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Culture Studies on Four Selected Phytoplankton Isolated from Freshwater Tributaries Connected to Carigara Bay

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Abstract

Carigara Bay is one of the successful fishing areas in the entire Philippines because of its multi-gear fishery system. Filipinos living around the bay consider its marine life as one of the sources of their livelihood. However, the biodiversity and biological importance of phytoplankton are still unexplored as evidenced by the dearth of published data in scientific journals, thus, this study was undertaken. Sampling sites are the freshwater tributaries namely Lindog River [Brgy. Uyawan], Bislig River [Brgy. Bislig] situated in Carigara, Himanglos River [Brgy. Hilaba], Canomantag River [Brgy. Canomantag] in Barugo, stream located in Brgy, Libertad, Capoocan, Caraycaray River [Brgy. Caraycaray], Lipasan falls [Brgy. Pinarigusan] in San Miguel and Tula-an falls [Brgy. Tula-an], Busay falls [brgy. Busay] in Babatngon. Water samples from the sampling sites were collected using a 2.5-3L plexi glass sampler, transferred in three [3] 1L cap bottle and brought in the laboratory for processing. All data were subjected to statistical analyses such as two-way analysis of variance [ANOVA] and *Post-hoc* test. Culture studies were conducted on selected phytoplankton namely the *Asterococcus* cf *limneticus; Chlorococcum* cf *humicola; Anabaena* cf *azollae* and *Oscillatoria* cf *limnetica.* The species were selected and further subjected to optimization due to their fast-growing ability in a span of time, less bacterial contamination compared with the others and their ability to withstand varying conditions of light, temperature and pH. The four phytoplankton were optimized in terms of light intensities, pH and temperature. Cell density was measured every two days using the haemacytometer method and specific growth rates were computed. Results show that the four phytoplankton have the highest specific growth rates at 40.5 *umol* m^{-2} s^{-1}, 25^{0}C and pH 7.

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These measurements form the basis of microalgae cultivation in great quantities for the production of natural food and processing of pharmaceutical and other industry products. The optimized microalgae namely *Asterococcus* cf *limneticus; Chlorococcum* cf *humicola; Anabaena* cf *azollae, Oscillatoria* cf *limnetica* have high ash, protein and lipid contents which are vital nutrients for food and supplement development.

Keywords: blue-green algae; green algae; freshwater tributaries; semi-large-scale cultures; natural food.

1. Introduction

Phytoplankton, the main focus of this study are polyphyletic assemblage of organisms that are diversely found in nature which can be in the form of one-celled, multi-celled, filamentous, made up of simple reproductive structures and can live in harsh, extreme conditions. These groups of organisms are the earliest and oldest forms of life on Earth [1] and considered as tiniest plant in the archipelago with sizes ranging from few to many micrometers [2,3]. From its biological diversity to industrial importance, microalgae are now gaining much attention for feed, food and other important products in various fields and industries [4] yet they are thought of as the most poorly studied group of aquatic organisms [5]. Noteworthy, some of the microalgae which exist already in the commercial market are *Spirulina, Chlorella, Haematococcus* and *Chaetoceros* [4,6]. Because of this, industrial microalga is a field that needs to be explored primarily to produce promising high-valued chemicals for nutraceuticals, functional food and living feed, and other feed additives [7]. Additionally, microalgae were visualized as the "food for the future" because of its many applications [8,9,10]. Historically, the first microalgae to be commercialized for food industry was the *Nostoc* sp. over many decades and consumed in China, Taiwan, Japan and other Southeast Asian nation [4,11]. Micro algal species are versatile which can invade different types of aquatic ecosystems from freshwater, marine or seawater, hypersaline lakes, deserts and arctic environment [12,13,14,15] as well as in many types of submerged vegetation and soil [16,17]. Carigara Bay, as one of the unexplored areas in the Philippines being the site of the study, is located in the Province of Leyte at Region 8 or the Eastern Visayas where it surrounds five coastal towns namely Carigara, Capoocan, Barugo, San Miguel and Babatngon. Freshwater tributaries such as streams and rivers of the Carigara Bay are present keeping the marine life moist in condition over many months [18]. The bay itself and its freshwater resources are abounding with diversity of organisms but poorly studied as shown by dearth of reports. In fact, the study on the assessment of water quality and initial identification of zooplankton and phytoplankton was solely the existing biological report about Carigara bay [19]. Noticeably, too, are current publications stating that most of the micro algal cultivation is sold in the market for animal food and pharmaceutical products [20] and the algal biomass is starting to have a high rate of demand for aquaculture industry including fish feed providing an initiative revenue for algae industry even microalgae cultivation is only a few decades old [21]. However, adequate studies on the suitability of these microalgae as animal feed are limited [4,22]. Micro-algal based bio-resources products necessary in daily living are the new trends of today's era and scientists dwell more attention on the said matter [23]. Thus, this study on the identification and assessment of microalgae in Carigara Bay and freshwater resources in its vicinity would serve as baseline information for future studies. The objectives of this study are to isolate and culture micro algal species; characterize and identify the micro algal species isolated from the freshwater tributaries of Carigara bay and optimize the phytoplankton in terms of light intensity, temperature and pH tests.

2. Methodology

Sampling sites. The island of Leyte where Carigara Bay is located and connected with freshwater tributaries, is a sister island of Samar and one of the ten [10] biggest land masses in the country. Fourteen [14] stations were established at the upper coastal towns adjoining the Carigara Bay. Station 1 was the Carigara Bay at Brgy. Libertad [Capoocan] [11°39'N 124°53'E]; Station 2 was a stream at Brgy. Libertad [Capoocan] [11°37'N 124°52'E]; Station 3 Carigara Bay at Brgy. Visoria [Carigara] [11°30'N 124°68'E]; Station 4 was the Lindog River at Brgy. Uyawan [Carigara] [11°27'N 124°66'E]; Station 5 was the Bislig River at Brgy Bislig [Carigara] [11°29'N 124°67'E]; Station 6 was the Carigara Bay at Brgy. Duka [Barugo] [11°36'N 124°78'E]; Station 7 was the Himanglos River at Brgy. Hilaba [Barugo] [11°31'N 124°73'E]; Station 8 was the Canomantag River at Brgy. Canomantag [Barugo] [11°30'N 124°71'E]; Station 9 was the Carigara Bay at Brgy. Mawod-pawod [San Miguel] [11°19'N 124°51'E]; Station 10 was the Caraycaray River at Brgy. Caraycaray [San Miguel] [11°20'N 124°52'E]; Station 11 was the Lipasan Falls at Brgy. Pinarigusan [San Miguel] [11°21'N 124°53'E]; Station 12 was the Carigara Bay at Brgy. Kalangawan Guti [Babatngon] [11°23'N 124°51'E]; Station 13 was the Tula-an Falls at Brgy. Tula-an [Babatngon] [11°24'N 124°52'E]; and Station 14 was the Busay Falls at Brgy. Busay [Babatngon] [11°25'N 124°53'E].

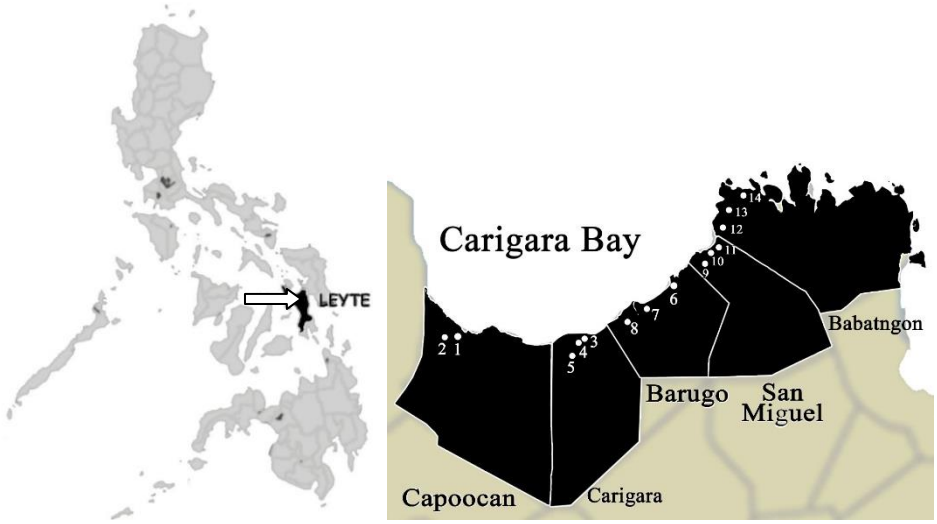

Figure 1: Fourteen [14] sampling stations at Carigara bay and its freshwater resources

Collection of water samples. The water samples were collected during July 2-8, 2018 which was considered as the 1st sampling, and on November 23-26, 2018 which was the 2nd sampling. Collection of samples from the freshwater tributaries of the bay, the water samples were collected in integrated manner, thus, from surface, middle and approximately 0.5 meter away from the bottom. Most have depths ranging from 0.75- 2 meters. Depths were measured using the plexi glass sampler attached to a rope with measurement in meters and/or the secchi disk. The three [3] L water samples from the river/falls were apportioned for the different analyses which 1 L was taken for phytoplankton analysis and fixed with Lugol's solution to preserve the cell wall of phytoplankton; one [1] L was kept cooled during the transport at University of Santo Tomas, Manila and immediately stored in freezer or placed in a refrigerated condition upon arrival in the laboratory for *ex situ*

nutrient determination of the water samples such as nitrate and phosphates. The other one [1] L was used as a live sample for the isolation and culture studies of microalgae.

Identification of Phytoplankton. In order to quantify and identify, isolate and separate micro algal species, standard protocol on washing and plating techniques were done to ensure the isolation of all the microalgae components from water samples collected [24,25,26]. For qualitative determination of phytoplankton, several references on algal taxonomy and biodiversity were used such as the following: Taxonomy and Ecology of Algae in Fishponds and Fishpens of Laguna and some Physiological Studies of Navicula accomoda Hust [27]; Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity [28]; Illustrations of the Freshwater Plankton of Japan [29]; Algae of the Western Great Lakes Area [30]; and How to Know the Freshwater Algae [31]. Also, established keys for micro algal species were also used in the identification [32,]. Moreover, several online websites were also consulted: River Diatoms of the United States [http://westerndiatoms.colorado.edu/]; Common Freshwater Diatoms of Britain and Ireland [http://craticula.ncl.ac.uk/EADiatomKey/html/]; and algaebase [http://www.algaebase.org/]. The verification of the identification of phytoplankton was also made by Dr. Susana F. Baldia. The identification is up to genus level only.

Isolation of Phytoplankton. For the isolation, BRSP medium [Binangonan Research Station Pantastico medium] was used. The medium consists of the following nutrients, [125.8g $CaNO_3$, 64.5g $MgCl_2$, 45.0g $MgSO_4 \cdot 7H_2O$, 19.1g KCl, 81.2g NaCl, 186.1g Na_2SiO_3, 257.3g $NaNO_3$, 22.9g Na_2HPO_4, 0.3g $FeCl_2$ and with trace elements [mg/200 mL], 400mg H_3BO_3, 300mg $MnCl_2 \cdot H_20$, 40mg $ZnSO_4 \cdot 7H_2O$, 20 mgCaCl $5H_2O$, 2mg $NaMoO_4$ in 1L stock solution] After growth colonies and establishment of uni-algal cultures, the isolates were maintained in each test tubes containing 10 ml of BRSP media at a temperature of 25 ± 2ºC, pH of 7.27 and light intensity of 3,000 lx.

Culture Studies. Optimization tests were conducted for the four [4] selected microalgae namely Genera *Asterococcus*, *Chlorococcum*, *Anabaena* and *Osillatoria*. For the light intensity test, the growth of the selected micro algal species was tested at different light intensities: 1500lx, 3000lx, 6000lx, and 9000lx. The temperature was kept constant at 25 ± 2ºC and pH of the BRSP medium at 7.27. One [1] ml aliquot from the stock culture in each of the phytoplankton was placed in 100 ml of BRSP medium. The micro algal species were replicated thrice in each of the different treatments. The light intensities were measured and monitored using Topcon IM-2D Lux meter. For the temperature test, four [4] different temperatures, 20, 30, 35^OC and room temperature, 25 ± 2ºC were assessed for the different selected micro algal species. For 20^OC set-up, the replicates were put in an air-conditioned room. For the 30^OC set up, the algal cultures were put in a water bath with a heating probe. For the 35^OC, the replicates of the algal cultures were kept in lighted incubators. Flasks containing 1 ml aliquot from the stock cultures and 100 ml of BRSP liquid medium and each of the micro algal species were triplicated. The replicated micro algal species were kept in the known optimum light intensity and pH obtained from the previous tests. For the pH test, the pH was manipulated by adding concentrated 11.00M HCl or 6.00M NaOH. pH ranges of 7, 8, and 9 were managed to observe the optimum pH of the algal culture. The replicates of cultures were kept in the known optimum light intensity obtained from the preceding experiment and room temperature of 25 ± 2ºC. For each planktonic species isolated, three replicates were made for the experimental set-up; wherein 100 ml of BRSP liquid medium was inoculated with 1 ml of the unialgal species.

Evaluation of Algal Growth. A 0.5 ml of sample from the different treatments was taken and preserved with 0.1 ml Lugol's solution. This was done every 2 days for the duration of the culture period until growth of cells was observed to decline. Cell density [cells/ml] was determined using a Neubauer Germany Hemacytometer. Cells were counted under a Compound light microscope [CH20 Olympus] and computed using the formula of Martinez and his colleagues 1975.

Data Measurements. The following measurements were determined such as specific growth rate, maximum growth yield and doubling per day.

Calculation of Specific Growth Rates

The formula for calculating the specific growth rate [u] is:

$$u=\frac{\ln N_t-\ln N_o}{t}$$

where u is expressed as the specific growth rate, N_o is the population size at the start of the time interval, N_t is the population size at the end of the time interval and Δt is the length of the time interval $[t_t-t_o]$ expressed in days at the log or exponential phase only.

Doubling per day [k] was calculated with the use of the formula:

$$k=\frac{u}{0.6931}$$

Statistical Analyses. Mean and standard deviations were used to summarize the data in optimized results for the light intensities, temperature and pH desired by the isolated microalgae. One-way ANOVA was applied for the analysis on the optimization results such as light intensities, temperature and pH of the microalgae.

3. Results

Phytoplankton were isolated in all the freshwater stations established in the sampling site. Tables 1 and 2 show the list of the successfully isolated phytoplankton sampled in the month of July and November 2018, respectively. Most of the isolates are under the Phyla Chlorophyta and Cyanophyta and only two genera are from Phylum Bacillariophyta. Table 5 specifies that bacillariophytes or diatoms were not observed and isolated in the five sampling stations in July 2018. The said table further details that green and blue-green algae dominated the area during the said sampling periods, *Asterococcus* cf *limneticus*, *Chlorococcum* cf *humicola*, *Anabaena* spp. and *Synechococcus* sp. were seen in all stations.

Table 1: List of successfully isolated phytoplankton during the month of July 2018.

Stations	Bacillariophyta	Chlorophyta	Cyanophyta
2 [Stream, Brgy Libertad, Capoocan]		• *Asterococcus limneticus* G.M. Smith	• *Nostoc* sp. • *Anabaena* sp. • *Anabaena azollae* Strasburger
4 [Lindog River, Brgy. Uyawan, Carigara]		• *Chlorococcum humicola* [Nageli] Rabenhorst • *Stigeoclonium attenuatum* [Hazen] Collins	• *Synechococcus* sp.
5 [Bislig River, Brgy. Bislig, Carigara]		• *Chlorella ellipsoidea* Gerneck • *Chlorococcum humicola* [Nageli] Rabenhorst	• *Nostoc* sp. • *Chroococcus limneticus* Lemmermann
7 [Himanglos River, Bgry. Hilaba, Barugo]		• *Chlorella vulgaris* Beyerinck • *Asterococcus limneticus* G.M. Smith	• *Synechococcus* sp.
8 [Canomantag River, Brgy. Canomantag, Barugo]		• *Chlorella vulgaris* Beyerinck	• *Anabaena* sp. • *Synechococcus* sp.

Table 2 summarizes the isolated phytoplankton from the four sampling stations established during the month of November 2018. Green and blue-green algae dominated the area during the sampling period where *Chlorococcum* cf *humicola* and *Oscillatoria* cf *limnetica* respectively were observed in all stations. *Fragilaria* cf *brevistrata* and *Navicula* cf *cincta* which are under Phylum Bacillariophyta were also noted and documented.

Table 2: List of successfully isolated phytoplankton during the month of November 2018.

Stations	Bacillariophyta	Chlorophyta	Cyanophyta
10 [Caray-caray River, Brgy. Caray-caray, San Miguel		• *Gloeocystis ampla* Kutzing • *Chlorococcum humicola* [Nageli] Rabenhorst	• *Oscillatoria* sp.
11 [Lipasan Falls, Brgy. Pinarigusan, San Miguel]	• *Fragilaria brevistrata* Grun • *Navicula* sp.		• *Chroococcus dispersus* [Keissler] Lemmermann
13 [Tula-an Falls, Brgy. Tula-an, Babatngon	• *Navicula cincta*[Ehrenberg] Ralfs	• *Gloeocystis ampla [*Kuetz] Lagerheim • *Chlorococcum infusionum* [Schrank] Meneghini	
14 [Busay Falls, Brgy. Busay, Babatngon	• *Fragilaria brevistrata* Grun	• *Chlorococcum humicola* [Nageli] Rabenhorst • *Hormidium klebsii* G.M Smith	• *Oscillatoria* sp.

The table summarizes the isolated phytoplankton on the four [4] sampling stations established during the month of November 2018. Again, the green and blue-green alga dominated the area on the said sampling time where Genus *Chlorococcum* and *Genus Oscillatoria* respectively were seen in almost stations. Moreover, Genus

Fragilaria and *Navicula* which are bacillariophytes were noted and documented this time. The following are the 18 micro algal isolates arranged according to phylum. Phylum Chlorophyta has six [6] genus; Phylum Cyanophyta has five [5] genus and Phylum Bacillariophyta has two [2] genus.

Chlorophyta

Family Palmellopsidaceae

***Asterococcus limneticus* G.M. Smith [Figure 6]**. The micro algal cells are globose or subglobose, either solitary or in colonies of from 4 to 16 and in colorless homogenous envelopes of mucilage. It has chloroplast which is a stellate mass with radiate arms from a central core and contains a pyrenoid. It has an average measurement of 20.2um [L] and 10.3um [W].

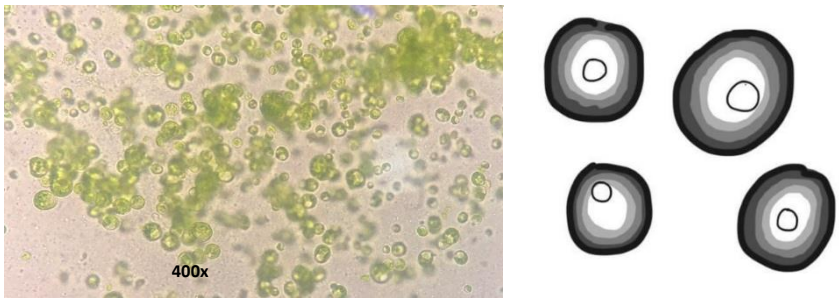

Figure 6

Family Chlorellaceae

***Chlorella ellipsoidea* Gerneck [Figure 7].** The cells are ellipsoidal and unsymmetrical where the chloroplast is folded over. The species produces 32 autospores during reproduction and 9-10um [L] and 7-8um [W] during vegetation. These microalgae are distributed in many lakes and ponds.

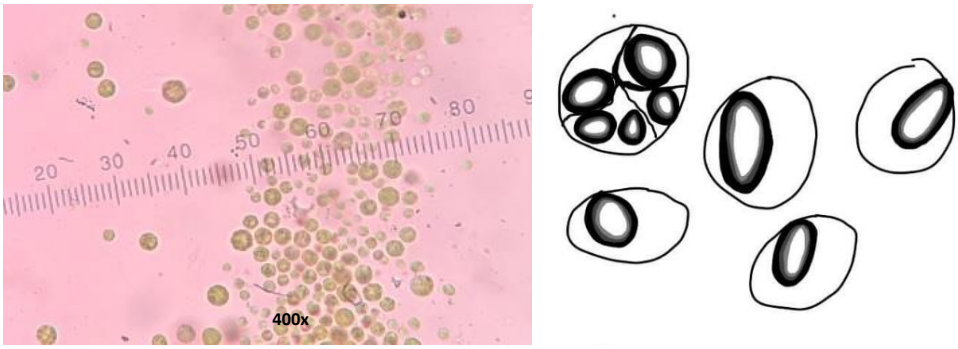

Figure 7

***Chlorella vulgaris* Beyerinck [Figure 8].** The cells are spherical in nature and sometimes occurring in almost pure growth. The chloroplast is like a parietal cup. The cell's width is approximately 5-10um. These species are found in small lakes and pools where there is concentrations of organic matter.

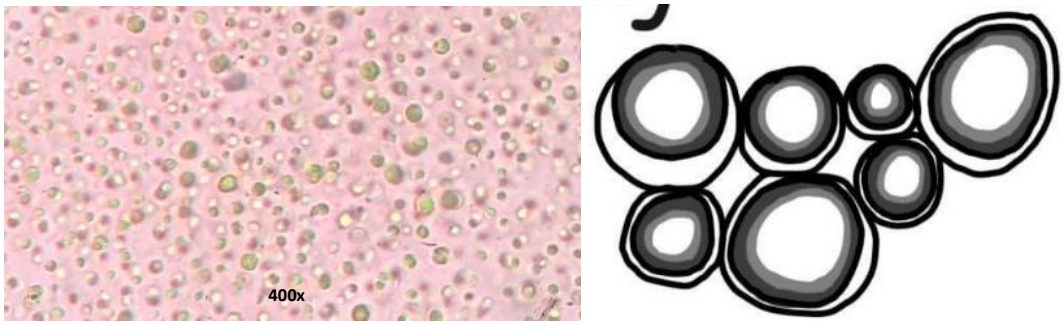

Figure 8

Family Chaetophoraceae

***Stigeoclonium attenuatum* [Hazen] Collins [Figure 9].** The filaments of this phytoplankton are elongated with upper branching mostly alternate and the branches either short or spine-like or long. The cells are cylindrical, with little or no constriction at the cross walls. The cells have sizes of 12-20um [L] and 5-7um [W].

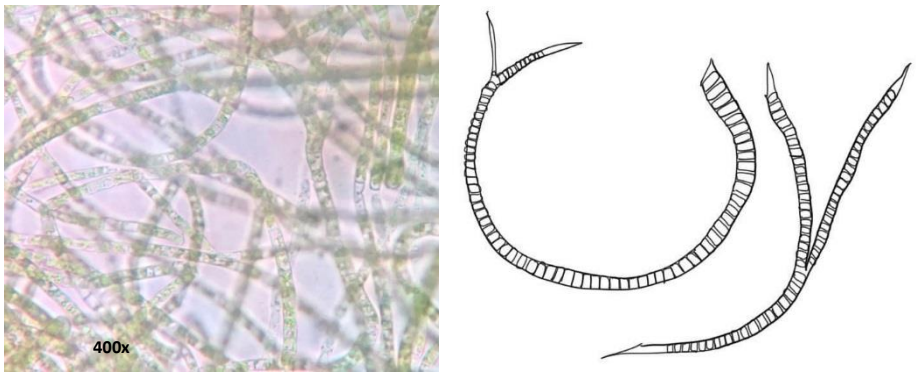

Figure 9

Family Chlorococcaceae

Figure 10

***Chlorococcum humicola* [Nageli] Rabenhorst [Figure 10].** The cells are spherical, solitary or a number of cells crowded together to form a stratum. Their chloroplast is a hollow sphere with a lateral notch and a single pyrenoid. Cells ranging from 2-25um in diameter.

Chlorococcum infusionum **[Schrank] Meneghini [Figure 11].** The cells are usually spherical, rarely ovoid or elongated and of variable dimension, solitary or in flat irregular colonies. Their chloroplast is like a hollow sphere with a notch on one side and with a single pyrenoid. Cells range from 10-109um, rarely up to 135u in diameter.

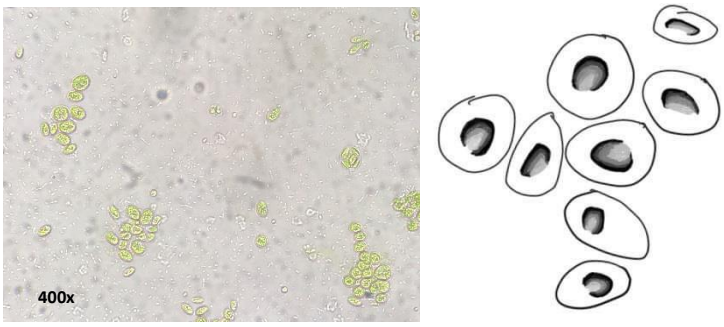

Figure 11

Family Prasiolaceae

Hormidium klebsii **G.M Smith [Figure 12].** This microalgae is composed of long unbranched filaments in which there is no basal-distal differentiation. The cells are cylindrical, not constricted at the cross walls and the chloroplast is a parietal plate covering only a small portion of the cells wall. Cells have sizes of 15.6-25um [L] and 5.8-6um [W].

Figure 12

Family Radiococcaceae

Gloeocystis ampla **[Kuetz] Lagerheim [Figure 13].** The cells are ovoid or oblong, arranged in amorphous or somewhat globular colonies and embedded in copious unlamellated gelatinous envelopes and each cells is distinct and angular. The cell sizes have 10-14um [L] and 5.8-6um [W].

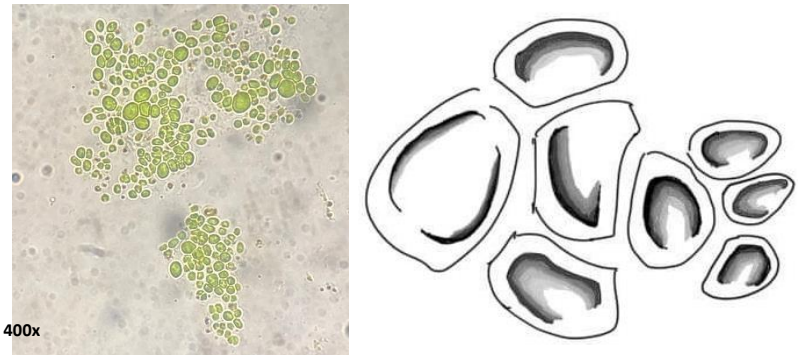

Figure 13

Cyanophyta

Family Nostocaceae

***Anabaena* Bory [Figure 14].** The trichomes are uniformly broad throughout but apices somewhat attenuated; may be straight or irregularly contorted but of less definite form than *Nostoc*. Their cells are usually spherical or barrel-shaped, rarely cylindrical, and never discoid; protoplasts either homogenous, granulose, or filled with numerous pseudovacuoles. The akinetes occur singly or in very short catenate series and may develop next to heterocysts.

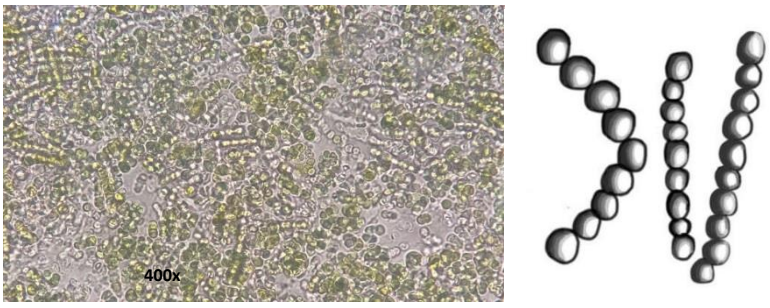

Figure 14

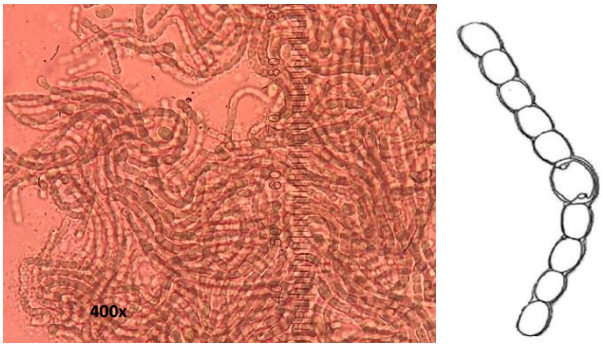

Figure 15

***Anabaena azollae* Strasburger [Figure 15].** Each trichomes is straight or coiled, often in small clusters but

more frequently solitary inhabiting the tissues of *Azolla*. The micro algal cells are subglobose to ellipsoid, the contents are granular and 4-5um in diameter, 6-8um long. Each heterocysts is ovate, and 9-10um [L] and 6-9.5um [W].

***Nostoc* Vaucher [Figure 16].** The trichomes are chain-like or very much contorted, enclosed by copious, thick mucilaginous sheath giving the colony a fixed, definite shape. The cells are spherical, depressed- spherical, barrel-shaped or cylindrical and heterocysts are intercalary but in young filaments terminal. Moreover, akinetes are globular or oblong, either solitary or in series; usually more akinetes formed than Anabaena.

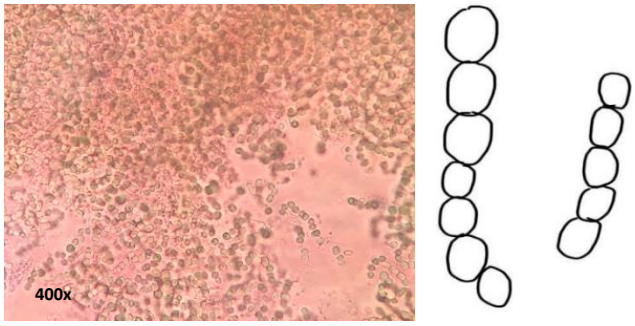

Figure 16

Family Chroococcaceae

***Chroococcus dispersus* [Keissler] Lemmermann [Figure 17].** This organism is a free-floating, flattened, ovate or irregularly-shaped colony of 4-16 spherical cells which are either single or arranged in small clusters. The cell contents are bright blue-green or gray- green sometimes and each cells has 3-4.5um in diameter [Prescott, 1962].

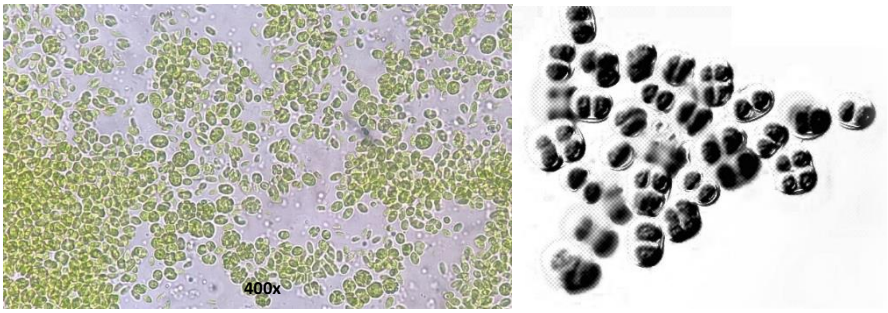

Figure 17

***Chroococcus limneticus* Lemmermann [Figure 18].** This phytoplankton is a free-floating, spherical or ovate colony of 4-32 spherical cells rather closely and evenly arranged, sometimes in groups of 2-4 cells as a result of rapid cell division. The cell contents dull to bright blue-green, not conspicuously granular and average size of 6-12um in diameter.

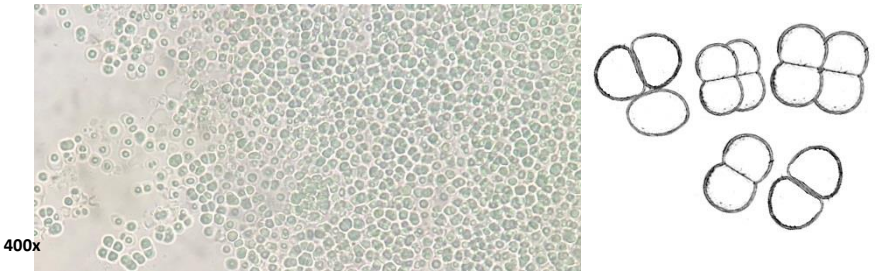

Figure 18

Family Synechococcaceae

***Synechococcus* Naegeli [Figure 19].** This microalgae is cylindrical, oblong or elliptical and unicellular or sometimes composed of 2 to 4 cells seriately united as a result of cell division in one plane. They are also free-floating without a sheath and their cell contents are pale blue-green or some shade of yellow, highly granular.

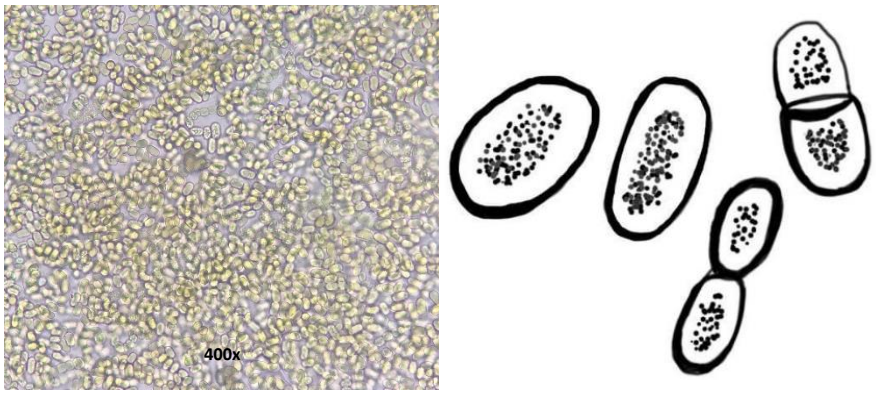

Figure 19

Family Oscillatoriaceae

***Oscillatoria* Vaucher [Figure 20].** A microalgae which is filamentous and elongated, without a sheath. Each trichomes is solitary and scattered, or forming expanded plant masses and slimy layers on submerged objects or on the bottom. It has an average measurement of 25.4um [L] and 3.4um [W].

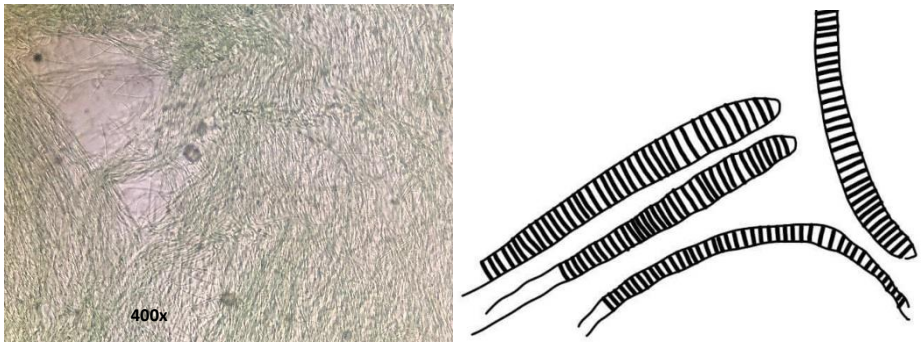

Figure 20

Bacillariophyta

Family Fragilariaceae

***Fragilaria brevistrata* Grun [Figure 21].** Their valves are linear-lanceolate and 15-22um gradually attenuated to rounded-truncate poles. These species are usually solitary, planktonic and common to freshwater.

Figure 21

Family Naviculaceae

***Navicula* Bory [Figure 22].** The valves may be boat-shaped, linear to elliptical, usually attenuated at their poles with either capitate, rounded or rostate apices. This microalgae has chromatophores commonly 2, laminate, each lying on opposite girdle, rarely 4 or 8. They are generally solitary, free-floating or maybe enclosed in mucous tubes forming clusters or chain-like colonies. This phytoplankton has an average sizes 10.5um [L] and 6.3um [W].

Figure 22

***Navicula cincta* [Ehrenberg] Ralfs [Figure 23].** Each cells of this microalgae has sizes ranging from 5.5-8.8um broad and 17.2-33um long. Their valves are linear to elliptic-lanceolate with rounded ends. There are transverse striations appearing as lines.

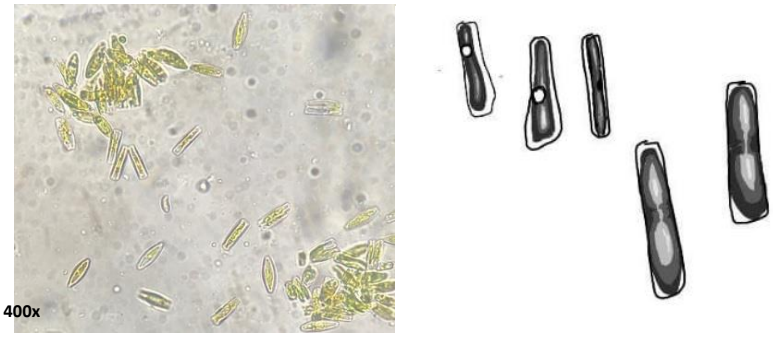

Figure 23

Six isolated phytoplankton were screened for their large-scale potential but only four survived. The chosen four organisms belong to only two Phyla namely Chlorophyta [green algae] and Cyanophyta [blue-green algae]. Bacillariophyta [diatoms] and Dinophyta [dinoflagellates] were not considered in the study because they are known for their difficulty to be cultured in the laboratory and maintain their growth conditions in a short period of time. Moreover, the medium that was used is not applicable to diatoms and dinoflagellates. Figure 2 shows the 4 selected phytoplankton with their respective descriptions.

Family Palmellopsidaceae

Asterococcus* cf *limneticus. The micro algal cells are globose or subglobose, either solitary or in colonies of from 4 to 16 and in colorless homogenous envelopes of mucilage. It has chloroplast which is a stellate mass with radiate arms from a central core and contains a pyrenoid. It has an average measurement of 20.2um [L] and 10.3um [W].

Family Chlorococcaceae

Chlorococcum* cf *humicola. The cells are spherical, solitary or a number of cells crowded together to form a stratum. Their chloroplast is a hollow sphere with a lateral notch and a single pyrenoid. Cells range from 2-25um in diameter.

Family Nostocaceae

Anabaena* cf *azollae. The trichomes are uniformly broad throughout but apices somewhat attenuated; may be straight or irregularly contorted but of less definite form than *Nostoc*. Their cells are usually spherical or barrel-shaped, rarely cylindrical, and never discoid; protoplasts either homogenous, granulose, or filled with numerous pseudovacuoles. The akinetes occur singly or in very short catenate series and may develop next to heterocysts.

Family Oscillatoriaceae

Oscillatoria* cf *limnetica. A microalga which is unbranched filamentous and elongated with a mucilaginous sheath. Each trichomes is solitary and scattered, or forming expanded plant masses and slimy layers on submerged objects or on the bottom. It has an average measurement of 25.4um [L] and 3.4um [W].

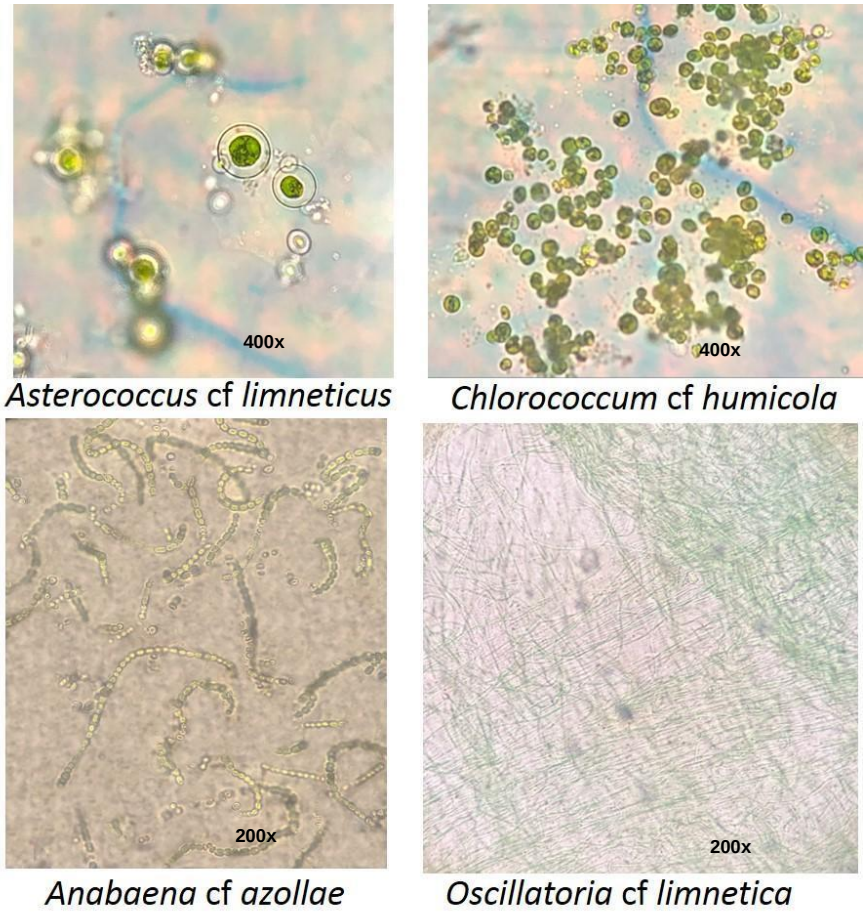

Figure 2: The four selected phytoplankton for culture studies and large-scale production

The following tables and figures demonstrate the mean specific growth rates of the four phytoplankton being studied as to various light intensities, pH and temperature.

Light Intensity Experiment

Table 3 presents the mean specific growth rates of the four phytoplankton subjected in various light intensities at constant pH and temperature.

Table 3: Mean Specific Growth Rate of Phytoplankton [per day] at different light intensities at constant pH and temperature

Phytoplankton	**Light Intensity [*umol m^{-2} s^{-1}*]**			
	20.25	40.5	81	121.5
Asterococcus cf *limneticus*	0.051 ± 0.01	0.125 ± 0.01	0.073 ± 0.004	0.099 ± 0.004
Chlorococcum cf *humicola*	0.079 ± 0.01	0.104 ± 0.004	0.079 ± 0.001	0.083 ± 0.02
Anabaena cf *azollae*	0.044 ± 0.01	0.079 ± 0.01	0.062 ± 0.01	0.079 ± 0.01
Oscillatoria cf *limnetica*	0.123 ± 0.01	0.058 ± 0.004	0.058 ± 0.03	0.087 ± 0.01

Values expressed as mean ± SEM, n = 3.

As shown in the table, the highest mean specific growth rates was obtained in *Asterococcus* cf *limneticus* grown at 40.5 *umol* m^{-2} s^{-1} [0.125 ± 0.01], followed by *Oscillatoria* cf *limnetica* at 20.25 *umol* m^{-2} s^{-1} [0.123 ± 0.001] and *Chlorococcum* cf *humicola* at 40.5 *umol* m^{-2} s^{-1} [0.104 ± 0.004]. All the rest have lower specific growth with *Anabaena* cf *azollae* demonstrating the lowest specific growth rate [0.044 ± 0.01] at 20.25 *umol* m^{-2} s^{-1}. Furthermore, Figure 12 displays the mean specific growth rates of the four phytoplankton subjected in the different light intensities which showed the standard error means. All of the light intensities showed no significant differences exhibited by the overlapping bars except in 20.25 *umol* m^{-2} s^{-1} where no overlapping bars were observed. However, two-factor analysis of variance [ANOVA] reveals that there were no significant differences in the mean specific growth rates of the four phytoplankton [F = 1.574, *p*=0.215].

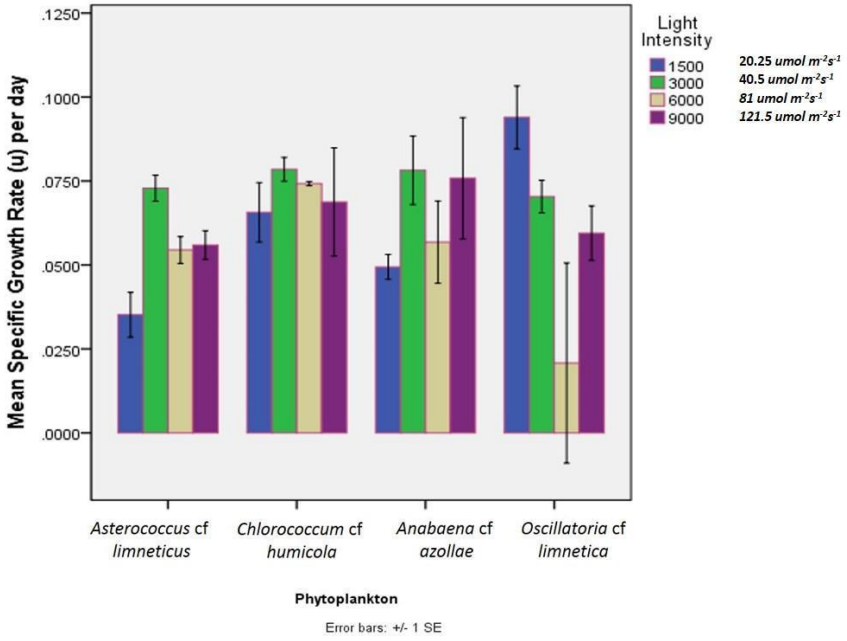

Figure 3: Mean specific growth rates of the 4 phytoplankton in the different light intensities

Also, there were no significant differences in the mean specific growth rates of the phytoplankton when grouped according to levels of light intensity [F = 2.859, *p*=0.052]. This data suggests that the four phytoplankton can utilize a wide range of light intensities. However, there was a significant interaction between phytoplankton and light intensity [F = 2.600, *p*=0.022], indicating that the combined effect of phytoplankton and light intensity to the mean specific growth rates of the four phytoplankton is significant. In depth analysis, *Post hoc* one-way analysis of variance of combinations of phytoplankton and light intensity showed that the mean specific growth rates of *Asterococcus* cf *limneticus* when subjected to 3000lux and *Anabaena* cf *azollae* in 20.25 *umol* m^{-2} s^{-1} had the highest and lowest specific growth rates, respectively. The rest of the combinations have the same specific growth rates.

Temperature Experiment

Meanwhile, Table 8 presents the mean specific growth rates of the four phytoplankton grown at different temperatures at constant pH and light intensity.

Table 4: Mean Specific Growth Rates of Phytoplankton [per day] grown at different temperature at constant pH and light Intensity

Phytoplankton	**Temperature [^{0}C]**			
	20	25	30	35
Asterococcus cf *limneticus*	0.113 ± .02	0.054 ± .01	0.062 ± .01	0.076 ± .01
Chlorococcum cf *humicola*	0.059 ± .01	0.036 ± .01	0.042 ± .003	0.085 ± .01
Anabaena cf *azollae*	0.058 ± .01	0.047 ± .01	0.052 ± .01	0.057 ± .004
Oscillatoria cf *limnetica*	0.077 ± .01	0.037 ± .01	0.59 ± .002	0.069 ± .01

Values expressed as mean ± SEM, n = 3.

The table emphasizes that the highest mean specific growth rates occurred in *Asterococcus* cf *limneticus* when subjected to 20^{0}C [0.113 ± .02]; followed by *Chlorococcum* cf *humicola* at 35^{0}C [0.085 ± .01] and *Oscillatoria* cf *limnetica* showed the lowest specific growth rate at 25^{0}C [0.036 ± .01]. Moreover, Figure 13 displays the mean specific growth rates of the four phytoplankton showing the standard error means. The graph explains that only *Asterococcus* cf *limneticus* in all the temperature tests are significantly different to the other three phytoplankton in all the temperature variables depicted by the non-overlapping lines of the said phytoplankton.

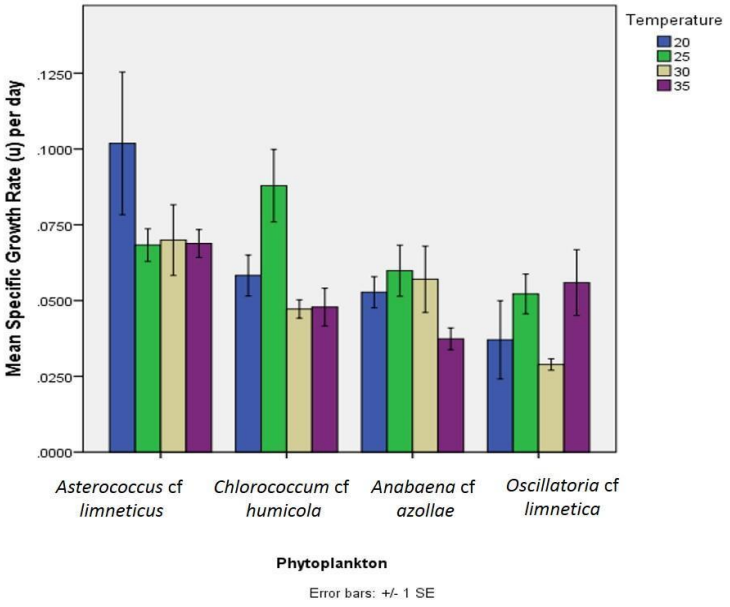

Figure 4: Mean specific growth rate of the 4 phytoplankton in the different temperature

This is proven by the two-factor analysis of variance [ANOVA] where there are significant differences in the mean specific growth rates of the 4 phytoplankton [F = 8.663, $p<.01$]. However, there were no significant differences in the mean specific growth rates of the phytoplankton when grouped according to different temperatures [F = 2.559, $p=0.072$]. This further expounds that in varying temperatures, all of the microalgae can adapt, survive, grow, and reproduce. In addition, there was a significant interaction between type of phytoplankton and temperature [F = 2.255, $p=0.044$], indicating the significant combined effects of phytoplankton type and temperature to the mean specific growth rates of the four phytoplankton. *Post hoc* one-way analysis of variance of combinations of phytoplankton and temperature shows that the mean specific growth rate of *Asterococcus* cf *limneticus* had significantly the highest specific growth rate at 20^0C while *Oscillatoria* cf *limnetica* had significantly the lowest at 25^0C compared to the rest of the groups. The remaining combinations have the same specific growth rates.

Varying Concentrations of pH

Finally, Table 5 presents the mean specific growth rates of the four phytoplankton grown at different pH at constant light intensity and temperature.

Table 5: Mean Specific Growth Rate of Phytoplankton [per day] subjected to different pH at constant light intensity and temperature

Phytoplankton	**Ph**		
	7	**8**	**9**
Asterococcus cf *limneticus*	0.052 ± 0.03	0.042 ± 0.03	0.037 ± 0.05
Chlorococcum cf *humicola*	0.103 ± 0.01	0.034 ± 0.002	0.025 ± 0.01
Anabaena cf *azollae*	0.033 ± 0.003	0.031 ± 0.02	0.057 ± 0.04
Oscillatoria cf *limnetica*	0.042 ± 0.02	0.026 ± 0.01	0.054 ± 0.002

Values expressed as mean ± SEM, n = 3.

Table 5 explains that the highest mean specific growth rates was obtained by *Chlorococcum* cf *humicola* at pH 7 [0.103 ± 0.01]; followed by *Anabaena* cf *azollae* at pH 9 [0.057 ± 0.04]; and the *Chlorococcum* cf *humicola* at pH 9 [0.025 ± 0.01]. Meanwhile, *Oscillatoria* cf *limnetica* at pH 8 showed the lowest mean specific growth rates [0.025 ± 0.01 and 0.026 ± 0.01]. Furthermore, Figure 14 shows the mean specific growth rates of all the phytoplankton which are not significantly different from each other represented by the overlapping error bars in all pH levels.

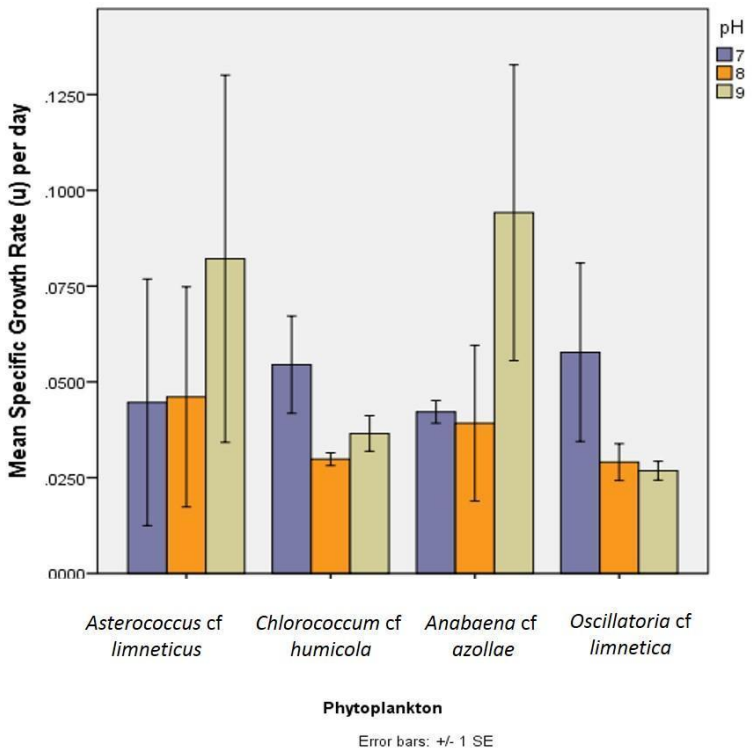

Figure 5: Mean specific growth rates of the 4 phytoplankton in the different pH level

This is further proven by the two-factor analysis of variance [ANOVA] showing that there were no significant differences in the mean specific growth rates of the four phytoplankton [F = 0.640, *p*=0.596]. In addition, there were no significant differences in the mean specific growth rates of phytoplankton when grouped according to pH levels [F = 1.008, *p*=0.380] and finally no significant interaction was observed between the four phytoplankton and pH [F = 0.757, *p*=0.610], indicating that all the phytoplankton tested can adapt to wide ranges of pH.

4. Discussions

The quantified and identified phytoplankton were isolated and purified for culture studies. These organisms were produced in semi large-scale and observed for their fast-growing activity in 3-5 days, lesser microbial contamination and adjustable to various culture conditions. Noteworthy, four phytoplankton namely *Asterococcus* cf *limneticus*, *Chlorococcum* cf *humicola*, *Anabaena* cf *azollae* and *Oscillatoria* cf *limnetica* were optimized for their light, temperature and pH levels. Light intensity is one of the essentials of phytoplankton for their growth and development. Results of this study show that chlorophytes and cyanophytes have an optimum light intensity of 3000lux or 40.5 *umol* $m^{-2}s^{-1}$. Green algae such as Genera *Asterococcus* and *Chlorococcum* reached its optimum growth rate at light intensity of 1000-10,000lux or 13.5-135 *umol* $m^{-2}s^{-1}$ [33,34]. Moreover, *Anabaena ambigua* was noted to have best growth performance when lighted with 7000lux or 94.5 *umol* $m^{-2}s^{-1}$ significantly at the 10^{th} day compared to 1500lux or 20.25 *umol* $m^{-2}s^{-1}$ and 4000lux or 54 *umol* $m^{-2}s^{-1}$. The observation ended at Day 18 [35]. These studies proved that a diverse array of phytoplankton can grow well in varied light intensities. On the other hand, results on temperature tests, another important condition, showed that

phytoplankton used in the study can grow well in 25^{O}C. In a study of *Anabaena ambigua* where it was evaluated with an optimum temperature of 35°C along with other species namely *Synechococcus* sp. and *Arthronema africanum* [36]. More recent, cyanophytes, *Oscillatoria* sp. along with *Lyngbya* sp. were observed to withstand the temperature of 23-25°C [37,38]. Lastly, the pH level where phytoplankton thrive, was also studied. The four phytoplankton used have an optimum pH condition of 7. Related studies reported that the cyanophyte, *Anabaena ambigua* was observed to have the highest growth rate at pH 7 [34,37]. Meanwhile, the blue-green algae, *Oscillatoria* sp. together with *Lyngbya* sp. were noted to have an optimum pH at 9.0 [39,40]. In general, Genera *Anabaena* and *Oscillatoria* had been considered and studied initially for their optimum light intensity, temperature and pH levels compared to Genera *Asterococcus* and *Chlorococcum* where there is a scarcity of studies on their optimized conditions.

5. Summary, Conclusion and Recommendations

During the isolation, chlorophytes and cyanophytes dominated where four phytoplankton were purified and semi-scaled up for culture studies namely *Asterococcus* cf *limneticus, Chlorococcum* cf *humicola, Anabaena* cf *azollae* and *Oscillatoria* cf *limnetica*. Results show that the four microorganisms have the highest mean specific growth rates at 40.5 *umol* $m^{-2}s^{-1}$, 25^{0}C and pH 7. The freshwater tributaries of Carigara bay located in the five upper shoreline municipalities of the province of Leyte are still under study in terms of phytoplankton diversity and applications. Indeed, the freshwater resources are home to numerous species of phytoplankton which can be isolated, identified and mass-produced to generate natural food necessary for many aquatic organisms in their fry stage or post fry stage. *Asterococcus* cf *limneticus* and *Chlorococcum* cf *humicola*, both chlorophytes, showed potential as sources of natural food for common carp fry from the isolation, optimization, and proximate chemical composition analysis up to feeding experiment.

The researcher recommends the following:

1. Purification of the other isolated phytoplankton such as *Stigeoclonium* cf *attenuatum, Gloeocystis* cf *ampla, Hormidium* cf *klebsii, Chroococcus* cf *disperses* and *Synechococcus* sp.
2. Culture studies on the other phytoplankton present during the isolation and purification namely *Stigeoclonium* cf *attenuatum, Gloeocystis* cf *ampla, Hormidium* cf *klebsii, Chroococcus* cf *disperses* and *Synechococcus* sp.
3. Culture studies of Phylum Bacillariophyta such as *Fragilaria* cf *brevistrata* and *Navicula* cf *cincta* which were also isolated in the sampling stations.

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