**Proportions**
Zinc chloride ($ZnCl_2$)	2.10 g
Cobalt (II) chloride hexahydrate ($CoCl_3.6H_2O$)	2.00 g
Ammonium molybdate tetrahydrate ($(NH_4)_6MO_7O_{24}.4H_2O$)	0.90 g
Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$)	2.00 g
Zinc chloride ($ZnCl_2$)	2.10 g
Deionized/distilled water	1 L

Solution C-Vitamin's solution	
Compound name and molecular formula	**Proportions**
Thiamine, Vitamin B1	200 mg
Cyanocobalamin, Vitamin B12	10 mg
Deionized/distilled water	100 mL

3.7. Determination of growth curve

The isolated microalgae were cultivated using the Conway media (Stein, 1980). Cultures were grown at a temperature of 24 ± 1°C in a 350 mL culture volume of a sterile 500 mL borosilicate Erlenmeyer flask for each species with three replicates where 2% pure culture stocks were added in each flask. Microalgae cultures were maintained at 24 hr light condition at 150 $\mu Em\text{-}2s^{-1}$ intensities with continuous gentle aeration at a rate of 4.53 ± 0.53 mg/L. The experiment was continued until the death phase and finally completed the growth curve depending on cell density ($cells.ml^{-1}$) and optical density (absorbance).

3.7.1. Determination of cell density

Microalgae cell count was carried out every day by using a Neubauer hemacytometer (0.0025 mm^2, 0.1 mm deep chambers, Assistent, Germany). Before use, hemacytometer was cleaned with distilled water to make sure it is free from dust, lint and grease. To facilitate counting, Lugol's iodine was added to microalgae sample for fixation and

staining. Then small drop of properly mixed sample was placed into the counting chamber and the cells were allowed to settle 3-5 minutes for better counting. Under the magnification of 40X, microalgae cells were counted for both chambers of the hemacytometer. The cells were counted by using the following formulae (Lavens and Sorgeloos, 1996):

$$\text{Cell count (cell/ml) for 5 squares} = \frac{\text{Total number of cells counted}}{10\times4} \times 10^6$$

$$\text{Cell count (cell/ml) for 25 squares} = \frac{\text{Total number of cells counted}}{50\times4} \times 10^6$$

Where 10 and 50 = the squares of the 2 hemacytometer chambers and 4 x 10^{-6} = the volume of samples over the small square areas, that were equivalent to 0.004 mm^3 (0.2 mm x 0.2 mm x 0.1 mm), expressed in cm^3 (ml).

3.7.2. Determination of maximum absorbance (optical density)

Optical density (OD) was measured every day for the growth curve analysis. Conway media without any microalgae cells were used as blank. Maximum absorbance value for each microalga was used to perform the growth curve by OD. Maximum absorbance was measured at the wavelength of 430nm for *Gonyostomum* sp. and *Chlamydomonas* sp., 450nm for *Nannochloropsis* sp., 428nm for *Navicula* sp. as those wavelengths gave maximum absorbance when the culture samples were scanned between 300 to 700 nm, using a spectrophotometer (Nano Drop Spectrophotometer, Model-Nanoplus, Germany).

3.8. Culture of selected marine microalgae for pigment determination and analysis of proximate composition

In large sterile 2L borosilicate Erlenmeyer flasks, having 1.7L pure Conway media were used for this experiment. Each of the microalgae species was cultured to maintain similar environmental condition (Temperature: 24 ± 1°C; Light: 150 µEm-2s-1 intensity) until stationary phase. From the fresh cultured sample, carotenoid and chlorophyll were analyzed at the end of their exponential phase. As phycobiliprotein and proximate composition analysis required dry biomass, all the cultures were harvested at the end of their exponential phase by centrifugation (Hitachi* High-speed Refrigerated Centrifuge, himac CR 21g-II, China) depending on the growth curve experiment, and dried at 40°C temperature by using hot air oven and finally preserved at a refrigerator (4°C) for further use.

3.9. Determination of biomass

Biomass determination is prerequisite for productivity analysis. Biomass were determined by filtering of 1ml microalgae sample from each replication of individual microalgae through a pre-weighted (after marking of filter paper rinsed with 10ml distill water and dried at 100°C for 4hr in hot air oven) glass microfiber filter paper, which was further rinsed with 10ml distill water for three times. Then the filter paper with biomass was oven dried at 100°C for 4hours. After that, final weight of filter paper was taken followed by 15min of desiccation and dry biomass was calculated according to Ratha et al. (2016).

3.10. Determination of pigments

3.10.1. Extraction of microalgae for chlorophyll determination

For the extraction of microalgae, first 1ml of $MgCO_3$ solution (after proper shaking) was filtered through the filter paper (47 mm Ø Whatman® GF/C glass microfiber filter papers) by using filtering apparatus, so that most of the area of filter was covered. Then, 10ml of each algae sample was filtered and the edges which were not coated with residue were trimmed away. After that, the filter paper was fold and place into 15ml centrifuge tube where the middle of the filter is facing downward. Into the centrifuge tube, 2ml of 90% acetone was added and grind for 1 minute, again 8ml of 90% acetone was added and grind for 30 sec. Then, the sample was refrigerated in the dark for 1 hour. After 1 hour, sample was centrifuged at 3000 rpm for 10 minutes and transferred the acetone extract into another centrifuge tube and centrifuged at low speed (500 rpm) for 5 minutes. Lastly, the absorbance of acetone extract was measured against 90% acetone as blank.

3.10.2. Determination of chlorophyll

Chlorophyll concentration was determined according to Jenkins (1982). The clean extract was transferred to a 1cm cuvette and OD was measured at 750, 664, 647 and 630nm wavelength. OD at 664, 647, and 630 nm were used for chlorophyll determination where, OD at 750 nm was used as turbidity correction factor and subtracted from each of the pigments OD values before using them in the equations. Concentrations of chlorophyll a, b, and c in the extract were calculated by inserting the corrected optical densities in following equations (Jeffrey and Humphrey, 1975):

a) $C_a = 11.85(OD664) - 1.54(OD647) - 0.08(OD630)$

b) C_b = 21.03(OD647) - 5.43(OD664) - 2.66(OD630)

c) C_c = 24.52(OD630) - 7.60(OD647) - 1.67(OD664)

Where: C_a, C_b, and C_c = concentrations of chlorophyll a, b, and c, respectively, mg/L, and OD664, OD647, and OD630 = corrected optical densities (with a 1 cm light path) at the respective wavelengths. After the determination of the concentrations of pigments in the extract, the amount of pigments per unit volume was calculated as follows:

$$\text{Chlorophyll a, mg/m}^3 = \frac{\text{Ca} \times \text{extract volume, L}}{\text{volume of sample, m}^3}$$

3.11. Mass culture of microalgae in glass tanks

Sea water was collected from Cox's Bazar to prepare media for mass culture. To eliminate debris, the collected sea water was first stored in plastic tanks overnight to allow the solid particles to settle down appropriately, filtered with a filter bag and vacuum filter pump to separate fine particles from the water and then sterilized in an autoclave (for 15 mins in 121℃ and 15 lbs). Then, the growth media was prepared with required nutrients; salinity and pH were adjusted to 15 ppt and 7.8, optimum for the culture of *Chlamydomonas* sp. Pure strains from the stock were transferred to 20L glass tanks for mass culture at room temperature with a 12-hour light: 12-hour dark photoperiod, commencing with a volume of 5L and subsequently scaling up to 16L. Every two days, media was added to the culture tank until it reached the desired volume. Individual culture tanks were supplied with continuous aeration through a central air pump. Transparent polythene was used to cover the top sides of culture tanks to prevent contamination. Every week, samples from each tank were taken and examined under a light microscope for any signs of contamination. Temperature within the algal culture unit was measured daily.

3.12. Harvesting as dry algal biomass

A 15-day culture trial was used to determine the native strain's stationary phase in a previous investigation (Zahid et al., 2021). As a result, algal biomass was recovered by centrifugation (Muylaert et al., 2017) during the previously reported stationary phase and dried in a hot air oven (Natural Convention Oven LNO-150) at 60 °C overnight (Stramarkou et al., 2017). The dried biomass was pulverized using small sized mortar and pestle. The finely powdered algal biomass was then stored in airtight glass vials at 4°C temperature until it could be used in the production of test diets.

3.13. Experimental design for growth performance of Common Carp (*Cyprinus carpio*) fry

3.13.1. Test diet preparation

All of the formulated test diets were approximately iso-proteic and iso-lipidic (Crude protein-30% and lipid-6%). The necessary feed ingredients such as, processed feed grade fishmeal, soybean, wheat bran, wheat flour, barley meal, corn meal, starch, mineral premix and commercial feed were purchased from local stores in Chattogram City (Bangladesh). First, fishmeal and rice bran were sieved and oven-dried at 60ºC to reduce the moisture level. Then they were crushed in the grinder to make more fine powder. To make dough, fishmeal, rice bran and wheat flour were mixed together and then *Chlamydomonas* sp. powder was added to that mixture as required. For comparative study, dried *Chlamydomonas* sp. powder was incorporated into the diet as follows: control (no inclusion), 5% (C5), 10% (C10), and 15% (C15) for comparative study. The composition of the base ration used in the diet is shown in Table 2.

After mixing all of the ingredients together, an adequate amount of water was added to form a doughy texture. Then the doughy mixture was run through a grinder, dried, and granulated. The diets were sieved to ensure uniform granule size. Before final approval, all of the experimental diets were subjected to a chemical analysis. Crude protein (Kjeldahl Auto System) (ISO 5983–1987) and crude lipid (Soxtec HT6) of all the formulated feeds were determined to ensure homogenous nutritional quality. Finally, the prepared feeds were sealed in plastic bags, placed in airtight, labeled containers, and stored in a cool (4°C), dry place away from direct sunlight to preserve the feed's quality and prevent mould growth throughout the experiment.

Table 2: Percent ingredients and proximate composition of the experimental diets.

	Dietary group				
	Control	**Microalgae diet**			**Commercial**
	T0	**T1**	**T2**	**T3**	**T4**
Ingredients					
Fish Meal	25%	22%	19%	16%	-
Soybean	30%	28%	25%	22%	-
Wheat Bran	13%	12%	11%	10%	-
Wheat Flour	8%	9%	10%	10%	-
Barley Meal	10%	10%	10%	10%	-
Corn Meal	9%	9%	9%	10%	-
Starch	3%	3%	4%	5%	-
Mineral Premix	2%	2%	2%	2%	-
Chlamydomonas sp.	*0*	5%	10%	15%	-
Proximate composition					
Crude protein (%)	30.68%	30.74%	30.75%	30.83%	30.81%
Lipid (%)	6.39%	6.47%	6.45%	6.53%	6.42%

3.13.2. Collection and stocking of fish

Common carp fries (*C. carpio*) were purchased from a hatchery situated in Mymensingh and brought to the lab in aerated plastic bags. Afterwards, the fries were gently released in the previously prepared tank water (filtered, aerated and UV treated for 2 days) and kept there for three days to being acclimatized with the laboratory conditions. This will reduce the mortality rate during experimental period. Fries with an average length and weight of 1.83±0.03 cm and 120±10 mg respectively were selected for the study. Then the fries were divided into five treatment groups, each with a triplicate replication cohort of fifteen uniformly sized individuals. Then each group were kept in a rectangular glass tank (18× 12 × 14 inches) filled with 40L of water carrying 20fries in each replication.

3.13.3. Feeding and water quality management

Test diets were fed to the fish at 5% of their body weight twice a day in equally divided doses at 6 hour intervals (10:00 AM and 4 PM). Throughout the trial, each tank received continuous aeration. To maintain optimum water quality, every day 1/3 of the culture water was siphoned out from the tank bottom followed by adding new water to compensate the removed amount. A complete water exchange in the tanks was done twice per week.

Water temperature was maintained at 26±2℃ with a 12 h dark: 12 h light photoperiod, dissolved oxygen at 6.5±1 mg and pH at 7.7 to 8.4. Required amount of lime was used to maintain the pH of the culture tank whenever needed. Total ammonia and nitrite concentrations remained below 0.02 mg. This study was carried out over 60 days to observe the growth properly.

3.13.4. Daily monitoring and record keeping

On a daily basis, water quality parameters such as temperature, pH and dissolved oxygen (DO; mg/L) was measured with glass thermometer, a handheld pH meter (pHep-HI98107, HANNA, Romania), and dissolved oxygen meter (DO-5509, Lutron). Mortality rate and breeding status were observed on a daily basis. Fish were also routinely monitored for any occurrence of stress or disease outbreak. Dead fish were removed from the aquarium as soon as they were discovered.

3.14. Data collection and analyses

3.14.1. Assessment of growth parameters

At the termination of feeding trial, fish were starved for 24 hours before sampling to empty their gastrointestinal system and then anesthetized with clove oil (eugenol solution) to reduce the handling stress while sampling. Fish were randomly selected from each tank and weighed using a digital meter for growth study. The metrics utilized to evaluate fish growth performance included net weight gain, average daily gain, specific growth rate, length increment, condition factor, FCR, and FCE. Growth parameters were calculated using the following formula (Sarathchandra et al., 2018; Ayala et al., 2020)-

Survival rate (%) = (Number of harvested Fish/ Number of Fish Stocked) × 100

ADG (Average Daily Gain) = (Final Weight−Initial Weight)/ culture period

SGR (%) = ln (final weight) −ln (initial weight) / Duration of Experiment (days) ×100
Length increment= Final Total Length (cm) - Primary Total Length (cm)

FCR (g⁄g) = feed given (Dry weight) / Body weight gain (Wet weight).

3.14.2. Proximate composition analysis

Sample fish were dried in a hot air oven for 8 hours at 40°C to determine carcass proximate composition. The dried carcass was then finely ground and homogenized with a mortar and pestle prior to the analyses of crude protein, crude lipid and carbohydrate content.

3.14.2.1. Protein

Reactive 1 (1 percent potassium sodium tartrate) and Reactive 2 (2 g sodium carbonate per 100 ml 0.1 N NaOH) were prepared. Then, 1 mL of Reactive 1 was added to 50 mL of Reactive 2 to prepare a final volume of mixed reagent. For each sample analysis, 5, 6 mg of oven-dried sample was taken, and a 25 ml solution was made by combining it with deionized water. 0.5 ml of aliquot from each sample was collected from the generated 25 ml sample. 0.5 ml of sample was mixed with 0.5 ml of 1 N sodium hydroxide and maintained in a water bath at 100 °C for 5 minutes. It was then chilled in a cold water bath for 10 minutes before adding the prepared mixed reagent. Afterwards, 2.5 ml of the mixed solution was mixed with 0.5 mL Folin reagent and stored in the dark for 30 minutes. In a spectrophotometer (T80 UV/VIS

Spectrophotometer), absorbance of the mixed reagent was measured at 750 nm wavelength.

3.14.2.2. Lipid

Lipid content was examined by Bligh and Dyer (1959) and Folch et al. (1957). For each sample, aluminum dishes were constructed and labeled. Each labeled dish's initial weight was recorded. A pre-weighed 50 mg sample was taken in a centrifuge tube and diluted 5 times in deionized water. The sample was systematically mixed with 3 ml 1:2 chloroform:

methanol (v/v) using a tissue homogenizer. Then the mixed solution was centrifuged at 1000 rpm for 4 minutes at 4 °C temperature. Supernatants were transferred to a clean centrifuge tube and stored on ice using a Pasteur pipette. 3 ml of 2:1 methanol: chloroform (v/v) was consistently mixed with the sample. The tubes were centrifuged for one more time, and the supernatants were transferred to the old supernatant tubes. In this combined supernatant, 1.5 ml of 0.9 percent NaCl was mixed with a vortex mixture (VM-10). After that, the tubes were placed in the refrigerated for an hour at 4 °C. After an hour, the tubes were centrifuged at 4 °C for 10 minutes at 1000 rpm.Two distinct layer of solution were formed inside the centrifuge tube. First, the upper layer containing methanol and chloroform was removed with a Pasteur pipette and then the lower layer was transferred to the aluminium dish. The solvent in the dishes was evaporated in a hot air oven at 4°C temperature. The final weight was determined by weighing the aluminum plate. Finally, the lipid weight of the samples was calculated by subtracting the initial weight from the final weight.

3.14.2.3. Carbohydrate

Carbohydrate analysis was carried out using the method of Dubois et al. (1956). 5-6 mg of oven-dried microalgae sample was taken for each assay, and a 25 ml solution was made by mixing it with deionized water. Prior to the analysis, a 5 percent phenol solution and concentrated sulphuric acid were prepared. From the prepared sample, a milliliter aliquot was taken into the test tube. The test tube was filled with one milliliter of phenolic solution and five milliliters of strong sulphuric acid and placed in an ice bath to cool. After cooling, the optical density was measured with a spectrophotometer (T80 UV/VIS Spectrophotometer) at 488 nm wavelength.

3.15. Chemical analysis of water quality

3.15.1. Total ammonia nitrogen (TAN)

Total ammonia nitrogen was determined according to Parson et al. (1984). Standard stock solution was prepared by weighing 9.343 g of anhydrous grade $(NH_4)2\ SO_4$ (dried at 110^0C for 1 hour, cooled in desiccator before weighing) and dissolving in 1000 ml deionized water. From the stock solution (1000 mg L-1 of total ammonia-nitrogen), a series of standard solutions (0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5 and 1.0 mg L-1) were prepared by mixing with appropriate ratio of deionized water. Samples and standard solutions (10 ml) were placed in test tube and 0.4 ml of phenol solution (20 g of analytical grade phenol was dissolved in 200 ml of 95% v/v ethyl alcohol and 0.4 ml of sodium nitroprusside (1 g of $Na_2[Fe(CN)_5\ NO]_2H_2O$, dissolved in 200 ml of DDH_2O water) was added in sequence. Finally, 1 ml of oxidizing solution was added and allows cooling at room temperature (20-27 C) for 1 hour. The test tubes were covered with parafilm (the color is stable for 24 hours after the reaction period). The extinction was measured at 640 nm with Shimadzu spectrophotometer model UV-1601. Oxidizing solution was prepared by mixing 100 ml of alkaline reagent (dissolve 100 g of sodium citrate and 5 g of sodium hydroxide in 500 ml of DDH_2O) and 25 ml of sodium hypochlorite solution [commercial hypochlorite (e.g. clorox) which should be about 1.5 N].

3.15.2. Nitrite-nitrogen (NO_2-N)

Nitrite was determined according to Parsons et al. (1984). Standard stock solution was prepared by weighing 4.9259 g anhydrous grade $NaNO_2$ (dried at 110 for 1 hour, cooled in dessicator before weighing) and dissolving in 1000 mL deionized water. From the stock solution (1000 mg L-1 of NO_2-N), a series of standard solutions (0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5 and 1.0 mg L-1) were prepared by mixing with deionized water. Samples and standard solutions (10 ml) were placed in test tube. Then 0.2 ml of sulfanilamide solution (5 g of sulfanilamide was dissolved in a mixture of 50 mL of concentrated hydrochloric acid and dilute to 500 ml with DDH_2O) was added. After more than 2 minutes but less than 10 minutes, 1 ml of NED reagent (0.5 g of the N- (1-napthyl)- ethylenediamine dihydrochloride was dissolved in 500 ml of distilled water) was added and mixed immediately. Between 10 minutes and 2 hours afterwards, the

extinction was measured at a wavelength of 543 nm by using the Shimadzu spectrophotometer model UV- 1601.

3.15.3. Soluble reactive phosphorous (SRP)

Soluble reactive phosphorous (SRP) was determined according to Parsons et al. (1984). Standard stock solution was prepared by weighing 4.3937 g of anhydrous grade potassium dihydrogen phosphate, KH_2PO_4 (dried at 110OC for 1 hour, cooled in dessicator before weighing) and dissolving in 1000 ml deionized water. From the stock solution (1000 mg L-1of PO_4-P) a series of standard solutions (0.01, 0.03, 0.05, 0.07, 0.1, 0.3, 0.5 and 1.0 mg L-1) was prepared by mixing with deionized water.

Ten milliliter of samples and standard solutions (10 ml) were placed in test tubes and 1 ml of mixed reagent was added. After 5 minutes and preferably within the first 2-3 hours, the extinction was measured at 885 nm by using Shimadzu spectrophotometer model UV- 1601. Mixed reagent was prepared by mixing 100 ml of ammonium molybdate (dissolve 15 g of analytical reagent grade ammonium paramolybdate $(NH_4)_6Mo_7O_{24}$ in 500 ml of distilled water), 250 ml sulfuric acid, 100 ml ascorbic acid (dissolve 27 g of ascorbic acid in 500 ml of distilled water) and 50 ml of potassium antimonyl-tartrate solution (dissolve 0.34 g of potassium antimonyl-tartrate (tartar emetic) in 250 ml of water.

3.16. Fatty acid determination

Fish lipids must contain fatty acids since they are vital to many physiological activities. They provide energy, aid in the formation of the cell membrane, and function as building blocks for the manufacture of critical substances including hormones and signaling chemicals. The extraction of lipids from fish samples is usually the first step in the process of fatty acid determination. The Folch or Bligh and Dyer procedures, which entail combining the fish tissue or oil with organic solvents to separate the lipids from other components, can be used to accomplish this (Spiric et al., 2010). The extracted lipids are then purified and concentrated to obtain a representative sample. Following the process of segregation, the lipids must next undergo a procedure known as transesterification in order to become their methyl ester derivatives. Fatty acid methyl esters (FAMEs) are created when the ester linkages in lipids are broken down by a powerful acid, such as sulfuric acid or methanolic hydrochloric acid (Guler et al.,2008). Due to their higher volatility, FAMEs may be analyzed with the use of gas

chromatography (GC) or high-performance liquid chromatography (HPLC) methods. In order to separate and quantify fatty acid methyl esters, gas chromatography is frequently used. The FAMEs are introduced into a GC apparatus with a capillary section, where they are vaporized and separated according to their molecular characteristics, such as chain span and degree of saturation. Individual fatty acids can be precisely identified and quantified by detection utilizing a flame ionization detector (FID) or mass spectrometry (MS). Alternative methods for determining fatty acids include high-performance liquid chromatography (HPLC). To quantify the separated fatty acids, a variety of detection techniques, including ultraviolet (UV) and refractive index detectors, can be used (Farkas et al., 2018). The basic method for identifying and quantifying certain fatty acids in fish samples is to contrast the retention periods or peak areas of the FAMEs with those of common reference compounds. For calibration purposes, fatty acid standards or commercially available standard mixes are frequently utilized. Researchers and nutritionists may learn a lot about the nutritional value and health advantages of various fish species by studying the fatty acid makeup of fish. Fish species can differ greatly in their fatty acid composition, especially in terms of omega-3 and omega-6 fatty acids. This can have an impact on human health, notably in terms of cardiovascular health, inflammation, and brain function.

3.17. Amino acid determination

Amino Acid Analysis (AAA) affords an absolute quantitative measure of protein content of a sample independent of an external protein reference standard. The use of AAA for protein identification has mostly been replaced by automated Edman sequencing and MS methods. The main use of AAA in contemporary biotechnology labs is to determine protein absorptivity constants, which are then used as a foundation for basic, everyday measurements of protein concentration at 280 nm (Simpson et al., 1976). The use of AAA as a standard concentration assay is possible in some circumstances, such as when a pharmaceutical peptide or protein contains few or no 280 nm-absorbing residues (Tyrosine, cystine), or when a specific modified amino acid needs to be measured in peptides, proteins, or conjugates that have undergone chemical modification (Parsons et al., 1984). Additionally, AAA can be helpful for protein quantification when formulation excipients or UV-absorbing prosthetic groups (covalent or noncovalent) obstruct UV readings. Maybe a less time-consuming approach would be to first determine the concentration for the peptide or protein

reference material batch using AAA, and then use this reference material as the standard curve for a typical protein concentration test like absorbance or a colorimetric assay. Since the 1950s, HPLC methods have been employed to quantify amino acids. Deproteinization and derivatization, which may be done before or after separation, are necessary for amino acid detection by absorption or fluorescence (Mai et al.,1980).

3.18. Analysis of blood parameters

Towards the end of the feeding trial, ten fish were randomly selected from each culture tank and clove oil (eugenol solution) was used to anesthetize the fish in order to measure the blood parameters. The fish's neck was poked with a sterile syringe filled with one milliliter of blood. Blood samples were quickly transferred into a non-heparinized 2ml (EDTA K3 PROVEN vacuum tube) for hematology and serum testing. Additionally, samples were taken into heparinized (CURE clot activator tube) tubes. The extracted blood plasma was transferred into a 1.5 ml Eppendorf tube using a micropipette and it was then kept there at -20^0C pending further analysis. Hematological analysis of blood parameters including Red Blood Cells (RBC), Hemoglobin (Hb), Hematocrit (Hct), White Blood Cells (WBC), Lymphocytes (LYM), and Platelets (PLT) was completed concurrently using a hematology analyzer (NIHON KOHDEN, India). Fish blood serum included total serum protein, albumin, globulin, triglyceride, cholesterol, blood glucose, urea, and blood urea nitrogen (BUN), which were all determined using a biochemistry analyzer (Humalyzer 3000, Germany).

3.19. Statistical analysis

The mean and standard error of the mean of all the data were calculated in MS Excel and reported throughout the text as means and standard error. The IBM SPSS (v. 26.0) software was used to perform all statistical analyses related to the survival rate, growth characteristics, proximate composition, pigments, and oxidative stress. Descriptive statistics were computed for each treatment, followed by a test for homogeneity of variance. A one-way analysis of variance was used to examine the acquired data (ANOVA). Tukey's multiple comparison tests were used to look for significant differences among treatments at 95% confidence interval level. To distinguish between groups, a post- hoc test was performed. Because of insufficient sample, it was not possible to provide replication in case of amino acid and blood parameters analysis. Otherwise, all the associated data were analysed by using MS Excel and IBM SPSS.

Chapter-4: Results

4.1. Sampling site

4.1.1. Water quality parameters of the sampling sites

Water quality parameters of the sample water collected from different seawater sites:

Parameters	**Teknaf**	**Sonadia**
Temperature (°C)	33℃	29.5℃
DO (mg/L)	7.5	7.3
Ph	8.5	8.1
Salinity	15 ppt	25 ppt
Total Ammonia nitrogen (TAN) (mg/L)	0.003	0.004
Soluble reactive phosphate (mg/L)	0.3365	0.005
Nitrite-Nitrogen (mg/L)	0.085	0.037

4.1.2. Chlorophyll (µg/ml)

Sampling Station	**Chl-a**	**Chl-b**	**Chl-c**
Teknaf	0.2552	0.1706	0.3248
Sonadia	0.9343	0.1864	0.1796

4.2. Isolated Microalgae

Teknaf	**Sonadia**
Gonyostomum sp.	*Navicula* sp.
Chlamydomonas sp.	*Nannochloropsis* sp.

4.3. Morphological characteristics of isolated microalgae

4.3.1. *Gonyostomum* sp.

Taxonomic classification:

Phylum: Ochrophyta

Class: Raphidophyceae

Order: Raphidomonadales

Family: Vacuolariaceae

Genus: *Gonyostomum* (Diesing, 1866)

Morphological characteristics:

The drop-shaped, vegetative cells of G. semen are up to 100 µm long, but can vary quite a bit in form and size as they are only surrounded by a cell membrane instead of a cell wall. Like other heterokont algae, the planktonic cells of G. semen possess two differently shaped flagella which enable them to actively swim around in the water column. Under physical stress, small organelles that sit under the cell membrane and are called trichocysts, explode and release slimy threads.

Figure 4: *Gonyostomum* sp.

4.3.2. *Chlamydomonas* sp.

Taxonomic classification:

Kingdom: Plantae

Phylum: Chlorophyta

Class: Chlorophyceae

Order: Chlamydomonadales

Family: Chlamydomonadaceae

Genus: *Chlamydomonas* (Ehrenberg, 1833)

Morphological characteristics:

Motile unicellular algae. Generally oval and/or circular. Cell wall is made up of a glycoprotein and non-cellulosic polysaccharides instead of cellulose. Two anteriorly inserted whiplash flagella are found. Each flagellum originates from a basal granule in the anterior papillate or non-papillate region of the cytoplasm. Each flagellum shows a typical 9+2 arrangement of the component fibrils. Prominent cup or bowl-shaped chloroplast is present.

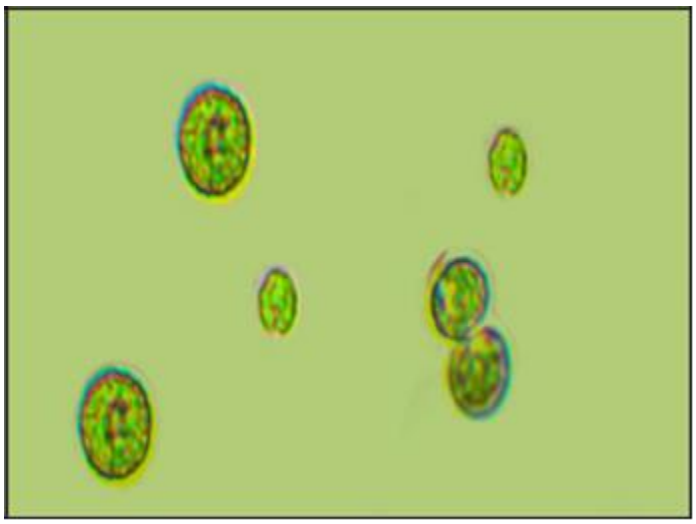


Figure 5: *Chlamydomonas* sp.

4.3.3. *Navicula* sp.

Taxonomic classification:

Kingdom: Chromista

Phylum: Ochrophyta

Class: Bacillariophyceae

Order: Naviculales

Family: Naviculaceae

Genus: *Navicula* (Bory de Saint-Vincent, 1822)

Morphological characteristics:

Navicula sp. is a raphed, pennate diatom with boat-shaped cells that may exist singly or in ribbons. The valves are symmetrical both apically and trans apically, and may have rounded, acute, or capitate ends. Striations vary and may or may not be visible under light microscope. The central area may be thickened but without a banded stauros shape. Most species are found in valve view, but can be found in girdle view.

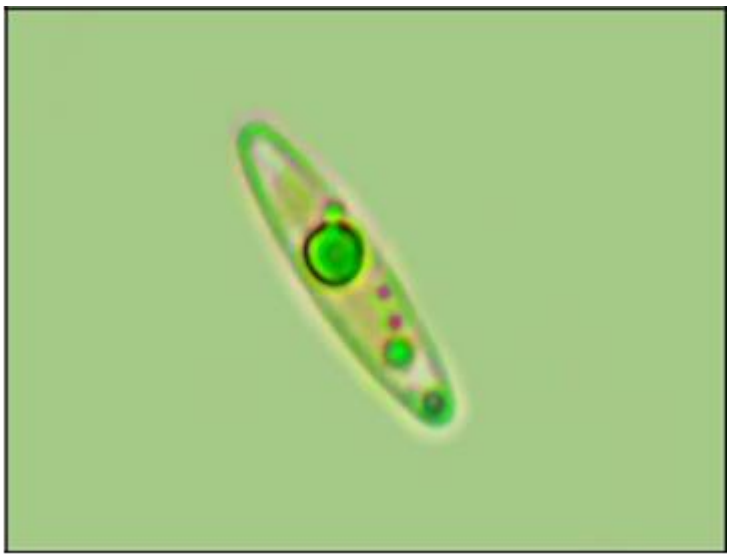


Figure 6: *Navicula* sp.

4.3.4. *Nannochloropsis* sp.

Taxonomic classification:

Kingdom: Chromista

Phylum: Ochrophyta

Class: Eustigmatophyceae

Order: Eustigmatales

Family: Monodopsidaceae

Genus: *Nannochloropsis* (Hibberd, 1981)

Morphological characteristics:

Unicells lacking flagella (no zoospores are produced), spherical to ovoid, 2 – 4 µm diameter. One plastid, no pyrenoid. All of the species are small, nonmotile spheres which do not express any distinct morphological features that can be distinguished by either light or electron microscopy.

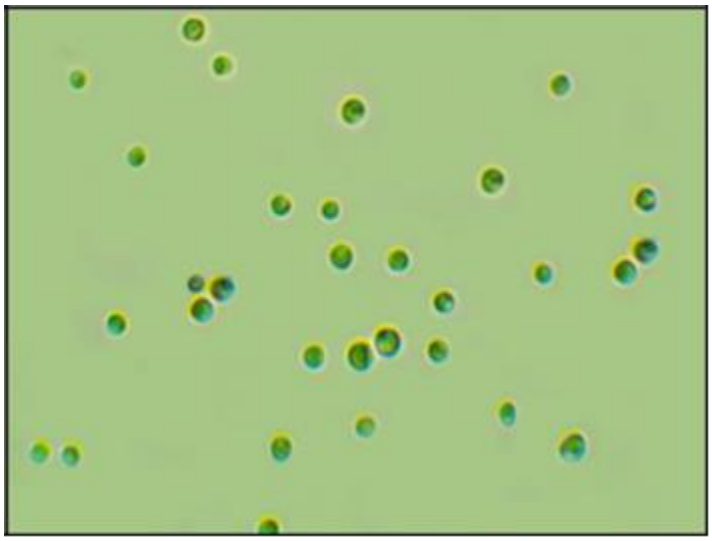
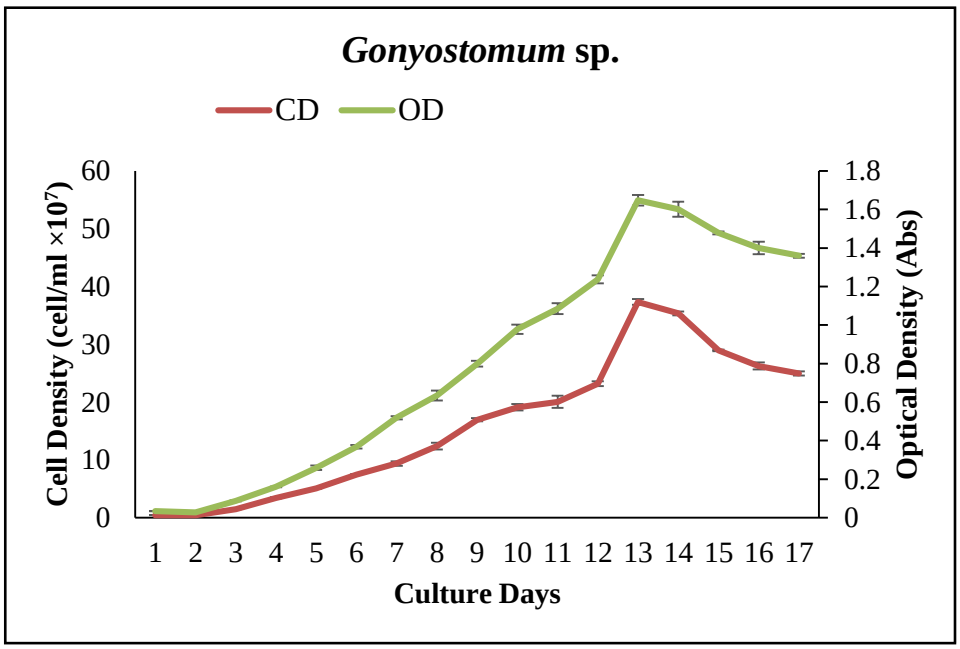


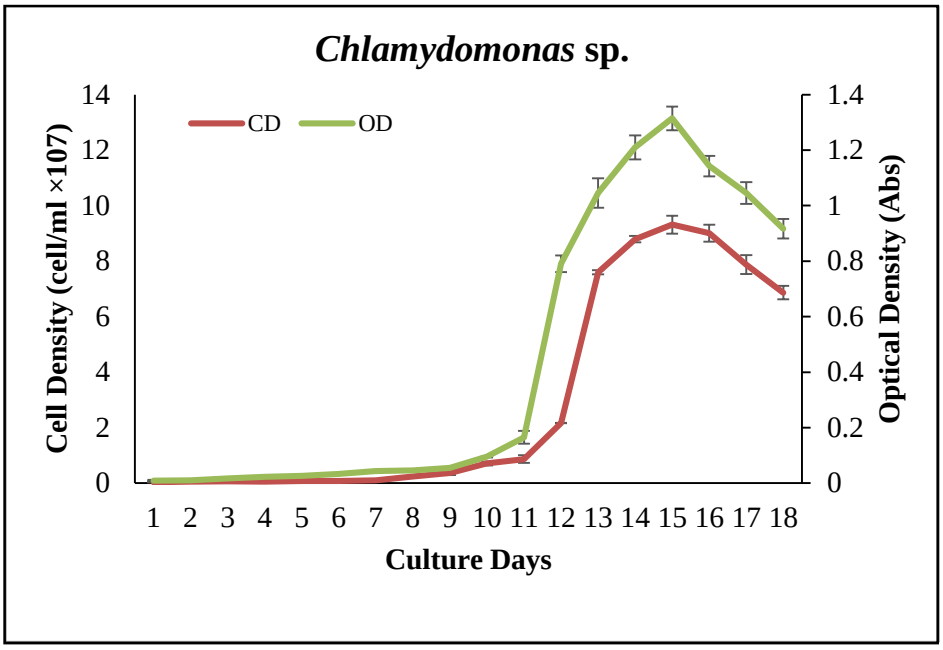
Figure 7: *Nannochloropsis* sp.

4.4. Growth phases of isolated microalgae

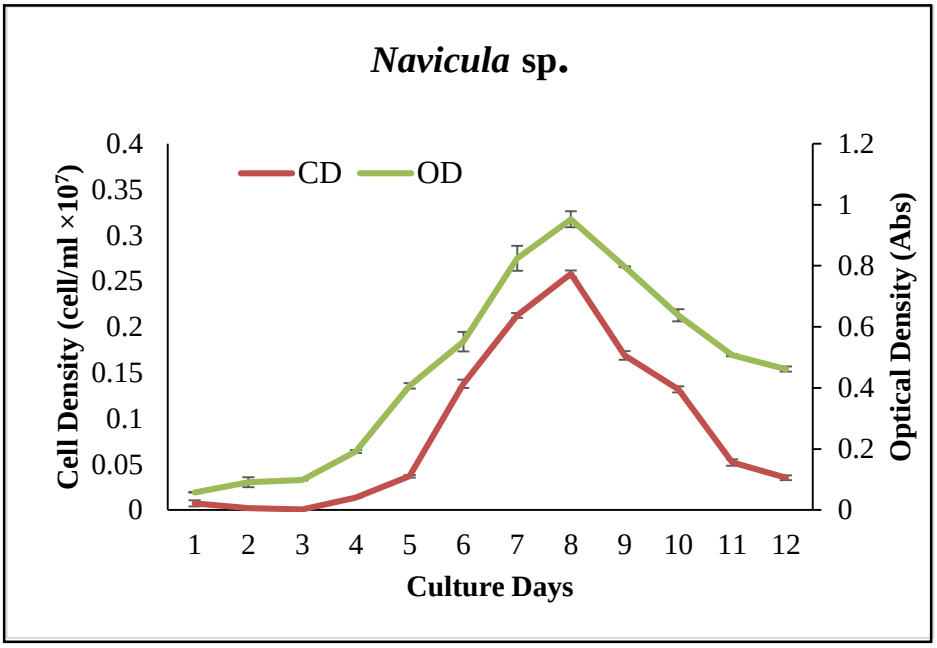
In the present study, different microalgae showed different cell concentration and proximate composition as those can vary from species to species. The figure illustrates the cell density and optical density versus the cultivation time of each of the four species (*Gonyostomum* sp.-a, *Chlamydomonas* sp.-b, *Navicula* sp.-c, *Nannochloropsis* sp.-d) during the cultivation in conway media. Observation results of growth showed that onset of stationary phase (8-15 days) varied among the four species. The growth phases are almost same in *Gonyostomum* sp. and *Nannochloropsis* sp. that showed the lag phase on days 1 to 3, exponential phase on day 4 to12, the stationary phase on day 11 and 12 and finally the phase of death from 12 th days. The lag phase recorded in *Chlamydomonas* sp. is 1 to 8, exponential phase 9 to 15, stationary phase on 14 to 15 and death phase started from day 16. *Navicula* sp. showed the lag phase on day 1 to 3, exponential phase on 3 to 8, stationary phase on 7 to 8 and death phase from day 8. In the stationary phase, cell density was significantly higher ($p< 0.05$) in *Gonyostomum* sp. (37.33×10^7 cells/ml) compared to the other microalgae. Significantly lower ($p<0.05$) cell density (0.25×10^7 cells/ml) and optical density (0.95 Abs) were observed in *Navicula* sp.



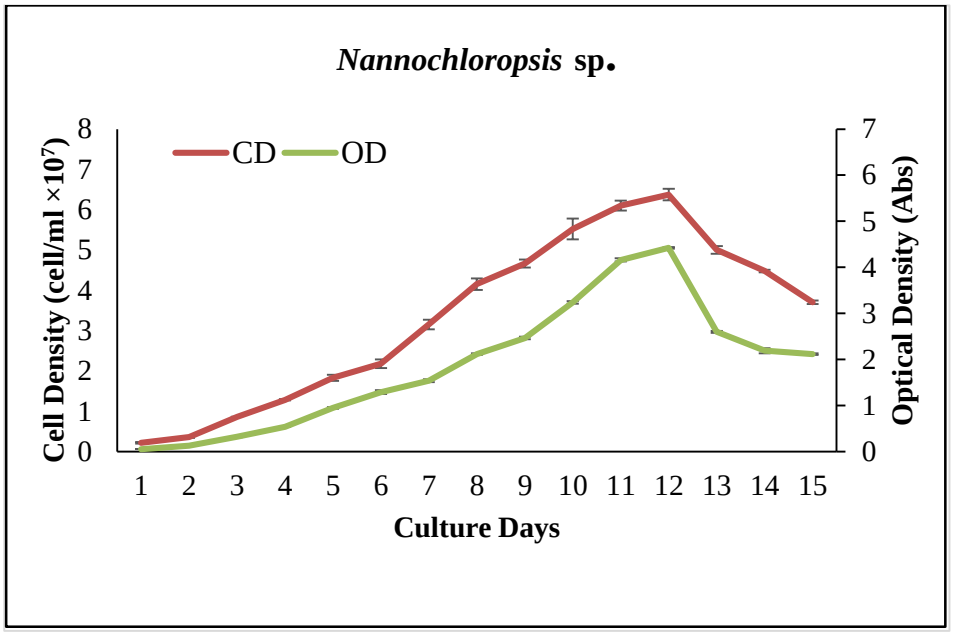
(a)


Chlamydomonas sp.
CD
OD
Cell Density (cell/ml ×107)
Optical Density (Abs)
Culture Days
0
2
4
6
8
10
12
14
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

(b)


Navicula sp.
CD
OD
Cell Density (cell/ml ×10⁷)
Optical Density (Abs)
Culture Days
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0
0.2
0.4
0.6
0.8
1
1.2
1 2 3 4 5 6 7 8 9 10 11 12

(c)

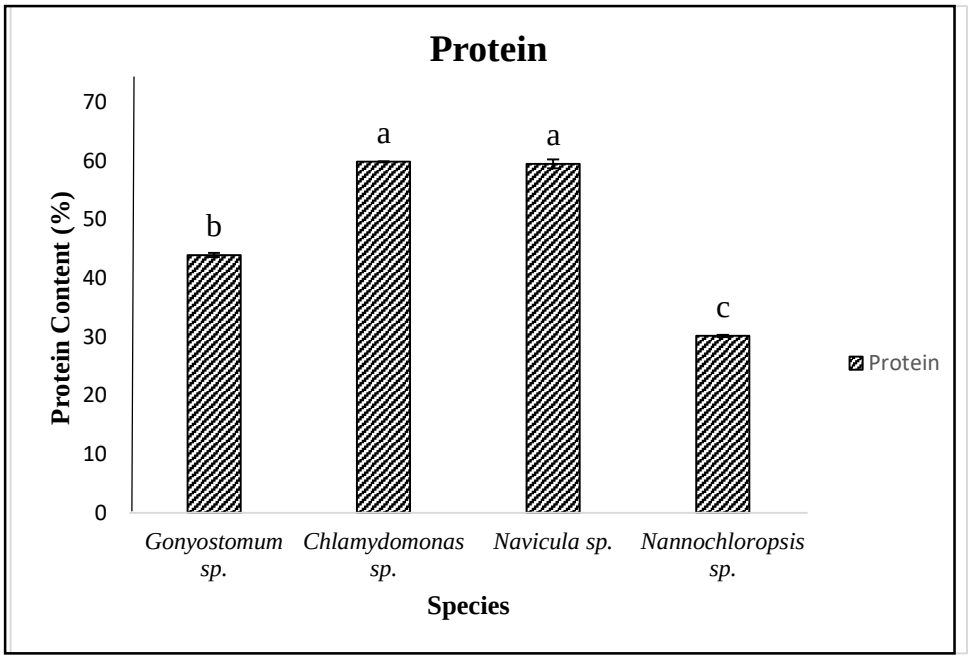


(d)

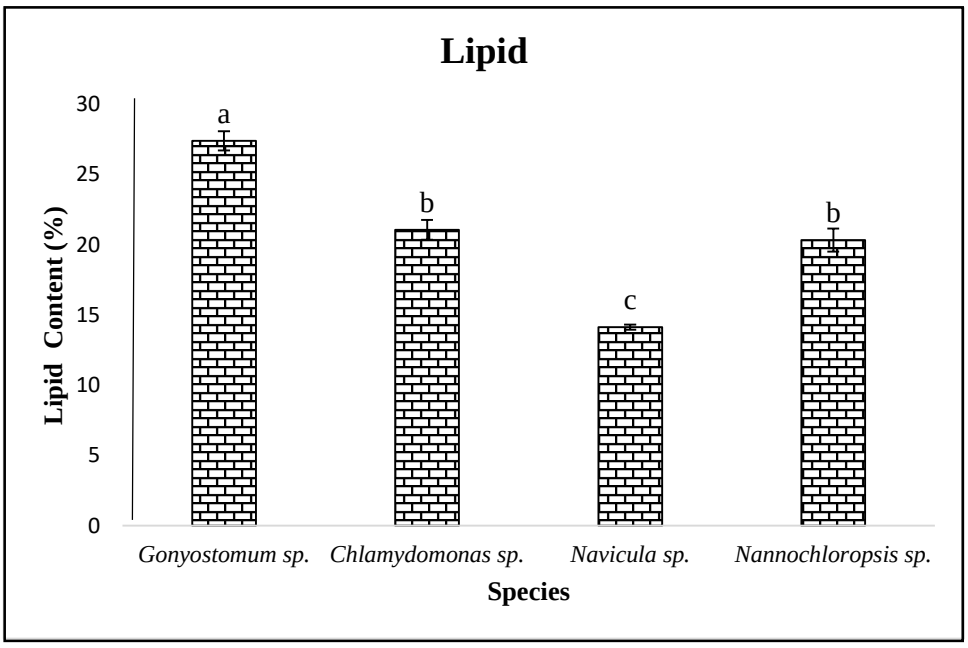
Figure 8: Growth curve in terms of cell density (cells/ml×10^7) and optical density (Absorbance) of marinewater microalgae *Gonyostomum* sp. (a), *Chlamydomonas* sp. (b), *Navicula* sp. (c), *Nannochloropsis* sp. (d). Values are means ± standard error. CD and OD represent cell density and optical density respectively.

4.5. Proximate composition of isolated microalgae

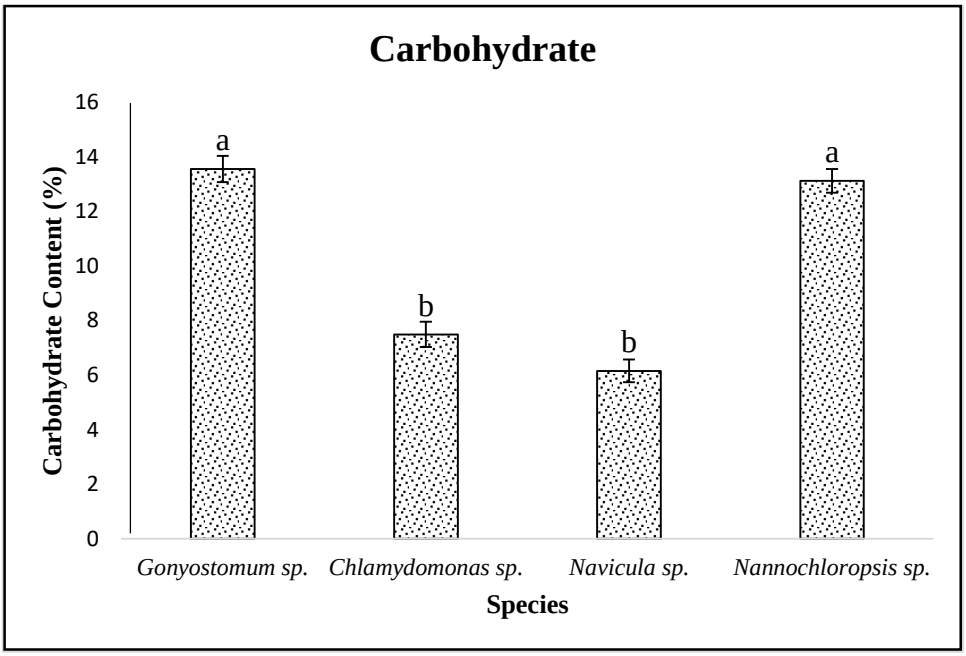
In this study, different marinewater tropical microalgae species were cultured in Conway media providing uniform parameters to determine the difference in their biochemical composition. The figure shows the variation in protein content for the four microalgae species ranged from 30.16 ± 0.18 to 59.51 ± 0.78% dry weight. The percentage of protein was significantly higher ($p<0.05$) in *Navicula* sp. and lower in *Nannochloropsis* sp. All the four microalgal species demonstrated significant difference ($p<0.05$) in terms of protein content. *Chlamydomonas* sp. showed the higher content ($p<0.05$) of lipid which is 21.07 ± 0.71% on the basis of dry weight. Lipid content was significantly lower ($p<0.05$) in *Navicula* sp. Carbohydrate production in various microalgae also differ from genus to genus which ranges from 6.15 ± 0.42 to 13.56 ± 0.48% dry weight. Highest carbohydrate was recorded in *Gonyostomum* sp. and lowest was observed *Navicula* sp.


Protein
Protein Content (%)
70
60
50
40
30
20
10
0
a
a
b
c
Protein
Gonyostomum sp.
Chlamydomonas sp.
Navicula sp.
Nannochloropsis sp.
Species

(a)


Lipid
Lipid Content (%)
30
25
20
15
10
5
0
a
b
c
b
Gonyostomum sp.
Chlamydomonas sp.
Navicula sp.
Nannochloropsis sp.
Species

(b)



(c)

Figure 9: Proximate composition of isolated microalgae. (a) Protein content, (b) Lipid content, (c) Carbohydrate content. Values are presented in (% dry weight) (means ± standard error) of isolated microalgae cultured in Conway media. Values with the different letters within each series indicate significant differences ($p<0.05$) among the species.

4.6. Fatty acid composition of isolated microalgae

Saturated, mono unsaturated and poly unsaturated fatty acids (PUFAs) are three kinds of fatty acids. Omega-3 andomega-6 series are two significant groups of PUFAs regarded as the most important bioactive molecules for living species (Li et al.,2014). Several dietary components such as minerals, vital fatty acids of omega-3 and omega-6 and other useful nutrients are predominantly found in microalgae (Tokusoglu and Unal, 2001). Among PUFAs, notably for EPA and DHA, omega-3 fatty acids are universally acknowledged and proved to be beneficial (Siriwardhana et al., 2012; Tocher, 2015) in prevention or treatment of a variety of illness of human beings.

In the current study, four microalgae showed significant variation ($p<0.05$) of useful fatty acids which include SAFA, MUFA, n3-PUFA, n6-PUFA etc. Highest total amount of SAFA was recorded in *Chlamydomonas* sp. (30.11±10.2%) and lowest was in *Nannocholropsis* sp. (17.01±2.44%). *Gonyostomum* sp. showed highest amount of MUFA and while *Navicula* sp. showed the least. The total amount of n-3 PUFA ranges from 2.08±0.24% to 6.62±2.49 where highest was observed in *Nannochloropsis* sp. and lowest in *Gonyostomum* sp. In case of n-6 PUFA *Navicula* sp. demonstrated highest level and *Nannochloropsis* sp. possessed lowest content.

Table 3: Fatty acid composition of isolated microalgae (*Gonyostomum* sp., *Chlamydomonas* sp., *Navicula* sp., *Nannochloropsis* sp.)

Carbon	**Fatty Acid Methyl Esters**	***Gonyostomum* sp.**	***Chlamydomonas* sp.**	***Navicula* sp.**	***Nanno chloropsis* sp.**
		Conc. (ppm)			
C8:0	Methyl Octanoate	1.63±0.02	2.04±2.01	1.52±0.04	1.11±0.03
C10:0	Methyl Decanoate	1.47±0.02	1.39±1.37	1.16±0.04	0.87±0.02
C12:0	Methyl Laurate	5.05±0.02	5.86±5.85	4.27±0.06	3.85±0.06
C13:0	Methyl Tridecanoate	0.40±0.00	1.11±0.01	0.01±0.00	0.48±0.00
C14:0	Methyl Myristate	0.17±0.02	1.07±0.06	0.05±0.00	0.11±0.00
C16:0	Methyl Palmitate	2.22±0.03	4.30±0.06	2.51±0.13	3.08±0.71
C18:0	Methyl Stearate	0.57±0.26	4.71±0.04	6.95±0.15	1.76±0.14
C20:0	Methyl Arachidate	9.73±0.96	2.62±0.92	4.31±0.08	4.31±0.99
C17:0	Methyl Heptadecanoate	4.24±0.02	3.99±0.02	3.53±0.00	1.29±0.28
C21:0	Methyl Heneicosanoate	0.02±0.01	0.34±0.08	0.05±0.02	0.11±0.11
C22:0	Methyl Behenate	0.003±0.01	2.68±0.17	0.02±0.00	0.003±0.00
C23:0	Methyl Tricosanoate	0.18±0.1	ND	ND	0.05±0.05
C24:0	Methyl Lignocerate	ND	ND	ND	ND
	ΣSAFA	**25.67±2.22^{a}**	**30.11±10.2^{a}**	**24.38±2.47^{a}**	**17.01±2.44^{a}**
C16:1	Methyl Palmitoleate	8.19±0.04	6.05±0.34	1.02±0.08	6.60±0.51
C18:1	Methyl Oleate	0.31±0.00	0.26±0.00	2.64±0.05	0.47±0.00
C20:1	Methyl cis-11-eicosenoate	0.01±0.00	0.004±0.00	0.004±0.00	0.09±0.00
C22:1	Methyl Erucate	0.19±0.12	0.51±0.00	0.09±0.09	1.00±0.01
C24:1	Methyl Nervonate	0	0.07±0.00	0.09±0.04	0.01±0.01
	ΣMUFA	**8.70±0.07^{a}**	**6.89±0.34^{b}**	**3.84±0.18^{c}**	**8.18±0.5^{ab}**
C18:2n-6	Methyl Linoleate	2.71±0.51	2.12±0.12	4.59±0.48	2.02±0.16
C20:3n-6	Methyl 11-14-17-Eicosatrienoate	0.24±0.03	1.91 ±0.07	1.98±0.09	0.48±0.03
C20:4n-6	Methyl Arachidonate	0.28±0.09	0.77±0.20	0.21±0.07	0.26±0.63
	Σn6-PUFA	**3.23±9.57^{b}**	**4.79±0.39^{c}**	**6.78±16.3^{a}**	**2.76±0.81^{d}**
C18:3n-3	Methyl Linolenate	1.26±0.10	0.49±0.02	3.20±0.00	2.47±0.40
C20:5n-3	**Methyl icosa-5,8,11,14,17-pentaenoate**	**0.60±0.02^{a}**	**0.72±0.09^{a}**	**0.003±0.00^{a}**	**2.55±2.14^{a}**
C22:5n-3	Methyl Docosapentaenoate	0.12±0.12	0.90±0.02	0.01±0.00	1.41±0.05

C22:6n-3	**Methyl Docosahexanoate**	**0.10±0.00^{a}**	**0.37±0.02^{a}**	**0.25±0.01^{a}**	**0.19±0.01^{a}**
	Σn3-PUFA	**2.08±0.24^{a}**	**2.49±0.08^{a}**	**3.46±0.01^{a}**	**6.62±0.49^{a}**
	ΣPUFA	**3.03±0.33^{b}**	**5.21±0.39^{b}**	**6.73±0.32^{a}**	**2.65±0.67^{d}**
	Σn3/ Σn6	**0.64±0.00^{b}**	**0.52±0.00^{b}**	**0.51±0.00^{b}**	**2.40±0.78^{ab}**
	DHA/EPA	**0.16±0.00^{b}**	**0.51±0.04^{b}**	**0.78±0.16^{a}**	**0.07±0.21^{b}**
	SAFA/TUFA	**0.07±0.01^{b}**	**0.13±0.05^{b}**	**0.04±0.00^{b}**	**0.87±0.22^{b}**
	SAFA/TFA	**0.07±0.01^{c}**	**0.12±0.04^{c}**	**0.03±0.00^{c}**	**0.46±0.06^{b}**
	TUFA/TFA	**0.93±0.01^{a}**	**0.88±0.04^{a}**	**0.97±0.00^{a}**	**0.54±0.06^{b}**

Fatty acid values are expressed as mean of the duplicates. Here, **SAFA** means Saturated Fatty Acids, **MUFA**= Monounsaturated fatty acids, **n6-PUFA**= ω-6 polyunsaturated fatty acids, **n3-PUFA**= ω-3 polyunsaturated fatty acids, **DHA**= Docosahexaenoic acid, **EPA=** Eicosapentaenoic acid, **TUFA**= Total unsaturated fatty acids, **TFA**= Trans fatty acids.

Values with different small uppercase within individual column are as mean±SE (Standard Error) and showing significance ($p<0.05$) followed by Tukey's multiple range test.

4.7. Amino acid profile of isolated microalgae

Table 4 shows the percentage of both essential and non-essential amino acid present in four microalgal species. The total amount of essential amino acid was higher in *Navicula* sp. (38.73%) followed by *Chlamydomonas* sp. (38.25%), *Gonyostomum* sp. (36.92%) and *Nannochloropsis* sp. (33.04%). Among the essential amino acids, leucine seemed to be predominant in the microalgae except *Nannochloropsis* sp. where leucine was absent. Highest percentage of Leucine was recorded in *Gonyostomum* sp. (7.91%). In terms of non-essential amino acid, highest total content was observed in *Nannochlorpsis* sp. (66.96%) and lowest in *Navicula* sp. (61.27%). alanine, aspartic acid and glutamic acid were prevalently found in the microalgal species. Highest alanine, aspartic acid and glutamic acid were recorded in *Nannochloropsis* sp. (12.69%), *Navicula* sp. (13.44%) and *Navicula* sp. (15.66%) respectively.

Table 4: Amino acid profile of selected microalgae isolated from marine water

Compound Name (570 nm)	**Types**	***Gonyostomum* sp.**	***Chlamydomonas* sp.**	***Navicula* sp.**	***Nanno chloropsis* sp.**
		Amount (%)			
Histidine	EAA	3.58	4.70	5.39	4.32
Isoleucine	EAA	2.21	1.97	2.96	2.73
Leucine	EAA	7.91	7.34	7.15	0
Lysine	EAA	5.31	6.84	3.69	5.17
Methionine	EAA	1.66	2.06	3.21	2.55
Phenylalanine	EAA	4.15	3.84	3.75	3.73
Threonine	EAA	4.91	4.72	5.47	6.37
Tyrosine	EAA	3.52	3.73	3.26	3.81
Valine	EAA	3.65	3.04	3.85	4.36
Alanine	NEAA	10.60	11.30	10.51	12.69
Arginine	NEAA	7.44	5.67	5.97	6.98
Aspartic acid	NEAA	12.68	12.33	13.44	12.26
Glutamic acid	NEAA	13.48	13.51	15.66	14.07
Glycine	NEAA	7.74	6.70	6.21	7.02
Cysteine	NEAA	0.28	0.15	0.28	0.44
Serine	NEAA	5.48	5.47	5.61	5.94
Proline	NEAA	5.38	6.62	3.59	7.57
∑EAA		**36.92**	**38.25**	**38.73**	**33.04**
∑NEAA		**63.08**	**61.75**	**61.27**	**66.96**

4.8. Selection of suitable microalgae for feeding trial

Following the evaluation of proximate composition of different microalgal species, *Chlamydomonas sp.* appeared to be a promising source of both protein and lipid which accounted for 58.96 ±0.56% and 21.07±0.70%. Moreover, in accordance with review of literature, there is a scope to investigate how this species performs as a substitute for fish meal in the diet of common carp.

4.9. Physical and chemical parameters of culture water

No significant differences($p<0.05$) were observed in the results of temperature, DO and pH of the different treatments. Values of TAN, NO_2-N and SRP of different treatments have significant differences among each other ($p<0.05$) and in comparison to control treatment, highest TAN and NO_2-N concentration obtained by C15 (0.68 ± 0.00), (0.61 ± 0.01) respectively and highest SRP recorded in control (0.16 ± 0.00). Moreover, lowest TAN and NO_2-N concentration was observed in C5 (0.54 ± 0.01**)** control group **(**0.46 ± 0.01) respectively. C15(0.11 ± 0.00) showed lowest SRP concentration.

Table 5: Physical parameters measured in common carp fry reared in tanks during 60 days' experimental period

Treatment	**Parameter**		
	Temperature (°C)	**DO (mg L^{-1})**	**pH**
Control (0%)	27.92 ± 1.6[a]	6.43 ± 0.7[a]	8.32 ± 0.09[a]
5%	27.64 ± 1.2[a]	6.60 ± 0.6[a]	8.27 ± 0.07[a]
10%	27.73 ± 0.9[a]	6.55 ± 0.3[a]	8.18 ± 0.14[a]
15%	27.97 ± 1.5[a]	6.58 ± 0.6[a]	7.92 ± 0.10[a]
CMF	27.44 ± 1.2[a]	6.156 ± 0.5[a]	8.24 ± 0.08[a]

Table 6: Chemical parameters measured in common carp fry reared in tanks during 60 days' experimental period

Treatment	**Parameter**		
	TAN (mg/l)	**NO_2-N (mg/l)**	**SRP (mg/l)**
Control (0%)	0.57 ± 0.01[c]	0.47 ± 0.01[d]	0.16 ± 0.00[a]
5%	0.54 ± 0.00[d]	0.52 ± 0.00[c]	0.13 ± 0.00[b]
10%	0.59 ± 0.00[b]	0.57 ± 0.01[b]	0.13 ± 0.00[b]
15%	0.68 ± 0.00[a]	0.61 ± 0.01[a]	0.11 ± 0.00[d]
CMF	0.57 ± 0.00[c]	0.47 ± 0.01[d]	0.12 ± 0.00[c]

Mean ± SE (Standard Error) along with different small uppercase letters of chemical parameters within same are statistically significant ($p< 0.05$); On the contrary same small uppercase letters of physical parameters within each column defining no significance ($p> 0.05$).

4.10. Growth performance of Common Carp (*C. carpio*) fry

At the end of feeding trial, the common carp appeared healthy, with significant differences in SGR or FCR across all diets. Average daily gain was in the range of 0.009 – 0.017 mg and SGR ranged from 1.96 to 2.82%. Feed conversion ratios (FCR) were within the range of 2.4 – 3.7%. Length increment was within the range of 0.96-1.66 cm. There were significant differences ($p<0.05$) observed among the all five treatments in terms of ADG whereas C15(0.017 ± 0.00 g) and C10(0.015 ± 0.00 g) were found to be significantly higher ($p<0.05$) respectively. In case of SGR, significant differences ($p<0.05$) were recorded where C15 (2.82 ± 0.03%) showed the highest SGR among the five treatments. Length increment was higher in C15, C10, CMF, C5 and control respectively which demonstrated significantly higher ($p<0.05$) growth performance in 15% and 10% microalgae induced feed. The result revealed the least FCR value ($p<0.05$) in C15 (2.4 ± 0.003) and highest FCR value in control group (3.7 ± 0.05).

Table 7: Different growth indices of common carp fry fed with experimental diets

	Diet				
	Control(0%)	**5%**	**10%**	**15%**	**CMF**
Initial length (cm)	1.83 ± 0.03^{a}	1.83 ± 0.04^{a}	1.83 ± 0.04^{a}	1.83 ± 0.03^{a}	1.83 ± 02^{a}
Final length (cm)	2.79 ± 0.01^{e}	2.92 ± 0.01^{d}	3.32 ± 00^{b}	3.49 ± 00^{a}	3.24 ± 0.04^{c}
LI (cm)	0.96 ± 0.02^{e}	1.09 ± 0.03^{d}	1.49 ± 0.03^{b}	1.66 ± 0.03^{a}	1.41 ± 0.02^{c}
Initial weight (g)	0.24 ± 0.62^{a}	0.29 ± 0.2^{a}	0.28 ± 0.78^{a}	0.23 ± 0.14^{a}	0.31 ± 0.53^{a}
Final weight (g)	0.78 ± 0.63^{e}	0.95 ± 0.65^{d}	1.19 ± 0.57^{b}	1.25 ± 0.39^{a}	1.03 ± 0.78^{c}
ADG (g)	0.009 ± 0.00^{e}	0.011 ± 0.00^{d}	0.015 ± 0.00^{b}	0.017 ± 0.00^{a}	0.012 ± 0.00^{c}
SGR (%)	1.96 ± 0.01^{e}	1.97 ± 0.02^{d}	2.41 ± 0.02^{b}	2.82 ± 0.03^{a}	2.00 ± 0.02^{c}
FCR (%)	3.7 ± 0.05^{a}	3.4 ± 0.003^{b}	2.7 ± 0.04^{d}	2.4 ± 0.003^{e}	3.1 ± 0.04^{c}

Note: Values are means of ± SE of three replicate groups (n = 3); LI, Length increment; ADG, Average daily gain; SGR, Specific growth rate; FCR, Feed conversion ratio

4.11. Survival rate of Common Carp (*C. carpio*) fry

After the experiment, total number of live fish from each treatment tanks were recorded. The results showed that survival rate was higher in the groups fed with *Chlamydomonas* sp. incorporated diets. Highest survival rate was found in C15 followed by C10, CMF and C5 respectively (81.67%, 71.67%, 63.33%, 58.33%) compared with the control group (43.2%). The survival rate was found to be very low in the control group (43.2%).

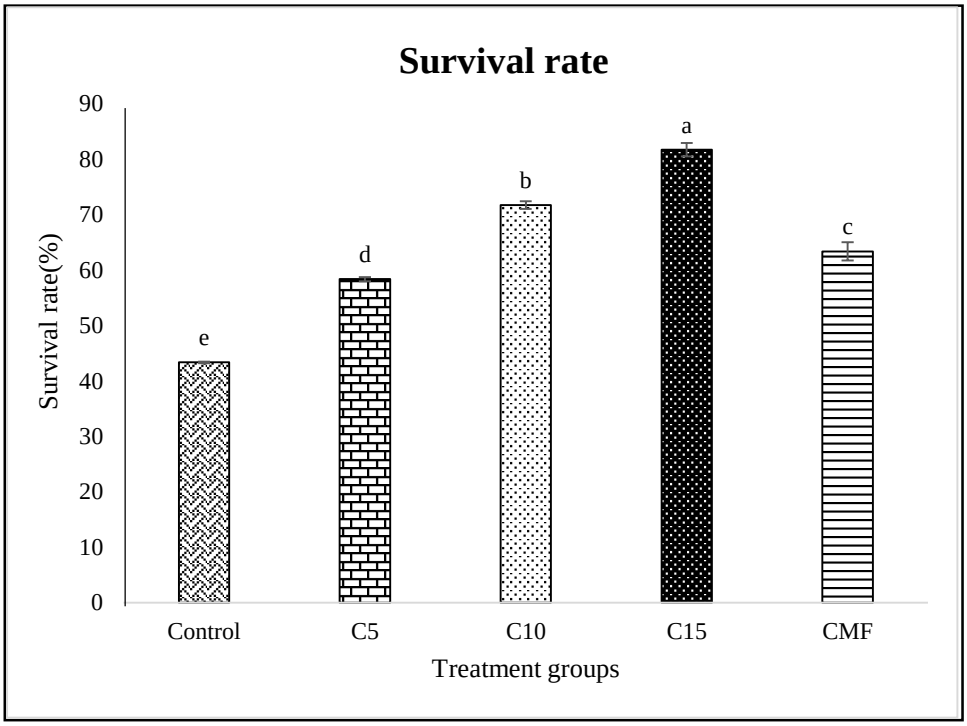


Figure 10: Survival rate (%) of common carp fry fed with different concentration of microalgae

4.12. Carcass proximate composition of Common Carp (*C. carpio*) fry

The data illustration showed that all the treatment groups possess significant differences in terms of proximate composition. The protein content gradually ranges higher to lower from C15(35.6%), C10(33.2%), CMF (29.8%), C5(25.2%) and control (24.8%). C15 group has the highest lipid value (15.7%) and control group has the lowest value (8.4%). The results also indicate that the value of carbohydrate was significantly lower (9.2%) in control and higher (12.7%) in C10 treatment group.

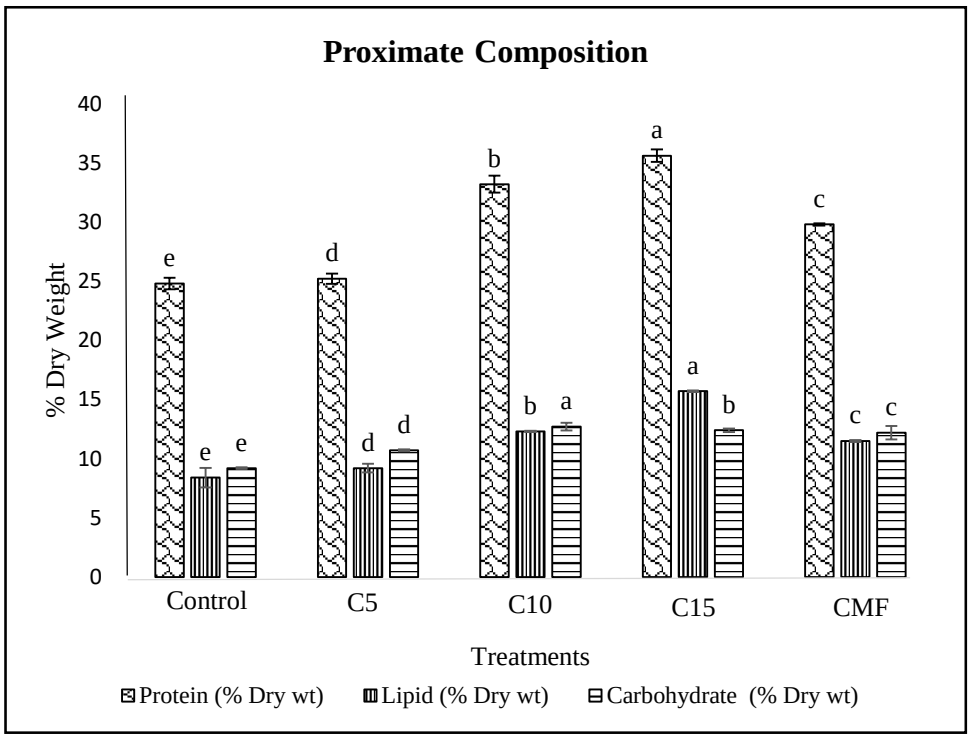


Figure 11: Proximate composition (% dry weight) of whole fish body fed with different concentration of microalgae

4.13. Fatty acid composition of Common Carp (*C. carpio*)

EPA and DHA are mostly found in fish (Tocher, 2015). In aquaculture, fish meal and fish oil are considered as unsustainable source to obtain the EPA and DHA in fish. The supply of fishmeal and fish oil will be insufficient to fulfill the demand because of the fast growth of aquaculture. To partially replace fish meal and fish oil, alternatives such as soybean oil and soybean meal have frequently been employed due to their ease of harvesting (Wang et al., 2014). In this aspect microalgae also can be a potential substitute to mitigate the essential fatty acid supply demand.

In the current study, the addition of *Chlamydomonas* sp. in diet has shown a significant positive impact on the fatty acid profile of whole common carp. According to the presented data, the most abundant SAFA, palmitic acid (C16:0); MUFA, oleic acid (C18:1) were found in C15 treatment. Essential PUFAs like linoleic acid (18:2n-6), EPA (C20:5n-3) and DHA (C22:6n-3) were found dominant in C15, C10 and CMF treatment respectively.

Table 8: Fatty acid composition of Common Carp (*Cyprinus carpio*) fry fed with different experimental diets (Control, C5, C10, C15, CMF)

Carbon	**Fatty Acid Methyl Esters**	**Control**	**C5**	**C10**	**C15**	**CMF**
		Conc. (ppm)				
C8:0	Methyl Octanoate	1.26±0.02	0.05±0.00	0.06±0.00	0.73±0.01	1.47±0.00
C10:0	Methyl Decanoate	1.02±0.01	4.48±0.20	1.18±0.05	0.48±0.00	0.50±0.05
C12:0	Methyl Laurate	4.88±0.05	0.04±0.00	5.89±0.08	1.99±0.03	6.37±0.08
C13:0	Methyl Tridecanoate	0.49±0.00	2.30±0.09	0.53±0.01	0.28±0.00	0.42±0.01
C14:0	Methyl Myristate	0.14±0.00	3.47±0.18	0.17±0.00	0.08±0.01	1.31±0.00
C16:0	Methyl Palmitate	6.66±0.09	6.33±0.63	5.28±0.09	6.56±0.10	5.76±0.09
C18:0	Methyl Stearate	0.09±0.01	0.14±0.03	3.13±0.15	2.67±0.03	0.23±0.15
C20:0	Methyl Arachidate	5.20±0.13	4.84±0.02	1.87±0.17	9.98±0.76	4.17±0.17
C17:0	Methyl Heptadecanoate	0.02±0.01	0.04±0.00	0.08±0.07	2.79±0.02	0.08±0.07
C21:0	Methyl Heneicosanoate	2.16±0.91	0.57±0.09	4.43±0.18	0.04±0.00	1.51±0.18
C22:0	Methyl Behenate	ND	0.51±0.04	1.13±0.03	2.75±0.05	1.66±0.03
C23:0	Methyl Tricosanoate	ND	ND	ND	0.06±0.01	ND
C24:0	Methyl Lignocerate	ND	ND	ND	ND	ND
	ΣSAFA	**21.92±1.86^{a}**	**22.76±1.17^{a}**	**23.73±1.4^{a}**	**28.41±0.76^{a}**	**23.48±1.4^{a}**
C16:1	Methyl Palmitoleate	6.02±0.22	1.17±0.09	20.48±0.97	35.72±0.50	10.79±0.09
C18:1	Methyl Oleate	0.38±0.00	2.10±0.06	0.83±0.01	0.47±0.02	0.5±0.06
C20:1	Methyl cis-11-eicosenoate	0.01±0.01	0.04±0.02	0.08±0.01	0.15±0.15	0.01±0.02
C22:1	Methyl Erucate	0.10±0.03	4.47±0.18	1.12±0.2	0.26±0.00	0.29±0.18
C24:1	Methyl Nervonate	0.02±0.01	0.21±0.11	0.05±0.03	0.01±0.00	0.02±0.11
	ΣMUFA	**6.53±0.24^{b}**	**7.98±0.29^{b}**	**22.57±5.9^{ab}**	**36.61±0.37^{a}**	**11.6±0.29^{b}**
C18:2n-6	Methyl Linoleate	1.14±0.33	1.39±0.41	1.19±0.99	2.11±0.38	1.29±0.8
C20:3n-6	Methyl 11-14-17-Eicosatrienoate	0.62±0.02	0.15±0.06	0.84±0.11	1.44 ±0.12	0.89±0.02
C20:4n-6	Methyl Arachidonate	0.13±0.01	0.49±0.03	0.89±0.07	0.43±0.04	0.27±0.00
	Σn6-PUFA	**1.89 ±0.7^{d}**	**2.03±4.12^{c}**	**2.92±1.17^{b}**	**3.98±0.30^{a}**	**2.45±7.7^{b}**
C18:3n-3	Methyl Linolenate	14.29±0.33	0.25±0.00	4.47±0.77	4.67±0.02	1.99±0.03
C20:5n-3	**Methyl icosa-5,8,11,14,17-pentaenoate**	**1.03±0.7^{ab}**	**0.03±0.00^{b}**	**1.07±0.13^{ab}**	**1.86±0.6^{a}**	**0.46±0.03^{ab}**

C22:5n-3	Methyl Docosapentaenoate	0.01±0.12	0.00±0.00	3.66±3.65	0.01±0.01	1.04±0.01
C22:6n-3	**Methyl Docosahexanoate**	**0.16±0.00^{a}**	**0.14±0.00^{a}**	**0.25±0.25^{a}**	**0.11±0.01^{a}**	**0.12±0.03^{a}**
	Σn3-PUFA	**2.08±0.82^{a}**	**2.42±0.00^{b}**	**7.5±0.31^{ab}**	**9.45±0.65^{ab}**	**6.60±0.75^{ab}**
	ΣPUFA	**1.37±0.16^{d}**	**2.03±0.12^{c}**	**2.48±0.14^{b}**	**3.63±0.94^{a}**	**2.38±0.75^{b}**
	Σn3/ Σn6	**1.10±0.00^{a}**	**1.19±0.00^{b}**	**2.57±0.02^{b}**	**2.37±0.00^{b}**	**2.69±0.00^{b}**
	DHA/EPA	**0.11±0.01^{b}**	**0.13±0.04^{a}**	**0.18±0.27^{b}**	**0.27±0.01^{b}**	**0.22±0.00^{b}**
	SAFA/TUFA	**0.12±0.01^{a}**	**0.15±0.00^{a}**	**0.13±0.01^{ab}**	**0.07±0.00^{b}**	**0.14±0.00^{ab}**
	SAFA/TFA	**0.11±0.01^{a}**	**0.13±0.00^{a}**	**0.12±0.01^{ab}**	**0.06±0.00^{b}**	**0.12±0.05^{ab}**
	TUFA/TFA	**0.89±0.01^{b}**	**0.87±0.00^{b}**	**0.88±0.01^{ab}**	**0.94±0.00^{a}**	**0.88±0.05^{ab}**

Fatty acid values are expressed as mean of the duplicates. Here, **SAFA** means Saturated Fatty Acids, **MUFA**= Monounsaturated fatty acids, **n6-PUFA**= ω-6 polyunsaturated fatty acids, **n3-PUFA**= ω-3 polyunsaturated fatty acids, **DHA**= Docosahexaenoic acid, **EPA=** Eicosapentaenoic acid, **TUFA**= Total unsaturated fatty acids, **TFA**= Trans fatty acids.

Values with different small uppercase within individual column are as mean±SE (Standard Error) and showing significance ($p<0.05$) followed by Tukey's multiple range test.

4.14. Amino acid profile of Common Carp (*C. carpio)* fry

Table 9 shows the percentage of both essential and non-essential amino acid present in the muscle of common carp after 60 days of feeding trial. The total amount of essential amino acid was higher in C15 (49.57%) followed by C10 (48.94%), CMF (47.54%), C5 (47.23%) and control (47.07%). Among the essential amino acids, valine was predominant in the common carp. Highest percentage of valine was recorded in C15(10.44%). In terms of non-essential amino acid, highest total content was observed in control group (52.94%) and lowest in C15 (50.43%). Aspartic acid, arginine, alanine were prevalently found in the fish. Highest aspartic acid, arginine and alanine were observed control (13.31%), C10 (10.27%) and CMF (5.65%) respectively.

Table 9: Amino acid profile of Common Carp (*Cyprinus carpio*)

Compound Name (570 nm)	**Types**	**Common Carp (*Cyprinus carpio*)**				
		Control	**C5**	**C10**	**C15**	**CMF**
		Amount (%)				
Histidine	EAA	6.76	6.78	7.88	7.69	7.33
Isoleucine	EAA	4.61	4.61	4.41	4.25	5.23
Leucine	EAA	4.62	4.72	5.47	5.31	4.92
Lysine	EAA	4.12	4.11	2.05	2.09	2.01
Methionine	EAA	3.02	3.19	3.80	3.70	3.83
Phenylalanine	EAA	3.06	2.98	4.24	4.48	3.30
Threonine	EAA	5.36	4.85	4.21	4.70	4.86
Tyrosine	EAA	6.33	6.56	6.89	6.91	6.41
Valine	EAA	9.19	9.43	9.99	10.44	9.65
Alanine	NEAA	5.56	5.64	5.08	5.47	5.65
Arginine	NEAA	8.18	8.03	10.27	9.90	8.91
Aspartic acid	NEAA	13.31	13.18	11.56	11.17	12.68
Glutamic acid	NEAA	5.91	5.96	4.94	5.18	5.65
Glycine	NEAA	6.12	6.20	4.62	4.61	5.71
Cysteine	NEAA	0.74	0.44	0.09	0.14	0.24
Serine	NEAA	4.86	5.32	4.63	4.43	4.71
Proline	NEAA	8.26	7.99	9.86	9.52	8.93
∑EAA		**47.07**	**47.23**	**48.94**	**49.57**	**47.54**
∑NEAA		**52.93**	**52.77**	**51.06**	**50.43**	**52.46**

4.15. Hematological index of Common Carp (*C. carpio)* fry

Hematological assessments are common practices in which the quality of fish and other existing organisms living in temporal places is determined. In this study, effect of microalgae fed diet on fish hematology was recorded with different nutritional state acquired through different inclusion rate of *Chlamydomonas* sp. RBC, hb, Hct, WBC, LYM, PLT are the essential components of blood hematology which can act like biomarkers because significant changes of these blood parameters can indicate the physiological changes of fish also. RBC, Hb, Hct, WBC and LYM were recorded higher in 15% inoculation of *Chlamydomonas* sp. which are ($1.84\times10^6/\mu l$), (9.71 g/dl), (32.62%), ($26.22\times10^3/\mu l$), (59.74%) followed by C10, CMF and C5 compared to the control. In contrast, significantly higher level of PLT was recorded in CMF($66.2\times10^3/\mu l$) followed by 15%, 10% and 5% inoculation of microalgae.

Table 10: Hematological index of common carp fry

Parameters	**Unit**	**Control**	**5%**	**10%**	**15%**	**CMF**
RBC	$10^6/\mu l$	1.42	1.52	1.77	1.84	1.67
Hb	g/dl	7.14	7.8	9.17	9.71	8.63
Hct	%	25.6	26.26	29.88	32.62	28.27
WBC	$10^3/\mu l$	22.46	23.55	25.61	26.22	24.98
LYM	%	49.94	52.5	56.62	59.74	55.58
PLT	$10^3/\mu l$	52.5	57.9	61.5	63.3	66.2

4.16. Biochemical index of blood serum of Common Carp (*C. carpio)* fry

Changes in blood serum component helps to identify certain functional disorders of body organ and health status of fish such as, total protein, albumin, globulin and A/G ratio detects blood nutritional status. Through triglyceride and cholesterol measurement, fat composition stored in blood can be identified. Blood glucose change indicates the liver function, urea and blood urea nitrogen represents kidney disorders present in fish. Analysis of these serum parameters defines the overall immunity level of fish. This study identified significant variation in overall blood serum profile of common carp fish as an effect of *Chlamydomonas* sp. microalgae inclusion in diet.

Discernible variation in all the values of total protein, albumin, globulin etc. were noticed. Replacement of fish meal with 15% and 10% inclusion of microalgae reported higher amount of total protein (8.52 g/dl), (7.83 g/dl) respectively followed by CMF (7.46 g/dl), C5(6.82 g/dl) and control (6.27g/dl). Highest level of albumin and globulin were recorded in C15(5.26 g/dl), (3.26 g/dl) respectively. The control group showed lowest level of albumin (3.95 g/dl) and globulin (2.32 g/dl).

CMF demonstrated highest level of glucose (396.11 mg/dl), cholesterol (247.24 mg/dl) and triglyceride (567.14 mg/dl). Lowest glucose, chloesterol and triglyceride were observed in control (210.62 mg/dl), C15 (190.52 mg/dl) and C15(328.49 mg/dl) respectively.

Highest amount of urea was recorded in C15 (40.81 mg/dl) and lowest in control (35.10 mg/dl). CMF showed maximum BUN (19.14 mg/dl) and C15 showed minimum BUN (16.32 mg/dl).

Table 11: Biochemical index of blood serum of common carp fry

Name of the ID	**Total Protein**	**Albumin**	**Globulin**	**Glucose**	**Cholesterol**	**Triglyceride**	**Urea**	**BUN**
	g/dl			**mg/dl**				
Control	6.27	3.95	2.32	210.62	245.15	345.98	35.10	18.37
C5	6.82	4.34	2.48	276.27	224.36	343.13	35.21	16.40
C10	7.83	4.92	2.91	331.76	212.50	337.67	36.62	17.10
C15	8.52	5.26	3.26	308.55	190.52	328.49	40.81	16.32
CMF	7.46	4.57	2.89	396.11	247.24	567.14	40.50	19.14

Chapter-5: Discussion

5.1. Water quality parameters of the sampling sites

Macro and micro nutrients availability plays a significant role on the growth and biochemical composition where sufficient amount of nutrients mainly nitrogen, phosphorus is mandatory to achieve optimum growth rates in microalgal cells (Xia et al., 2013). The growth rate reduced when the metabolic requirements and supplied nutrients are not balanced properly (Zarrinmehr, 2019). Some other factors like temperature, light, salinity, pH etc. also play a major rule in growth and biochemical compositions of microalgae (Yeh and Chang, 2012). According to Santhosh and Singh (2007) pH should be between 6.5 and 9.0, which were observed from all sampling site. On the other hand, DO >5mg/L is essential to support good fish production (Bhatnagar and Singh, 2010). DO of Naf estuary, Sonadia and Maheshkhali were in optimal range. Temperature of the sampling sites was in ideal level where the optimum growth temperature is mostly between 20 and 30˚C for most marine microalgae (Chisti, 2008). Optimum phosphorus concentration for microalgae is between 0.001 g/L to 0.179 g/L (Roopnarain et al., 2014), where TAN concentration must be less than 0.5 mg/L and desirable range of nitrite-nitrogen is 0-1 mg/L (Stone and Thomforde, 2004). In the entire sampling site, nitrogen and phosphorus concentration was in ideal range that is required for plankton growth.

5.2. Characterization of isolated microalgae

In Bangladesh, huge numbers of algal species were reported to occur in freshwater, brackish water and marine habitats (Ahmed et al., 2008) that could be a potential source of feed for aquaculture, biofuel production, pharmaceuticals and nutraceuticals industry. Characterization of isolated microalgae was done according to John et al. (2002) and Belcher and Swale (1976) and isolated microalgae were *Gonyostomum* sp., *Chlamydomonas* sp., *Navicula* sp., *Nannochloropsis* sp. Along with this, in respect of Diesing (1866); Ehrenberg (1833); Bory de saint-vincent (1822); Hibberd DJ (1981) isolated microalgae: *Gonyostomum* sp., *Chlamydomonas* sp., *Navicula* sp., *Nannochloropsis* sp. belong to the class Raphidophyceae, Chlorophyceae, Bacillariophyceae, Eustigmatophyceae, Cyanophyceae respectively.

5.3. Growth phases of isolated microalgae

In case of *Nannochloropsis* sp., Ermavitalini et al. (2019) reported almost similar growth pattern (lag, exponential and stationary phase) in *Nannochloropsis* sp. in the combined treatment media of Indole 3-acetic acid (IAA) and 6-Benzyl Amino Purine (BAP). Cell density was also similar in accordance with the earlier study found 4.877 $\times 10^7$ cells mL^{-1} in Conway media reported by Khatoon et al. (2014). A previous study done by Lin et al. (2019) found slightly declined cells number in *Navicula* sp. (HDMA-20) after 18 days in BG-11 medium, whereas, in the present study highest cell density of *Navicula* sp. was detected after day 8. The findings of the current study suggested that *Navicula* sp. can flourish by using Conway media. No previous growth data available on *Gonyostomum* sp. The growth phases are almost same in *Gonyostomum* sp.and *Nannochloropsis* sp. that showed the lag phase on days 1 to 3, exponential phase on day 4 to12, the stationary phase on day 11 and 12 and finally the phase of death from 12 th days. Similar orientation of growth phase was observed in *Chlamydomonas* sp. which resulted the lag phase, exponential phase, stationary phase and death phase on days 1 to 8, 9 to 15, 14 to 15 and from day 16 respectively. The same trend of performance in cell density was observed by Rochaa et al. (2017) who reported that *Chlamydomonas* sp. shows maximum cell density at day 15 in G-11 medium but in the present study cell density was higher which suggested that *Chlamydomonas* sp. productivity can be enhanced by culturing in Conway media.

5.4. Proximate composition of isolated marine water microalgae

The present study showed that the protein and lipid content in *Chlamydomonas* sp. were significantly higher in Conway media and unanimous with the study done by Renaud et al. (2017) who claimed that in the late-logarithmic growth phase, microalgae hold approximately 30 – 40 %(w/w) protein, 10 – 20 %(w/w) lipids and 5 – 15 %(w/w) carbohydrates. As *Chlamydomonas* sp. contains about 21% lipid, it may have potentiality in biofuel production because high oil yield of many microalgae species has been exploited in biofuel production (Hussain et al., 2017). The protein and lipid content in *Nannochloropsis* sp. in the present study is lower than the earlier study reported by Khatoon et al. (2014) where *Nannochloropsis* sp. contained about 44.3%, protein, 32% lipid in 30ppt salinity (dry basis). The biochemical composition of microalgae varies with different medium compositions and under different culture conditions (Chen et al., 2011), hence this could be the reason for the differences. Due

to its higher protein and lipid content *Nannochloropsis* sp. can be extensively used as animal feed in aquaculture (Rodolfi et al., 2003). The results of the study were supported by Reyes et al. (2012) opined that *Navicula* sp. contains nearly 45% - 60% protein and 17% - 25% lipid, in relation to the dry weight of biomass. In the present study, lipid content of *Navicula* sp. is lower at stationary phase in Conway media than BG11 reported by Dhup and Dhawan (2014). The decrease in lipid content usually occurs due to different culture condition (Yoo et al., 2010); this may justify the differences detected herein for the variation in the concentration of nutrients in culture media at different culture days. On the other hand, no previous data has been reported regarding biochemical composition of *Gonyostomum* sp., while in our study, comparatively higher percentage of lipid and protein content was recorded from *Gonyostomum* sp. and can be a potential species in feed industry for fish and other animals as Bleakley and Hayes (2017), reported that, higher protein containing microalgae can be a potential source for fish and animal feed industry.

5.5. Fatty acid composition of isolated microalgae

Microalgae are potential sources of certain fatty acids and generate high levels of -3 PUFAs when grown on a big scale (Irmak and Arzu, 2020). In addition to their numerous applications in medicines and nutraceuticals, PUFAs have garnered a lot of attention lately. According to Tsuzuki *et al*. (2010), *Navicula* sp. can produce more omega-3 fatty acids than green algae. In the present study, PUFAs were found higher in *Navicula* sp. The data analysis demonstrated that long-chain fatty acids (LCFAs; longer than 12 carbons) made up almost 82% of the total fatty acid levels in the *Chlamydomonas* sp. species. Medium-chain fatty acids (MCFAs; 8 to 12 carbons) made up about 8% of the overall fatty acids. *Chlamydomonas* sp., in contrast, lacked any short-chain fatty acids (SCFAs; those with less than 6 carbons). *Chlamydomonas* sp. contains 37.74% SFA, 57.96% MUFA, and 4.3% PUFA, according to Irmak and Arzu (2020). *Chlamydomonas* sp. exhibits similarities to the author's data in this study's SFA and PUFA cases, and it exhibits substantial variability in MUFA cases, which may be caused by differences within the species. In this research, the total amount of n-3 PUFA ranges from 2.08±0.24% to 6.62±0.49 where highest was observed in *Nannochloropsis* sp. and lowest in *Gonyostomum* sp. Maltsev and Maltseva (2021) also recorded similar range of n-3 PUFA in *Nannochloropsis* sp.

5.6. Amino acid profile of isolated microalgae

The total amino acid composition includes free amino acids, amino acid salts, and protein building blocks. Methionine, leucine, isoleucine, lysine, phenylalanine, threonine, tryptophan, and valine are the eight essential amino acids that are used to determine the quality of proteins (Lourenço et al., 2002). The overall amount of non-essential amino acids was found to be higher in the current investigation than the total sum of essential amino acids. The total amount of essential amino acid was higher in *Navicula sp.* (38.73%) followed by *Chlamydomonas sp.* (38.25%), *Gonyostomum sp.* (36.92%) and *Nannochloropsis sp.* (33.04%) which correspond with the range of Darwish et al. (2020).

Total sum of essential amino acids of *Chlamydomonas* sp. was found lower (38.24 mg/g) than a study done by Darwish et al. (2020). Moreover, in this study non-essential amino acids of *Chlamydomonas* sp. was found higher than essential amino acids. Amino acid content of *Nannochloropsis* sp. was observed lower than the investigation done by Hasan et al. (2022) and these changes may occur due to variations in the growth condition. In the present study, Among the essential amino acids, leucine seemed to be predominant in the microalgae except *Nannochloropsis* sp. This finding is quite similar with the result of James et al. (2013). In terms of non-essential amino acid, highest total content was observed in *Nannochlorpsis* sp. (66.96%). Marti-Quijal et al., (2019) also recorded higher non-essential amino acid in *Nannochloropsis* sp. rather than its counterpart.

5.7. Water quality of culture tank

In commercial fish hatchery and other culture systems, increased TAN and NO2-N level with culture time are important factors that affect the survival, health and growth performance of fish larvae and juvenile (Abdulrahman et al., 2014). In this study, the inclusion of *Chlamydomonas* sp. into the feed leads to the improvement and maintenance of good water quality throughout the culture period. Significantly lower TAN, NO2-N and PO4-P were found in water samples from the tank of fish supplemented with *Chlamydomonas* sp. compared to control during the culture. This result was in accordance with Kiron et al. (2012), who showed that microalgae could stabilize and improve the water quality of culture. Although dried microalgae were used in this study, there are still some viable microalgal cells present even if it is at a very

low level (Allam, 2007). Thus, there is a possibility of microalgal cells from leftover feed to start growing again in the tank. Throughout the common carp culture period, a slow increase in green water concentration in the tank was observed. It was contributed by the increase in microalgae concentration which absorbs the nutrients available, especially in tank C15, C10 and C5. Meanwhile, there was no significant difference in the physical parameters (temperature, DO and pH) of the water for all treatments during the whole experimental period (Table 5).

5.8. Growth performance of Common Carp (*C. carpio*) fry

In this 60 days feeding experiment, all of the test diets were well accepted by the fish in all treatment groups. Iso-nitrogenous and isoproteic feed were formulated in the laboratory to perform the experiment (Allam, 2007). The microalgae inclusion levels of 5%, 10% and 15% were determined based on the results of previous studies on *Chlamydomonas* sp. feeding trials in zebrafish (Darwish et al., 2020). The algal inclusion diet resulted in significant changes in terms of growth rates or voluntary feed intake. However, the control (0%) feed fed groups showed a significant reduction in growth performance compared with the others. The values of average daily gain (mg) and SGR significantly differed among the feeding groups (Table 7). The C15 was found to have a significantly higher growth rate. Darwish et al. (2020) also reported that there was discernible weight gain of zebrafish (*Danio rerio*) fed diets with algal inclusion. The result is slightly dissimilar to that of Allam (2007) who showed that *Chlamydomonas* sp. inclusion of more than 10% tends to result in lower weight gain and SGR. But some researchers established that, in case of algal feed formulation, the growth rate is optimum when partial replacement of dietary components is done up to 10 to 15% of the total feed content, depending on the species and their feed utilization efficacy (Valente et al., 2019).

5.9. Survival rate of Common Carp (*C. carpio*) fry

After the experiment, total number of live fish from each treatment tanks were recorded. In this investigation, the inclusion of *Chlamydomonas* sp. into the diet has shown a significantly higher survival compared to the groups fed with control and commercial feed after 60 days of culture period (Figure 11). The excessive production of reactive oxygen species (ROS) and the preservation of the cell membrane by antioxidants against the production of free radicals are directly related to the performance and health

of aquatic organisms (Mazurkiewicz, 2009). The increased survival rate of common carp fed with *Chlamydomonas* sp. was observed in this study which resembles the outcome of Darwish et al. (2020).

5.10. Nutritional Composition of Common Carp (*C. carpio*) fry

Significant differences were observed in protein content among the experimental groups fed with *Chlamydomonas* sp. These results are similar to those reported in other reaches (Nandeesha et al.,2001) suggesting that dietary inclusion of microalgae in feed affect digestible nitrogen intake and gain, modifying body protein content. But the value of carcass lipid content was found to be dissimilar from the previous reports. The total lipid content of common carp was higher in groups fed with test diets compared with the control one. This may be because all the test diets were iso-lipidic and the apparent digestibility coefficient (ADC) of *Chlamydomonas* sp. is high in lipids (68.24%) and eicosapentaenoic fatty acid (71.47%) (Mazurkiewicz, 2009). A previous investigation on lipid availability of different marine microalgae have suggested that, *Chlamydomonas* sp. contains high amount of lipid levels (18.6 percent) and can accumulate high levels of polyunsaturated fatty acids (Hussein et.al., 2012). Also the proximate composition can differ with different life stages of the fish and also influenced by feeding frequency, feed composition and environmental parameters like exogenous factors (Hussein et.al., 2012). These might be the reasons why the algal inclusion diet fed groups retained more lipid in their bodies. The results also indicate that the value of carbohydrate was significantly lower (9.2%) in control and higher (12.7%) in C10 treatment group which is similar to the findings reported by Nandeesha et al. (2001). It should be also noted that the nutrient retention values couldn't be discussed here as the data on proximate composition of initially stocked fish was not recorded which might provide some valuable information about nutrient retention efficiencies of fish fed with *Chlamydomonas* sp. inclusion diet.

5.11. Fatty acid composition of Common Carp (*C. carpio)* fry

Chlamydomonas sp. has been demonstrated to significantly improve the fatty acid profile of common carp when added to the diet. The most abundant SAFA, palmitic acid (C16:0) and MUFA, oleic acid (C18:1) were observed in the C15 treatment to be the most prevalent, according to the data provided. In the C15, C10, and CMF treatments, respectively, it was discovered that essential PUFAs such as linoleic acid

(18:2n-6), EPA (C20:5n-3), and DHA (C22:6n-3) were prominent. The results of this study are similar to the previous research of Pyz Lukasik et al. (2015) who revealed that palmitic acid, oleic acid, alpha-linoleic acid, EPA are consequently the most abundant SFA, MUFA, PUFA and HUFA in *Chlamydomonas* sp. among all types of fatty acids. However, significant changes in n-3/n-6 ratio were observed in different treatments (Table 8). Higher n-3/n-6 ratio was found in CMF group. This result is might be due to the comparatively higher total n-3 PUFA and presence of lowest total n-6 PUFA in the control diet. The maintenance of n-6 to n-3 PUFAs are considered as indispensable for human health, their physical development, homeostasis and psychological health. In this experiment, common carp received SAFAs, MUFAs and essential PUFAs from *Chlamydomonas* sp. inoculated diet. Previous researchers like Nandeesha et al. (2001) fed *Spirulina* sp. to common carp and found elevated level of PUFAs especially EPA which is confirmed in the present research. In this regard, our data show that *Chlamydomonas* sp. may be useful in enhancing the fatty acid profile of whole fish composition.

5.12. Amino acid profile of Common Carp (*C. carpio)* fry

The amount of amino acids in a protein dictates its quality. This quality is measured by contrasting the protein under test with a standard protein's necessary amino acid composition. The standard protein proposed by the FAO Expert Consultation on Protein Quality Evaluation in Human Nutrition from 2011 is now the preferred amino acid scoring pattern for assessing protein quality (FAO, 2013).

In the current experiment, the total amount of essential amino acid was higher in C15 (49.57%) followed by C10 (48.94%), CMF (47.54%), C5 (47.23%) and control (47.07%) which resembles the findings of Kiron et al. (2015) where microalgae induced fish diet showed greater retention of essential amino acid in common carp. Among the essential amino acids, valine was predominant in the common carp which doesn’t match the results found by Pyz Lukasik et al. (2020) in which lysine was most abundant in carp fish. In terms of non-essential amino acid, highest total content was observed in control group (52.94%) and lowest in C15 (50.43%). Aspartic acid, arginine, alanine were prevalently found in the common carp fish. Hussein et al. (2012) recorded higher glutamic acid and serine content found in cultured carp fish. The difference can be caused owing to the variation of feed ingredients uptaken by fish.

5.13. Hematological index of Common Carp (*C. carpio*) fry

In the current study, inclusion of *Chlamydomonas* sp. in diet showed variation in the hematological parameters where RBC, Hb, Hct, WBC were recorded higher in 15% inoculation of *Chlamydomonas* sp. including the value of ($1.84\times10^6/\mu l$), (9.7 g/dl), (32.6%), ($26.2\times10^3/\mu l$) followed by C10, CMF and C5 compared to the control.

The ability of teleost fish species to transport oxygen corresponds with RBC. As the level of RBC in the control group declines, less of the oxygen-binding molecule Hb is available in the blood, which limits the fish's ability to supply enough oxygen to the tissues and causes a decline in physical activity. In fish exposed to toxicants, Hb loss is perilous for oxygen transmission and any type of erythrocyte deterioration, according to Javed et al. (2016). Red blood cell loss may also be influenced by high levels of NH_3 that are produced by TAN in water.

Counting of WBCs is an indispensable indicator of fish health. It was found that Microalgae like *Chlamydomonas* sp. are enriched with essential vitamins such as ascorbate, β-carotene, tocopherol, thiamine, rivoflavin, pyridoxine, cobalamin and biotin (Allam, 2007). Presence of vitamins in microalgae has acted like immunostimulant in common carp and it led to the increment of WBCs.

Significantly higher level of PLT was recorded in CMF ($66.2\times10^3/\mu l$) followed by 15%, 10% and 5% inoculation of microalgae which contradicts the output of Souza et al. (2020) who reported the highest PLT in control group. Platelets in blood is responsible for the development of protective barrier (Amira et al., 2021). However, n-3 PUFA has the quality to cease the platelet coagulation (Javed et al., 2016) and fish get PUFA from microalgae which resulted in low amount of platelet in microalgae induced fed fish.

5.14. Biochemical index of blood serum of Common Carp (*C. carpio)* fry

In this study, the results obtained for total serum protein, albumin, globulin, blood glucose and cholesterol are within the reference range provided by Hrubec et al. (2000). However, higher values of albumin, globulin, A/G ratio, cholesterol and urea nitrogen were recorded in the research of Mauel et al. (2007) for common carp. All the data for total protein, albumin, globulin, etc. showed noticeable variance. The quantity of total protein was greater when fish meal was substituted with 15% and 10% inclusions of

microalgae (8.52 g/dl and 7.83 g/dl, respectively), followed by CMF (7.46 g/dl), C5 (6.82 g/dl), and control (6.27 g/dl) which corresponds with the findings of Souza et al. (2020) who showed that 5% or 10% inclusion of *Chlamydomonas* sp. in fish diet elevated the total protein content in grass carp (*Ctenopharyngodon idella*).Albumin and globulin levels were highest in C15 (5.26 g/dl and 3.26 g/dl, respectively). The lowest levels of albumin (3.95 g/dl) and globulin (2.32 g/dl) were seen in the control group. The absence of microalgae in the diet of control group might be a possible reason for the difference.

Although cholesterol and triglyceride are the sources of structural cell membrane component and cellular storage energy for fish health respectively (Patriche et al., 2011), an increase in these two parameters in blood serum may reflect high storage of lipid and metabolic syndrome (Amira et al., 2021). CMF demonstrated highest level of cholesterol (247.24 mg/dl) and triglyceride (567.14 mg/dl). Lowest chloesterol and triglyceride were observed in C15 (190.52 mg/dl) and C15(328.49 mg/dl) respectively. Microalgae supplementation demonstrated a lowering impact of cholesterol and triglyceride level. Similarly, Nandeesha et al. (2001) revealed that supplementation of microalgae in the diet of carp fish reduced the cholesterol and triglyceride.

Glucose is an instant energy supply that involves heart and muscle activity of the body (Amira et al., 2021). CMF showed highest level of glucose (396.11 mg/dl) may be because of environmental stress such as high ammonia, nitrite nitrogen, soluble reactive phosphorus etc. Serum glucose concentration changes are particularly associated with the renal damage.

Urea is the main food protein and tissue protein metabolite. The liver generates nitrogen as the body metabolizes protein (Amira et al., 2021). Highest amount of urea and BUN were recorded in C15 (40.81 mg/dl) and CMF (19.14 mg/dl) respectively. BUN reflects only the nitrogen content of urea as waste product. The body removes urea from the urine which increases the BUN levels as the activity of the kidney reduces (Amira et al., 2021). Thus increase in protein rich diet increases blood urea too (Allam, 2007).

Chapter-6: Conclusions and Recommendations

Chlamydomonas sp. appeared in this study to be an intriguing resource in terms of fish growth, survival rate, water quality, hemato-biochemical index, and nutritional profile resulting from isolation and analysis of proximate composition. *Chlamydomonas* sp. infused feed improved the growth and resilience and survival of common carp fry. Additionally, it was shown that compared to control feed fed groups, adding complete *Chlamydomonas* sp. biomass as a modest-level (near to 15%) feed additive to a control diet might greatly increase fish performance overall without having any detrimental impacts on their growth. The study also supported the idea that fish may be greatly protected against increased synthesis of critical fatty acids and amino acids by eating a diet supplemented with optimum microalgal biomass.

In conclusion, it is discernible that the microalgal component used in our fish feed has the prospective to become a substitute dietary ingredient to be used for the manufacturing of organic feed with a stable and reasonably priced supply of wholesome protein and oil without endangering the oceans or the food security of people with limited resources. Furthermore, this exploratory study lends support for future research aimed at maximizing the amount of microalgal biomass in the diet, the duration of carotenoid supplementation, and identifying the potential of bioactive compounds present in *Chlamydomonas* sp. and their effects on various biological factors of other species used in aquaculture.

6.1 Recommendations and future prospects

Although a qualitative approach was followed to explore the objectives of the research, there are some limitations of the study which can be minimized by following recommendations:

- Mass culture of microalgae should be done in a cost-effective way for commercial purpose as the chemicals used in the Conway media are highly expensive.
- This study also recommends to lower the plant protein source to improve the palatability.
- Algal bloom is a major concern in this experiment. There is a scope to investigate the different techniques of feed formulation with a view to minimizing the algal bloom.

Future prospects of this study may include:

- The findings of growth, survival and water quality signify the idea of microalgae selection as food and for the wellbeing of fish in commercial and scientific purposes.
- Hemato-biochemical data will aid in diagnosing diseases and examining the degree of blood cell loss to assess health condition and physiological improvements with microalgal diet intake in effective and comprehensive indexes.
- The findings will help the biologists in understanding the systemic interactions between the homeostasis and physiological modifications consequently from diet and water quality so that standard reference values for the fish can be established.
- Impact of lipid and essential fatty acids enriched microalgae on fish will help to use microalgae as feed not only for gonadal development of different fishes but also ameliorating the consumer health.

Chapter-7: References

Abdulrahman NM, Ameen HJH. 2014. Replacement of fishmeal with microalgae *Spirulina* on common carp weight gain, meat and sensitive composition and survival. Pakistan Journal of Nutrition. 13(2): 93.

Abdulrahman NM, Hama HJ, Hama SR, Hassan BR, Nader PJ. 2019. Effect of microalgae *Spirulina spp*. as food additive on some biological and blood parameters of common carp *Cyprinus carpio*. Iraqi Journal of Veterinary Sciences. 33(1): 27-31.

Ahmed ZU, Begum ZNT, Hassan MA, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA, Haque EU. 2008. Encyclopedia of flora and fauna of Bangladesh. vol. 6. angiosperms: Dicotyledons (Acanthaceae-Asteraceae). Asiatic Society of Bangladesh, Dhaka. 2: 408.

Aldana-Aranda D, Patiño-Suárez V, Brul T. 2017. Nutritional potentialities of *Chlamydomonas coccoides* and *Thalassiosira fluviatilis*, as measured by their ingestion and digestion rates by the Queen Conch larvae (*Strombus gigas*). Aquaculture. 156(1-2): 9-20.

Alfasane MA, Chowdhury MMK, Mehnaz M. 2019. Molecular characterization and new reports of two green algae from Bangladesh. Bangladesh Journal of Plant Taxonomy. 26(1): 39-45.

Allam HY. 2007. Physiological effects of some additives on growth, blood constituents and immunity in Nile tilapia (*Oreochromis niloticus*) (Doctoral dissertation, Thesis, Faculty of Agriculture, University of Assiut).

Amira KI, Rahman MR, Khatoon H, Sikder S, Islam SA, Afruj J, Jamal F, Haque ME. 2021. Effects of microalgae inoculated diet on growth performance and blood parameters of Nile tilapia (*Oreochromis niloticus*). Bangladesh Journal of Fisheries. 33(2):243-54.

Andreeva A, Budenkova E, Babich O, Sukhikh S, Ulrikh E, Ivanova S, Prosekov A, Dolganyuk V. 2021. Production, purification, and study of the amino acid composition of microalgae proteins. Molecules. 26: 27-67.

Aris H, Su L, Roland H, Michael DK. 2010. Chlorophyll extraction from microalgae: A review on the process engineering aspects. International Journal of Chemical Engineering. 391632: 1-11.

Arun J, Varshini P, Prithvinath PK, Priyadarshini V, Gopinath K P. 2020. Enrichment of bio-oil after hydrothermal liquefaction (HTL) of microalgae *Chlorella vulgaris* grown in wastewater: Bio-char and post HTL wastewater utilization studies. Bioresource Technology. 261: 182-187.

Ashour M, Elshobary ME, El-Shenody R, Kamil AW, Abomohra AE. 2019. Evaluation of a native oleaginous marine microalga *Nannochloropsis oceanica* for dual use in biodiesel production and aquaculture feed. Biomass and Bioenergy. 120:439-447.

Ayala MD, Galián C, Fernández V, Chaves-Pozo E, García de la Serrana D, Sáez MI, Galafaz Díaz A, Alarcón FJ, Martínez TF, Arizcun M. 2020. Influence of low dietary inclusion of the microalga *nannochloropsis gaditana* (Lubián 1982) on performance, fish morphology, and muscle growth in juvenile gilthead seabream (*sparus aurata*). Animals. 10(12):2270.

Aytenfisu AH, Yang M, MacKerell AD. 2018. Drude polarizable force field for glycosidic linkages involving pyranoses and furanoses. Journal of Chemical Theory and Computation. 14: 3132-3143.

Barclay W, Apt K. 2013. Strategies for Bioprospecting Microalgae for Potential Commercial Applications. In: Richmond A., Hu Q, editors. Handbook of Microalgal Culture: Applied Phycology and Biotechnology. 2: 69-79.

Barsanti L, Coltelli P, Evangelista V, Frassanito AM, Passarelli V, Vesentini N, Gualtieri P. Oddities and curiosities in the algal world. In: Evangelista V, Barsanti L, Frassanito AM, Passarelli V, Gualtieri P. 2008. Editors. Algal toxins: nature, occurrence, effect and detection. Dordrecht. Springer. 353-391.

Basaca-Loya GA, Valdez MA, Enrı´quez-Guevara EA, Gutierrez-Milla´n LE, Burboa MG. 2009. Extraction and purification of B-phycoerythrin from the red microalga *Rhodosorus marinus, Ciencias marinas*. International Journal of Marine Science. 35: 359-368.

Becker EW. 2007. Microalgae as a source of protein. Biotechnology Advances. 25: 207-210.

Becker W. 2004. Microalgae in human and animal nutrition. In: Richmond A, editors. Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd, Cambridge. 5: 312-351.

Belcher H, Swale E. 1976. A beginner's guide to freshwater algae. London, HMSO. pp. 47.

Bellinger EG, Sigee DC. 2010. Freshwater algae: identification and use as bioindicators. JohnWilley and Sons, Ltd. DOI:10.1002/9780470689554.

Bellou S, Baeshen MN, Elazzazy AM, Aggeli D, Sayegh F, Aggelis G. 2014. Microalgal lipids biochemistry and biotechnological perspectives. Biotechnology advances. 32(8):1476-1493.

Bennett A, Bogorad L. 1973. Complementary chromatic adaption in a filamentous blue-green alga. Journal of Cell Biology. 58: 419-435.

Bhagavathy S, Sumathi P. 2012. Purification and characterization of carotenoids from green algae *Chlorococcum humicola* by HPLC-NMR and LC-MS-APCI. Biomedicine and Preventive Nutrition. 2: 276-282.

Bhatnagar A, Singh G. 2010. Culture fisheries in village ponds: a multilocation study in Haryana, India. Agriculture and Biology Journal of North America. 1(5): 961-968.

Bleakley S, Hayes M. 2017. Algal proteins: extraction, application, and challenges concerning production. Foods. 6(5): 33.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification, Canadian Journal of Biochemistry and Physiology. 37(8): 911-917.

Boroh AW, Lawou SK, Mfenjou ML, Ngounouno I. 2019. Comparison of growth curve models for predicting geochemical concentration of microalgae. Journal of African Earth Sciences. 195:104-662.

Bory de Saint-Vincent JBGM. 1822. Bacillarées. Dictionnaire Classique d' Histoire Naturelle. 2: 127-129.

Castro SM, Guerrero-Plata A, Suarez-Real G, Adegboyega PA, Colasurdo GN, Khan AM, Garofalo RP, Casola A. 2020. Antioxidant treatment ameliorates

respiratory syncytial virus–induced disease and lung inflammation. American journal of respiratory and critical care medicine. 174(12):1361-1369.

Chen M, Tang H, Ma H, Holland TC, Ng KS, Salley SO. 2011. Effect of nutrients on growth and lipid accumulation in the green algae *Dunaliella tertiolecta*. Bioresource Technology. 102: 1649-1655.

Chisti Y. 2007. Biodiesel from microalgae. Biotechnology Advances. 25: 294-306.

Chisti Y. 2008. Biodiesel from microalgae beats bioethanol. Trends in Biotechnology. 26(3): 126-131.

Choong YJ, Yokoyama H, Matsumura Y, Lam MK, Uemura Y, Dasan YK, Kadir WNA, Lim JW. 2020. The potential of using microalgae for simultaneous oil removal in wastewater and lipid production. Journal of Environmental Science and Technology. 17: 2755-2766.

Darwish R, Gedi MA, Akepach P, Assaye H, Zaky AS, Gray DA. 2020. *Chlamydomonas reinhardtii* is a potential food supplement with the capacity to outperform *Chlorella* and *Spirulina.* Applied Sciences. 10(19): 6736.

Deutsch L, Gräslund S, Folke C, Troell M, Huitric M, Kautsky N, Lebel, L. 2007. Feeding aquaculture growth through globalization: Exploitation of marine ecosystems for fishmeal. Global Environmental Change. 17(2): 238-249.

Dhup S, Dhawan V. 2014. Effect of nitrogen concentration on lipid productivity and fatty acid composition of *Monoraphidium* sp. Bioresource Technology. 152: 572-575.

Diesing KM. 1866. Revision der Prothelminthen. Abtheilung: Mastigophoren. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturewissenschaftliche Classe. Abt. 1, Mineralogie, Botanik, Zoolohie, Anatomie, Geologie und Paläontologie. 52: 287-401.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugars and related substances, Analytical Chemistry. 28: 350-356.

Ehrenberg C. 1833. Dritter Beitrag zur Erkenntniss grosser Organisation in der Richtung des kleinsten Raumes. Berlin Konigl. Akad. d. Wiss. 33: 145-336.

Elabd H, El-latif A, Shaheen A. 2020. Effect of curcumin on growth performance and antioxidant stress status of Nile tilapia (*Oreochromis niloticus*). Iranian Journal of Fisheries Sciences. 20(5):1234-1246.

Elystia S, Muria SR, Pertiwi SI. 2019. Pemanfaatan Mikroalga *Chlorella* sp. untuk produksi lipid dalam media limbah cair hotel dengan variasi rasio C: N dan panjang gelombang cahaya. Jurnal Sains and Teknologi Lingkungan. 11(1):25-43.

Eriksen NT. 2008. The technology of microalgal culturing. Biotechnology letters. 30:1525-36.

Ermavitalini D, Nurhatika S, Rahayu AE, Arifiyanto A. 2019. Growth profile of *Nannochloropsis* sp. with combination effect of Indole 3-Acetic Acid (IAA) and 6-Benzyl Amino Purine (BAP). Bioscience Research. 16(3): 2402-2408.

FAO. 1996. Manual on the production and use of live food for aquaculture. Lavens P and Sorgeloos P (Eds), FAO Fisheries Technical Paper. 3:61-67.

FAO. 2009. *Cyprinus carpio*. In Cultured aquatic species fact sheets. Text by Peteri, A. Edited and compiled by Valerio Crespi and Michael New. CD-ROM (multilingual).

FAO. 2013. Dietary protein quality evaluation in human nutrition: Report of an FAO expert consultation. Food and Nutrition Paper. 92: 1-66.

Farkas T, Csengeri I, Majoros F, Oláh J. 2018. Metabolism of fatty acids in fish. II. Biosynthesis of fatty acids in relation to diet in the carp, *Cyprinus carpio* Linnaeus 1758. Aquaculture. 14(1): 57-65.

Folch J, Lees M, Stanley GHS. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226.

Ganguly S, Mohanty A, Mitra T, Mohanty S, Das BK, Mohanty BP. 2018. Nutrient profiling and evaluation of fish as a dietary component. Food Research International. 103: 21-29.

Gao F, Li C, Yang Z, Zenga G, Feng L, Liu J, Liu M, Cai H. 2016. Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal. Ecological Engineering. 92: 55-61.

Garcia RJ, Kane AS, Petullo D, Reimschuessel R. 2008. Localization Of Oxytetracycline In *Chlamydomonas Reinhardtii* (Chlorophyceae). Journal of phycology. 44(5): 1282-1289.

Godoy-Hernández G, Vázquez-Flota FA. 2006. Growth measurements. Estimation of cell division and cell expansion. In: Loyola-Vargas VM, Vazquez-Flota FA, editors. Methods in Molecular Biology. Plant Cell Culture Protocols vol. 318. Humana Press Inc., Totowa, New Jersey. pp. 51-58.

Goh SS, Woodman OL, Pepe S, Cao AH, Qin C, Ritchie RH. 2010. The red wine antioxidant resveratrol prevents cardiomyocyte injury following ischemia-reperfusion via multiple sites and mechanisms. Antioxidants and redox signaling. 9(1):101-113.

Gong Y, Jiang M. 2011. Biodiesel production with microalgae as feedstock: from strains to biodiesel. Biotechnology Letters. 33: 1269-1284.

Granado-Lorencio F, Herrero-Barbudo C, AciénFernández G, Molina-Grima E, Fernández-Sevilla JM, Pérez-Sacristán B, Blanco-Navarro I. 2009. In vitro bioaccesibility of lutein and zeaxanthin from the microalgae *Scenedesmus almeriensis*. Food Chemistry. 114: 747-752.

Granado-Lorencio F, Herrero-Barbudo C, AciénFernández G, Molina-Grima E, Fernández-Sevilla JM, Pérez-Sacristán B, Blanco-Navarro I. 2009. In vitro bioaccesibility of lutein and zeaxanthin from the microalgae *Scenedesmus almeriensis*. Food Chemistry. 114: 747-752.

Guedes AC, Amaro HM, Pereira RD, Malcata FX. 2011. Effects of temperature and pH on growth and antioxidant content of the microalga *Scenedesmus obliquus*. Biotechnology progress. 27(5):1218-24.

Guler GO, Kiztanir B, Aktumsek A, Citil OB, Ozparlak H. 2008. Determination of the seasonal changes on total fatty acid composition and ω3/ω6 ratios of carp (*Cyprinus carpio*) muscle lipids in Beysehir Lake (Turkey). Food Chemistry. 108(2): 689-694.

Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S. 2010. Biofuels from algae: challenges and potential. Biofuels. 1(5): 763-784.

Haoujar I, Cacciola F, Abrini J, Mangraviti D, Giuffrida D, Oulad El Majdoub Y, Kounnoun A, Miceli N, Fernanda Taviano M, Mondello L, Rigano F. 2019. The contribution of carotenoids, phenolic compounds, and flavonoids to the antioxidative properties of marine microalgae isolated from Mediterranean Morocco. Molecules. 24(22):4037.

Hasan J, Islam Z, Rabby AF, Sonia SS, Aktaruzzaman M, Rahman T, Rahman S, Mahmud Y. 2022. Dataset describing the growth pattern, amino acid and fatty acid profile of five indigenous marine microalgae species of Bangladesh. Data in Brief. 45:108643.

Hibberd DJ. 1981. Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). Botanical journal of the linnean society. 82(2): 93-119.

Hodar AR, Vasava RJ, Mahavadiya DR, Joshi NH. 2020. Fish meal and fish oil replacement for aqua feed formulation by using alternative sources: a review. Journal of Experimental Zoology India. 23(1).

Hosikian A, Lim S, Halim R, Danquah MK. 2010. Chlorophyll extraction from microalgae: A review on the process engineering aspects, International Journal of Chemical Engineering. 632: 1-11.

Hosikian A, Lim S, Halim R, Danquah MK. 2010. Chlorophyll extraction from microalgae: A review on the process engineering aspects, International Journal of Chemical Engineering, 391632: 1-11.

Hrubec TC, Cardinale JL, Smith SA. 2000. Hematology and plasma chemistry reference intervals for cultured tilapia (Oreochromis hybrid). Veterinary clinical pathology. 29(1):7-12.

Hu H, Gao K. 2003. Optimization of growth and fatty acid composition of a unicellular marine picoplankton, Nannochloropsis sp., with enriched carbon sources. Biotechnology letters. 25:421-5.

Hu X, Yang X, Li L, Wu Y, Lin W, Huang H, Yang SH. 2015. Antioxidant properties of microalgae protein hydrolysates prepared by neutral protease digestion. Applied Mechanics and Materials. 707: 149-153.

Huang HJ, Yuan XZ, Zhu HN, Li H, Liu Y, Wang XL, Zeng GM. 2013. Comparative studies of thermochemical liquefaction characteristics of microalgae, lignocellulosic biomass and sewage sludge. Energy. 56:52-60.

Hussain F, Shah SZ, Zhou W, Iqbal M. 2017. Microalgae screening under CO_2 stress: Growth and micro-nutrients removal efficiency. Journal of Photochemistry and Photobiology B: Biology. 170: 91-98.

Hussein EE, Dabrowski K, El-Saidy DM, Lee BJ. 2012. Enhancing the growth of Nile tilapia larvae/juveniles by replacing plant (gluten) protein with algae protein. Aquaculture Research. 44(6): 37-49.

Hyka P, Lickova S, Přibyl P, Melzoch K, Kovar K. 2013. Flow cytometry for the development of biotechnological processes with microalgae. Biotechnology Advances. 31: 2-16.

Hyka P, Lickova S, Přibyl P, Melzoch K, Kovar K. 2013. Flow cytometry for the development of biotechnological processes with microalgae. Biotechnology Advances. 31: 2-16.

Indexmundi. 2021. World demographic Profile 2021.

Irmak ŞO, Arzu AB. 2020. Determination of the fatty-acid composition of four native microalgae species. GSC Advanced Research and Reviews. 4(1): 1-8.

Islam Z, Khatoon H, Minhaz TM, Rahman MR, Hasan S, Mahmud Y, Hossain MS, Sarker J. 2021. Data on growth, productivity, pigments and proximate composition of indigenous marine microalgae isolated from Cox's Bazar Coast. Data in Brief. 35: 106860.

James CM, Al-Hinty S, Salman AE. 2013. Growth and ω3 fatty acid and amino acid composition of microalgae under different temperature regimes. Aquaculture. 77(4):337-51.

Javed M, Ahmad I, Ahmad A, Usmani N, Ahmad M. 2016. Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in *Channa punctatus* (spotted snakehead) perciformes, channidae exposed to thermal power plant effluent. Springer Plus. 5:1-9.

Jeffrey SW, Humphrey GF. 1975. New spectrophotometric equations for determining chlorphylls a, b, and c, in higher plants, algae and natural phytoplankton, Bhiochemie und Physiologie der Pflanzen. 167: 191-194.

Jenkins SH. 1982. Standard methods for the examination of water and wastewater, Water Research. 16(10): 1495-1496.

John DM, Whitton BA, Brook AJ. 2002. The Freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae, Cambridge University Press, 2002.

Kaewpintong K. 2004. Cultivation of *Haeatococus pluvalis* in Airlift Bioreactor (Doctoral dissertation, Master Thesis in Chemical Engineering, Department of Chemical Engineering Faculty of Engineering. Chulalongkorn University).

Karlson B, Potter D, Kuylenstierna M, Andersen RA. 1996Ultrastructure, pigment composition, and 18S rRNA gene sequence for *Nannochloropsis granulate* (Monodopsidaceae, Eustigmatophyceae), a marine ultraplankter isolated from the Skagerrak, northeast Atlantic Ocean. Phycologia. 35(3):253-260.

Khan MI, Shin JH, Kim JD. 2018. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial Cell Factories. 17 (1): 36.

Khatoon H, Rahman NA, Banerjee S, Harun N, Suleiman SS, Zakaria NH, Lananan F, Hamid SHA, Endut A. 2014. Effects of different salinities and pH on the growth and proximate composition of *Nannochloropsis* sp. and *Tetraselmis* sp. isolated from South China Sea cultured under control and natural condition. International Biodeterioration and Biodegradation. 95: 11-18.

Khatoon H, Rahman NA, Suleiman SS, Banerjee S, Abol-Munafi AB. 2017. growth and proximate composition of *Scenedesmus obliquus* and *Selenastrum bibraianum* cultured in different media and condition. Proceedings of the National Academy of Sciences of the United States of America. 89: 251–257.

Kiron V, Phromkunthong W, Huntley M, Archibald I, De Scheemaker G. 2012. Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. Aquaculture Nutrition. 18(5): 521-531.

Kitaya Y, Kibe S, Oguchi M, Tanaka H, Miyatake K, Nakano Y. 2008. Effects of CO2 and O2 concentrations and light intensity on growth of microalgae (*Euglena gracilis*) in CELSS. Life Support and Biosphere Science. 5(2):243-247.

Kleinegris D, Janssen M, Brandenburg WA, Wijffels RH. 2009. The selectivity of milking of *Dunaliella salina*. Marine Biotechnology. 12: 14-23.

Laing I. 1991. Cultivation of marine, unicellualar algae. Laboratory Leaflet Number. 67: 1-32.

Lamers PP, Janseen M, De-Vos RCH, Bino RJ, Wijffels RH. 2012. Carotenoid and fatty acid metabolism in nitrogen-starved *Dunaliella salina*, a unicellular green microalga. Journal of Biotechnology. 162: 21-27.

Lavens P, Sorgeloos P. 1996. Manual on the production and use of live food for aquaculture. Rome: Food and Agriculture Organization of the United Nations.

Li HY, Lu Y, Zheng JW, Yang WD, Liu JS. 2019. Biochemical and genetic engineering of diatoms for polyunsaturated fatty acid biosynthesis. Marine Drugs. 12(1): 153-166.

Lin Y, Ge J, Zhang Y, Ling H, Yan X, Ping W. 2019. *Monoraphidium* sp. HDMA-20 is a new potential source of α-linolenic acid and eicosatetraenoic acid. Lipids in Health and Disease. 18(1): 56.

Linnaeus C. 1758. Systema naturae. Laurentii Salvii: Stockholm.

Lourenço SO. 2006. Cultivation of microalgae: principle of applications. São Carlos: Rima.

Lourenço SO. 2006. Cultivation of microalgae: principle of applications. São Carlos: Rima.

Lupatsch I, Kissil GW, Sklan D, Pfeffer E. 2018. Energy and protein requirements for maintenance and growth in gilthead seabream (*Sparus aurata*). Aquaculture Nutrition. 4(3):165-173.

Macas-Sanchez EI, Cárdenas-Pérez A, Benavides-Echeverría I, Chango-Galarza M, Mácas-Sánchez G. 2005. Axiología en las finanzas y su importancia en la disminución del riesgo moral. Apuntes de economía y sociedad. 2(2):07-20.

Mai J, Shetty JK, Kan TM, Kinsella JE. 1980. Protein and amino acid composition of select freshwater fish. Journal of Agricultural and Food Chemistry.28(4): 884-885.

Maltsev Y, Maltseva K. 2021. Fatty acids of microalgae: Diversity and applications. Reviews in Environmental Science and Bio-Technology. 20:515-47.

Marketwatch. 2020. Global Analysis, Industry Demand, Trends, Size, Opportunities, Forecast 2020-Market Watch.

Marti-Quijal FJ, Zamuz S, Tomašević I, Gómez B, Rocchetti G, Lucini L, Remize F, Barba FJ, Lorenzo JM. 2019. Influence of different sources of vegetable, whey and microalgae proteins on the physicochemical properties and amino acid profile of fresh pork sausages. LWT. 110:316-23.

Mathivanan K, Elumalai S, Kanna GR. 2015. Screening, extraction and estimation of phycobiliproteins from cyanobacteria. Golden Research Thoughts. 4(8): 1-4.

Matos MJ, Mura F, Vazquez-Rodriguez S, Borges F, Santana L, Uriarte E, Olea-Azar C. 2017. Study of coumarin-resveratrol hybrids as potent antioxidant compounds. Molecules. 20(2):3290-3308.

Mauel MJ, Miller DL, Merrill AL. 2007. Hematologic and plasma biochemical values of healthy hybrid tilapia (*Oreochromis aureus*× *Oreochromis nilotica*) maintained in a recirculating system. Journal of zoo and wildlife medicine. 8(3):420-4.

Mazurkiewicz J. Utilization of domestic plant components in diets for common carp. Fisheries and Aquatic Life. 2009;17(1):5-39.

Michael A. 2018. Morphology of microalgae. Microalgae in Health and Disease Prevention. 18: 23-72

Moheimani NR. 2005. The culture of coccolithophorid algae for carbon dioxide bioremediation (Doctoral dissertation, Murdoch University).

Molino A, Iovine A, Casella P, Mehariya S, Chianese S, Cerbone A, Rimauro J, Musmarra D. 2018. Microalgae characterization for consolidated and new application in human food, animal feed and nutraceuticals. International journal of environmental research and public health.15(11):2436.

Muylaert K, Beuckels A, Depraetere O, Foubert I, Markou G, Vandamme D. 2017. Wastewater as a source of nutrients for microalgae biomass production. Biomass and Biofuels from Microalgae: Advances in Engineering and Biology. 75-94.

Nagappan S, Das P, AbdulQuadir M, Thaher M, Khan S, Mahata C, Al-Jabri H, Vatland AK, Kumar G. 2021. Potential of microalgae as a sustainable feed ingredient for aquaculture. Journal of Biotechnology. 20 (341):1-20.

Nagappan S, Nakkeeran E. 2020. Biorefinery: a concept for co-producing biofuel with value-added products. Environmental Biotechnology. 2:23-52.

Nandeesha MC, Gangadhara B, Manissery JK, Venkataraman LV. 2001. Growth performance of two Indian major carps, catla (*Catla catla*) and rohu (*Labeo rohita*) fed diets containing different levels of *Spirulina platensis*. Bioresource Technology. 80(2):117-20.

Nayek S, Choudury IH, Jaishee N, Roy S. 2014. Spectrophotometric analysis of chlorophylls and carotenoids from commonly grown fern species by using various extracting solvents. Research Journal of Chemical Sciences. 4(9): 63-69.

Niccolai A, Zittelli GC, Rodolfi L, Biondi N, Tredici MR. 2019. Microalgae of interest as food source: Biochemical composition and digestibility. Algal Research. 42: 101-617.

Park D, Jagtap S, Nair SK. 2014. Structure of a PL17 family alginate lyase demonstrates functional similarities among exotype depolymerases. Journal of Biological Chemistry. 289: 8645-8655.

Parmar A, Singh NK, Pandey A, Gnansounou E, Madamwar D. 2011. Cyanobacteria and microalgae: a positive prospect for biofuels. Bioresource technology. 102(22):10163-10172.

Parsons T, Maita Y, Lalli C. 1984. A manual of chemical and biological methods for seawater analysis. Oxford: Pergamon Press. 122(5): 570.

Patriche T, Patriche N, Bocioc E, Coada MT. 2011. Serum biochemical parameters of farmed carp (Cyprinus carpio). Aquaculture, Aquarium, Conservation and Legislation. 4(2):137-40.

Patterson D, Gatlin DM. 2013. Evaluation of whole and lipid-extracted algae meals in the diets of juvenile red drum (*Sciaenops ocellatus*). Aquaculture. 416:92-98.

Phukan MM, Chutia RS, Konwar BK, Kataki R. 2011. Microalgae *chlorella* as a potential bio-energy feedstock. Applied Energy. 88: 3307-3312.

Posten C, Schaub G. 2009. Microalgae and terrestrial biomass as source for fuels-a process view. Journal of biotechnology.142(1): 64-69.

Prabha SP, Nagappan S, Rathna R, Viveka R, Nakkeeran E. 2020. Blue biotechnology: a vision for future marine biorefineries. In Refining Biomass Residues for Sustainable Energy and Bioproducts. Academic Press. 25:463-480

Probert I, Klaas C. 1999. Microalgae culturing. Retrieved February 2, 2017, from INA: The International Nannoplankton Association: http://ina.tmsoc.org/ codenet/culturenotes.htm.

Pugliese A, Biondi L, Bartocci P, Fantozzi F. 2020. *Selenastrum Capricornutum* a new strain of algae for biodiesel production. Fermentation. 6(2): 46.

Pulz O, Gross O. 2004. Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology. 65(6): 635-648.

Pyz-Łukasik R, Chałabis-Mazurek A, Gondek M. 2020. Basic and functional nutrients in the muscles of fish: A review. International Journal of Food Properties. 23(1):1941-50.

Qiao Y, Rong J, Chen H, He C, Wang Q. 2019. Non-invasive rapid harvest time determination of oil-producing microalgae cultivations for biodiesel production by using chlorophyll fluorescence. Frontiers in Energy Research.3:44.

Ratha SK, Rao PH, Govindaswamy K, Jaswin RS, Lakshmidevi R, Bhaskar S, Chinnasamy S. 2016. A rapid and reliable method for estimating microalgal biomass using a moisture analyser. Journal of Applied Phycology. 28(3): 1725-1734.

Renaud SM, Parry DL. 1994. Microalgae for use in tropical aquaculture II: Effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. Journal of Applied Phycology. 6:347-56.

Renaud SM, Thinh LV, Parry DL. 2017. The gross composition and fatty acid composition of 18 species of tropical microalgae for possible use in mariculture. Aquaculture. 170: 147-159.

Reyes y, Chenard G, Aranda DG, Mesquita C, Fortes M, Ora J, Baceller L. 2012. Biodiesel production by hydroesterification of microalgal biomass using hetergenous catalyst. National Science. 4: 778-783.

Ribeiro-Rodrigues LH, Arenzon A, Raya-Rodriguez MT, Fontoura NF. 2011. Algaldensity assessed by spectrophotometry: a calibration curve for the unicellular algae *Pseudokirchneriella subcapitata*. Journal of Environmental Chemistry and Ecotoxicology. 3(8): 225-228.

Rizwan M, Mujtaba G, Memon SA, Lee K, Rashid N. 2018. Exploring the potential of microalgae for new biotechnology applications and beyond: A review. Renewable and Sustainable Energy Reviews. 92: 394-404.

Rochaa RP, Machadoa M, Vaza MGMV, Vinson CC, Leited M, Richarde R, Mendese LBB, Araujo WL, Caldana C, Martinsd MA, Williamsc TCR, Nunes-Nesi A. 2017. Exploring the metabolic and physiological diversity of native microalgal strains (Chlorophyta) isolated from tropical freshwater reservoirs. Algal Research. 28: 139-150.

Rodolfi L, Zittelli GC, Barsanti L, Rosati G. 2003. Growth medium recycling in *Nannochloropsis* sp. mass cultivation. Biomolecular Engineering. 20(4–6): 243-248.

Roopnarain A, Gray VM, Sym SD. 2014. Phosphorus limitation and starvation effects on cell growth and lipid accumulation in *Isochrysis galbana* U4 for biodiesel production. Bioresource Technology. 156: 408-411.

Ryckebosch E, Bruneel C, Termote-Verhalle R, Goiris K, Muylaert K, Foubert I. 2012. Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. Food chemistry.160: 393-400.

Safi C, Charton M, Pignolet O, Silvestre F, Vaca-Garcia C, Pontalier P. 2013. Influence of microalgae cell wall characteristics on protein extractability and

determination of nitrogen-to-protein conversion factors. Journal of Applied Phycology. 25: 523-529.

Saha SK, Murray P. 2018. Exploitation of microalgae species for nutraceutical purposes: cultivation aspects. Fermentation. 4(46): 1-17.

Santhosh B, Singh NP. 2007. Guidelines for water quality management for fish culture in Tripura, ICAR Research Complex for NEH Region, Tripura Center, Publication no. 29.

Santos-Ballardoa DU, Rossib S, Hernándezd V, Gómezd RV, Rendón-Uncetad MC, Caro-Corralesa J, Valdez-Ortiz A. 2015. A simple spectrophotometric method for biomass measurement of important microalgae species in aquaculture. Aquaculture. 448: 87-92.

Sarathchandra MA, Mahaliyana AS, Coswatte AC, Jayamanne SC. 2018. Effect of Raw *Spirulina platensis* Supplement on the Growth Performance of Guppy Fish-Red Blonde (*Poecilia reticulata*).

Shetty P, Gitau MM, Maróti G. 2019. Salinity stress responses and adaptation mechanisms in eukaryotic green microalgae. Cells. 8(12):1657.

Siegelman H, Kycia J. 1978. In: Algal Bili-Proteins: Handbook of Psychological Method, Cambridge University Press, Cambridge. 5(1): 71-79.

Simpson R J, Neuberger MR, Liu TY. 1976. Complete amino acid analysis of proteins from a single hydrolysate. Journal of Biological Chemistry. 251(7): 1936-1940.

Singh P, Kumari S, Guldhe A, Misra R, Rawat I, Bux F. 2016. Trends and novel strategies for enhancing lipid accumulation and quality in microalgae. Renew Sustain Energy Reviews. 55: 1-16.

Souza FP, Lima EC, Urrea-Rojas AM, Suphoronski SA, Facimoto CT, Bezerra Junior JD, Oliveira TE, Pereira UD, Santis GW, Oliveira CA, Lopera-Barrero NM. 2020. Effects of dietary supplementation with a microalga (*Schizochytrium* sp.) on the hemato-immunological, and intestinal histological parameters and gut microbiota of Nile tilapia in net cages. PloS one. 5(1): e0226977.

Spiric A, Trbovic D, Vranic D, Djinovic J, Petronijevic R, Matekalo-Sverak V. 2010. Statistical evaluation of fatty acid profile and cholesterol content in fish

(common carp) lipids obtained by different sample preparation procedures. Analytica chimica acta. 672(1-2): 66-71.

Stein J. 1980. Handbook of Phycological methods. Culture methods and growth measurements. Cambridge University Press. 2: 448.

Stramarkou M, Papadaki S, Kyriakopoulou K, Krokida M. 2017. Effect of drying and extraction conditions on the recovery of bioactive compounds from Chlorella vulgaris. Journal of Applied Phycology. 29:2947-60.

Takagi M, Aoyama K, Motone K, Aburaya S, Yoshida T. 2006. Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. Journal of bioscience and bioengineering. 101(3):223-226.

Tarin NJ, Ali NM, Chamon AS, Mondol MN, Rahman MM, Aziz A. 2016. Optimizing *Chlorella vulgaris* and *Anabaena variabilis* growth conditions for use as biofuel feedstock. Journal of the Asiatic Society of Bangladesh Science. 42(2): 191-200.

Teresa MM, Antonio AM, Nidia S, and Caetano. 2010. Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews. 14: 217-232.

Thompson PA, Levasseur ME, Harrison PJ. 1989. Light-limited growth on ammonium vs. nitrate: what is the advantage for marine phytoplankton. Limnology and Oceanography. 34: 1014-1024.

Tsuzuki W, Matsuoka A, Ushida K. 2010. Formation of trans fatty acids in edible oils during the frying and heating process. Food Chemistry. 123(4):976-82.

Valente A, Iribarren D, Dufour J. 2019. How do methodological choices affect the carbon footprint of microalgal biodiesel-A harmonised life cycle assessment. Journal of Cleaner Production. 207:560-568.

Vílchez C, Forján E, Cuaresma M, Bédmar F, Garbayo I, Vega JM. 2011. Marine carotenoids: biological functions and commercial applications. Marine Drugs. 9: 319-333.

Villarruel-Lopez A, Ascencio F, Nuno K. 2017. Microalgae, a potential natural functional food source-a review. Polish Journal of Food and Nutrition Sciences. 67: 251-263.

Wahidin S, Idris A, Shaleh SRM. 2013. The influence of light intensity and photoperiod on the growth and lipid content of microalgae *Nannochloropsis* sp. Bioresource Technology. 129: 7-11.

Wan C, Alam MA, Zhao XQ, Zhang XY, Guo SL, Ho SH, Bai FW. 2019. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. Bioresource technology. 184: 251-257.

White DA, Pagarette A, Rooks P, Ali ST. 2013. The effect of sodium bicarbonate supplementation on growth and biochemical composition of marine microalgae cultures. Journal of Applied Phycology. 25: 153-165.

Wiedenmann J. 2008. Marine proteins. In: Walsh PJ, Smith SL, Fleming LE, Solo-Gabriele HM, Gerwick WH, editors. Oceans and human health. risks and remedies from the sea. Academic Press, St. Louis, MO.5: 469-495.

Williams PJB, Laurens LM. 2010. Microalgae as biodiesel and biomass feedstocks: review and analysis of the biochemistry, energetics and economics. Energy and Environmental Sciences. 3: 554-590.

Xia A, Cheng J, Lin R, Lu H, Zhou J, Cen K. 2013. Comparison in dark hydrogen fermentation followed by photo hydrogen fermentation and methanogenesis between protein and carbohydrate compositions in *Nannochloropsis oceanica* biomass. Bioresource Technology. 138: 204-13.

Yan Z, Zhong Y, Duan Y, Chen Q, Li F. 2010. Antioxidant mechanism of tea polyphenols and its impact on health benefits. Animal Nutrition. 6(2):115-23.

Yeh KL, Chang JS. 2012. Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31. Bioresource Technology. 105: 120-127.

Yoo C, Jun SY, Lee JY, Ahn CY, Oh HM. 2010. Selection of microalgae for lipid production under high levels carbon dioxide. Bioresource Technology. 101: 71-74.

Zahid MU, Pervaiz E, Hussain A, Shahzad MI, Niazi MB. 2021. Synthesis of carbon nanomaterials from different pyrolysis techniques: a review. Materials Research Express. 5(5):052002.

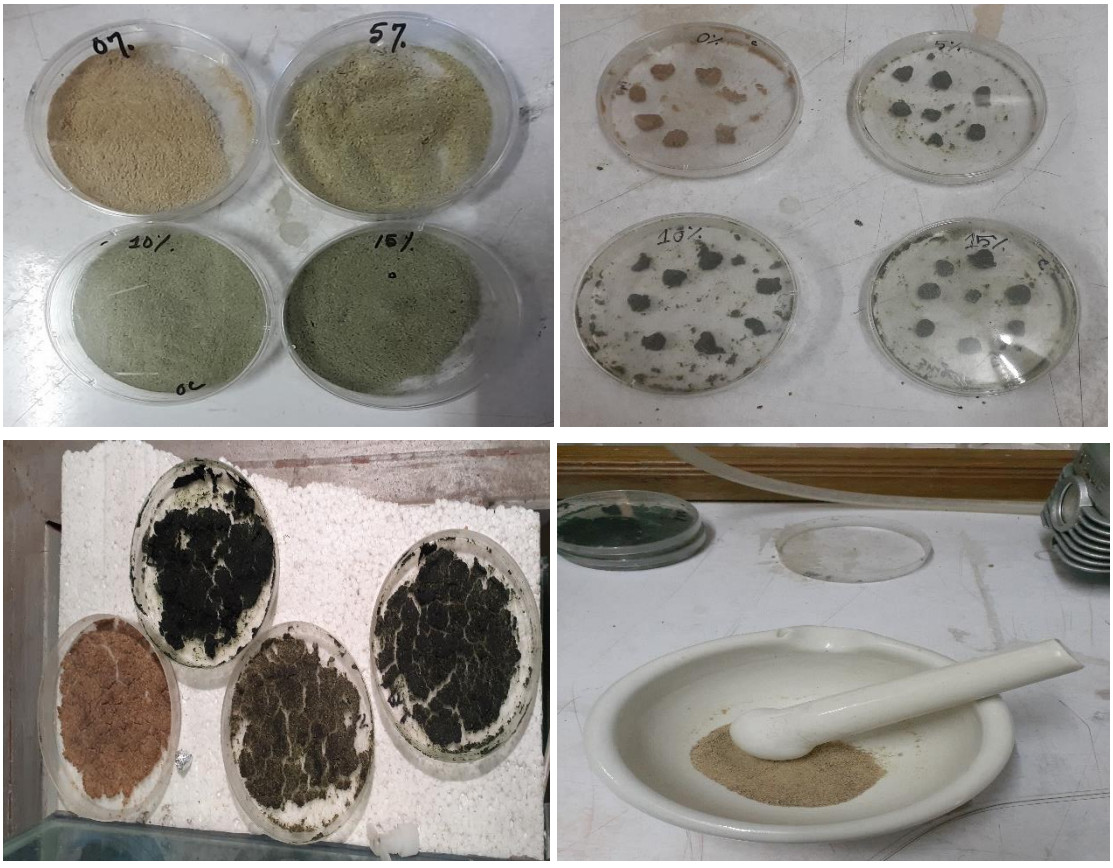
Zarrinmehr MJ, Farhadian O, Heyrati FP, Keramat J, Koutra E, Kornaros M, Daneshvar E. 2019. Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, *Isochrysis galbana*. The Egyptian Journal of Aquatic Research. 46(2).

Zhou Z, Liu X, Sun K, Lin C, Ma J, He M, Ouyang W. 2019. Persulfate-based advanced oxidation processes (AOPs) for organic-contaminated soil remediation: A review. Chemical Engineering Journal. 15 (372):836-851.

Appendices

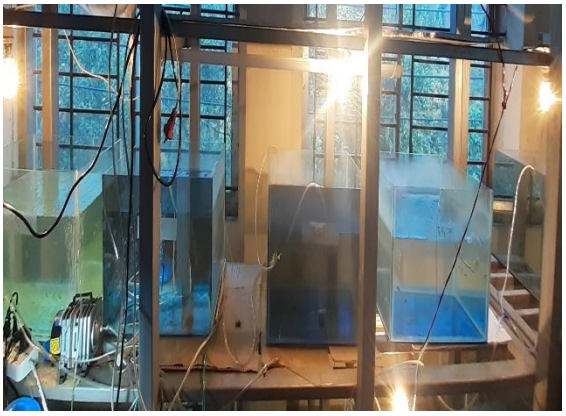
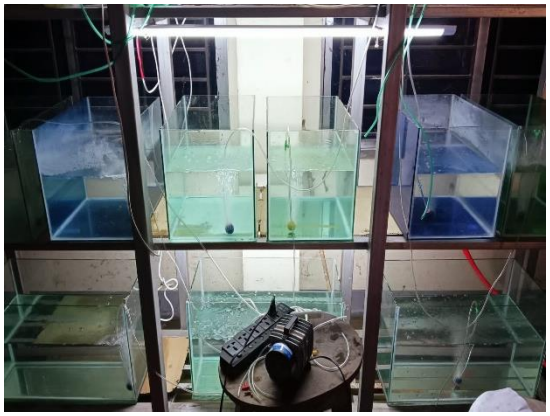
(a) (b)

Appendix A: (a) Isolated *Chlamydomonas* sp. (b) Mass culture of *Chlamydomonas* sp.

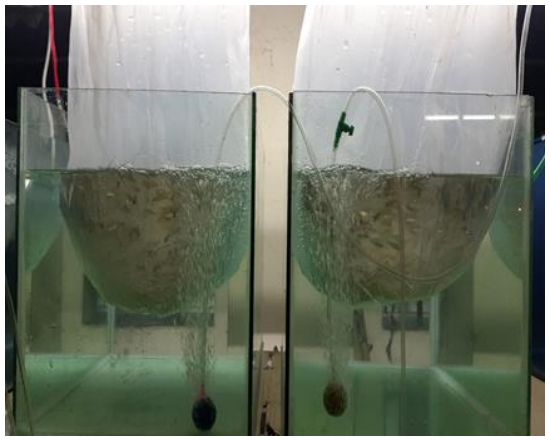


Appendix B: Test diet preparation for the feeding trial


Appendix C: Water purification through continuous aeration and UV radiation

Appendix D: Aquarium set up for feeding trial

Appendix E: Conditioning of fish fry before starting the experiment


Appendix F: Regular observation of physical parameters of the culture tanks

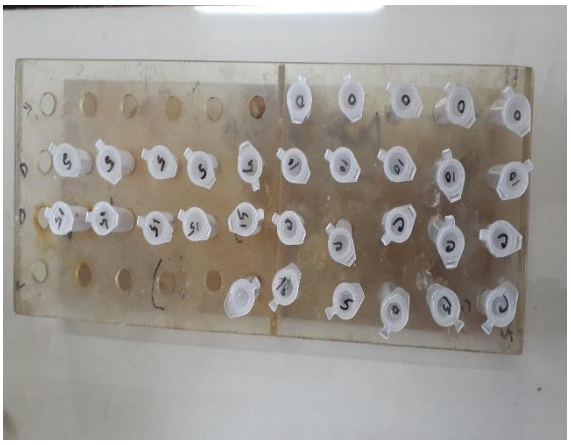
Appendix G: Regular siphoning and water exchange

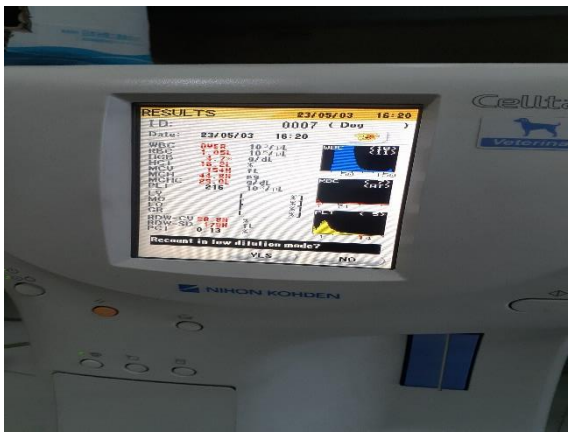
Appendix H: Weekly monitoring of the weight and length of fish fry

Appendix I: Harvesting of fish

Appendix J: Collection of blood after harvesting

Appendix K: Proximate analysis of common carp fry after harvesting





Appendix L: Hemato biochemical analysis of common carp fry

Appendix 1: One-way analysis of variance examining the difference in protein content among the isolated microalgae

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1794.897	3	598.299	3248.095	.000
Within Groups	1.474	8	.184		
Total	1796.371	11			

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1	3	43.8767	.05033	.02906	43.7516	44.0017
2	3	59.6033	.62268	.35951	58.0565	61.1502
3	3	59.3200	.44396	.25632	58.2171	60.4229
4	3	30.1467	.38657	.22318	29.1864	31.1070
Total	12	48.2367	12.77914	3.68902	40.1172	56.3561

Appendix 2: One-way analysis of variance examining the difference in lipid content among the isolated microalgae

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	264.827	3	88.276	70.620	.000
Within Groups	10.000	8	1.250		
Total	274.827	11			

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1	3	27.4000	1.20000	.69282	24.4190	30.3810
2	3	21.0667	1.22202	.70553	18.0310	24.1023
3	3	14.1333	.30551	.17638	13.3744	14.8922
4	3	20.3333	1.40475	.81104	16.8437	23.8229
Total	12	20.7333	4.99842	1.44292	17.5575	23.9092

Appendix 3: One-way analysis of variance examining the difference in carbohydrate content among the isolated microalgae

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	130.489	3	43.496	71.405	.000
Within Groups	4.873	8	.609		
Total	135.362	11			

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1	3	13.5567	.83500	.48209	11.4824	15.6309
2	3	7.4900	.80293	.46357	5.4954	9.4846
3	3	6.1533	.72501	.41858	4.3523	7.9543
4	3	13.1267	.75434	.43552	11.2528	15.0006
Total	12	10.0817	3.50794	1.01265	7.8528	12.3105

Appendix 4: One-way analysis of variance examining the difference in protein, lipid and carbohydrate content of whole fish body fed with different concentration of microalgae

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Protein	Between Groups	276.962	4	69.241	858.851	.000
	Within Groups	.806	10	.081		
	Total	277.769	14			
Lipid	Between Groups	95.468	4	23.867	317.184	.000
	Within Groups	.752	10	.075		
	Total	96.221	14			
Carbohydrate	Between Groups	25.445	4	6.361	991.891	.000
	Within Groups	.064	10	.006		
	Total	25.509	14			

Appendix 5: One-way analysis of variance examining the growth performance and survival rate of Common Carp (*Cyprinus carpio*) fry

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Initial_length	Between Groups	.000	4	.000	1.231	.358
	Within Groups	.001	10	.000		
	Total	.001	14			
Final_length	Between Groups	1.050	4	.263	1712.717	.000
	Within Groups	.002	10	.000		
	Total	1.052	14			
Length_increment	Between Groups	.993	4	.248	2189.618	.000
	Within Groups	.001	10	.000		
	Total	.994	14			
Initial_weight	Between Groups	.020	4	.005	32.283	.000
	Within Groups	.002	10	.000		
	Total	.021	14			
Final_weight	Between Groups	.475	4	.119	128.263	.000
	Within Groups	.009	10	.001		
	Total	.485	14			
ADG	Between Groups	.000	4	.000	35.579	.000
	Within Groups	.000	10	.000		
	Total	.000	14			
SGR	Between Groups	1.951	4	.488	150.523	.000
	Within Groups	.032	10	.003		
	Total	1.983	14			
FCR	Between Groups	3.320	4	.830	395.225	.000
	Within Groups	.021	10	.002		
	Total	3.341	14			

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2473.357	4	618.339	25828.703	.000
Within Groups	.239	10	.024		
Total	2473.596	14			

Appendix 6: One-way analysis of variance examining the fatty acid composition of isolated microalgae

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ΣSAFA	Between Groups	523.534	4	130.883	2.669	.155
	Within Groups	245.200	5	49.040		
	Total	768.734	9			
ΣMUFA	Between Groups	28.437	4	7.109	43.743	.000
	Within Groups	.813	5	.163		
	Total	29.250	9			
∑n6_$PUFA$	Between Groups	608718.383	4	152179.596	815.263	.000
	Within Groups	933.316	5	186.663		
	Total	609651.699	9			
Methyl_icosa_5_8_11_14_17_pentaenoate	Between Groups	11.420	4	2.855	1.561	.315
	Within Groups	9.143	5	1.829		
	Total	20.564	9			
Methyl_docosahexanoate	Between Groups	.083	4	.021	2.982	.131
	Within Groups	.035	5	.007		
	Total	.118	9			
∑$n3$_$PUFA$	Between Groups	28.035	4	7.009	2.159	.210
	Within Groups	16.230	5	3.246		
	Total	44.265	9			
ΣPUFA	Between Groups	603911.118	4	150977.779	813.069	.000
	Within Groups	928.444	5	185.689		
	Total	604839.562	9			
∑n3_∑n6	Between Groups	24.859	4	6.215	6.606	.031
	Within Groups	4.704	5	.941		
	Total	29.563	9			
DHA_EPA	Between Groups	16484.359	4	4121.090	36.715	.001
	Within Groups	561.224	5	112.245		
	Total	17045.583	9			
SAFA_TUFA	Between Groups	13.274	4	3.318	39.708	.001
	Within Groups	.418	5	.084		
	Total	13.691	9			
SAFA_TFA	Between Groups	.773	4	.193	77.915	.000
	Within Groups	.012	5	.002		

	Total	.785	9			
TUFA_TFA	Between Groups	.773	4	.193	77.915	.000
	Within Groups	.012	5	.002		
	Total	.785	9			

Appendix 7: One-way analysis of variance examining the fatty acid composition of Common Carp (*Cyprinus carpio*) fry

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ΣSAFA	Between Groups	51.277	4	12.819	4.266	.072
	Within Groups	15.023	5	3.005		
	Total	66.300	9			
ΣMUFA	Between Groups	1271.398	4	317.850	19.625	.003
	Within Groups	80.980	5	16.196		
	Total	1352.379	9			
∑n6_*PUFA*	Between Groups	35925.404	4	8981.351	359.470	.000
	Within Groups	124.925	5	24.985		
	Total	36050.329	9			
Methyl_icosa_5_8_11_14_17_pentaenoate	Between Groups	3.836	4	.959	6.053	.037
	Within Groups	.792	5	.158		
	Total	4.628	9			
Methyl_docosahexanoate	Between Groups	.026	4	.007	.262	.891
	Within Groups	.126	5	.025		
	Total	.152	9			
∑*n*3_*PUFA*	Between Groups	265.729	4	66.432	5.678	.042
	Within Groups	58.497	5	11.699		
	Total	324.226	9			
ΣPUFA	Between Groups	34300.235	4	8575.059	265.580	.000
	Within Groups	161.440	5	32.288		
	Total	34461.675	9			
∑n3_∑n6	Between Groups	.011	4	.003	12.978	.008
	Within Groups	.001	5	.000		
	Total	.012	9			

DHA_EPA	Between Groups	40.675	4	10.169	344.850	.000
	Within Groups	.147	5	.029		
	Total	40.822	9			
SAFA_TUFA	Between Groups	.004	4	.001	9.527	.015
	Within Groups	.000	5	.000		
	Total	.004	9			
SAFA_TFA	Between Groups	.002	4	.001	9.962	.013
	Within Groups	.000	5	.000		
	Total	.003	9			
TUFA_TFA	Between Groups	.002	4	.001	9.962	.013
	Within Groups	.000	5	.000		
	Total	.003	9			

Description of the Author

Sumit Kanti Dey is a postgraduate student at Department of Aquaculture, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU). He is the son of Swapan Kumar Dey and Shrabani Prava Dutta. He has been born and brought up in Chattogram. The passing years of his S.S.C. and H.S.C. examination are 2013 from Chattogram Collegiate School and 2015 from Chattogram College respectively. Sumit is fond of watching movies, travelling etc. to pursue his leisure time. His father has always been an inspiration and idol throughout his life.