

1
2 **PHYTOPLANKTON IDENTIFICATION, ISOLATION AND CULTURE OF**
3 **SELECTED MICRO ALGAL SPECIES FROM FRESHWATER**
4 **TRIBUTARIES OF CARIGARA BAY, Philippines**

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9 **ABSTRACT**

Carigara Bay is one of the successful fishing lands in the entire Philippines because of its multi-gear fishery system. Filipinos living around the bay considered its marine life as one of their livelihood. However, the biodiversity and biological importance of microalgae are still unexplored evident by the dearth of published data in scientific journals, thus, this study was undertaken. Sampling was conducted on the five (5) coastal towns surrounding the bay namely Carigara, Capoocan, Barugo, San Miguel and Babatngon, Its freshwater tributaries are 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 bay, stream, rivers and falls were collected using a 2.5-3L Plexi glass sampler, transferred in three (3) 1L cap bottle and brought in the laboratory for phytoplankton identification and micro algal culture. Physico-chemical parameters were also gathered in all sampling sites to correlate with the microalgae diversity. Phyla Bacillariophyta, Chlorophyta and Cyanophyta are 44, 37 and 19% of the total phytoplankton identified. Furthermore, there was a significant positive correlation between water pH ($r = 0.499$; $p = 0.001$), conductivity ($r=0.519$; $p=0.001$) and amount of phosphates ($r = 0.446$; $p = 0.003$). Moreover, six (6) genera namely *Asterococcus*, *Anabaena*, *Nostoc*, *Chlorococcum*, *Synechococcus* and *Oscillatoria* were isolated, cultured and semi-mass produced for optimization procedure.

10
11 *Keywords: freshwater tributaries, biodiversity, semi-large scale cultures*

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1. INTRODUCTION

Socio-cultural, economic and scientific evolution in the society nowadays gives a big difference and distinct impact on the food intake, balance diet and overall lifestyle of many people. Hence, nutritional content and value and feeding mechanisms play critical role in many developing and industrialized countries (Anderson and Alford 2014). This is also in support of the main goal of international agencies like World Health Organization (WHO) and Food and Agriculture Organization (FAO) which is healthy food for everyone in all nations (Nicoletti, 2016). Indeed, animal health is one of the struggles of the public today where synthetic and chemically produced food and feed for human and lower forms of animals, respectively are being manufactured and commercialized (Meena, 2013). The reason for this is the fast and growing population of humans where natural resources diminished proportionally (Ugoala et al., 2012). Consequently, in some instances, the products are dangerous due to the negative side effects that are being neglected while the synthetic ones brought positive effects. To address these issues, drugs, pigments and biochemicals excreted by natural aquatic weeds and grass, higher forms of flora, as well as macro and microalgae are being established and scrutinized for the development of products necessary for aquaculture, agriculture, and other industrially important applications for mankind and lower animals (Kaushik and Chauhan 2008; Voort et al., 2014). Algae have existing species of about 50,000 and more but only half of the total species are being experimented and analyzed on the said aspect and only 15 micro algal species are involved in current commercial production (Richmond 2004; Raja et al., 2008; Ugoala et al., 2012).

Generally, most of the microalgae have major attributes which are important for mass cultivation. One of these is the presence of high reproduction and growth rates, hence, can be cultured in simple ponds and complex bioreactors either indoor or outdoor (Duong et al., 2012; Chen et al., 2014). In order to grow, develop and reproduce in the said process, these organisms which are autotrophic in nature, can transform sunlight into glucose by photosynthesis through fixation of carbon dioxide with high production of dry matter supplementing its body in comparison to the self-feeder plants (Raja et al., 2008; Park et al., 2011; Karthikeyan 2012). However, algae can also be heterotrophic organisms where they use organic compounds as their source of carbon dioxide and light. To compare, micro algal species' efficiency to do photosynthesis is higher to plants by about 10-20% (Ferdowshi 2013). They are, indeed, fast growers which means that the species can duplicate its life for about 3 hours and can reproduce themselves very quickly in simple medium (Kovac et al., 2013). These microalgae can also tolerate varying concentrations of light and temperature, pH and oxygen concentration in different aquatic habitats which can eradicate the presence of pathogenic organisms. Microalgae can also tolerate extreme conditions of high salinity and large exposure to ultraviolet rays. In all of these, the microalgae produce high varieties of secondary metabolites with large potency and effectiveness for biological activities (Ibanez et al., 2012).

Carigara Bay, the location of the study, is located in the Province of Leyte at Region 8 or the Eastern Visayas where it is situated in five (5) 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 (Makabenta, 1995). The bay itself and its freshwater resources are abound 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 (Santos et al. 1999). Noticeably, too, are current publications stating that most of the micro algal cultivation is sold in the market for animal food and pharmaceutical products (Becker 2004) and the algal biomass is starting to have a high rate of demand for aquaculture industry including fish

65 feed providing an initiative revenue for algae industry even microalgae cultivation is only a
66 few decades old (Anemaet et al., 2010). However, adequate studies on the suitability of
67 these microalgae as animal feed are limited (Olaizola 2003; Sathasivam et al., 2017). Micro-
68 algal based bio-resources products necessary in daily living are the new trends of today's
69 era and scientists dwell more attention on the said matter (Feng et al., 2016). Thus this
70 study on the identification and assessment of microalgae in Carigara Bay and freshwater
71 resources in its vicinity, situated at the five (5) towns surrounding the bay would serve as
72 baseline information for future studies.

73 The objective of this study is to identify and quantify microalgae found in
74 Carigara bay and its freshwater tributaries; isolate and culture micro algal species;
75 characterize and identify the micro algal species isolated from the freshwater tributaries of
76 Carigara bay and screen, select and semi-mass produced micro algal species from the
77 freshwater tributaries of Carigara bay.

78 **2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY**

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81 The island of Leyte where Carigara Bay is sited and connected with freshwater
82 tributaries, is a sister island of Samar and one of the ten (10) biggest land masses in the
83 country. Sampling stations in the area were determined in coordination with Dr. Paciente A.
84 Cordero, Jr. with the supervision of the faculty members and staff of the College of Fisheries
85 at Eastern Visayas State University, Carigara Campus. Fourteen (14) stations were
86 established at the upper coastal towns adjoining the Carigara Bay. Station 1 was the
87 Carigara Bay at Brgy. Libertad (Capoocan); Station 2 was a stream at Brgy. Libertad
88 (Capoocan); Station 3 Carigara Bay at Brgy. Visoria (Carigara); Station 4 was the Lindog
89 River at Brgy. Uyawan (Carigara); Station 5 was the Bislig River at Brgy Bislig (Carigara);
90 Station 6 was the Carigara Bay at Brgy. Duka (Barugo); Station 7 was the Himanglos River
91 at Brgy. Hilaba (Barugo); Station 8 was the Canomantag River at Brgy. Canomantag
92 (Barugo); Station 9 was the Carigara Bay at Brgy. Mawod-pawod (San Miguel); Station 10
93 was the Caraycaray River at Brgy. Caraycaray (San Miguel); Station 11 was the Lipasan
94 Falls at Brgy. Pinarigusan (San Miguel); Station 12 was the Carigara Bay at Brgy.
95 Kalangawan Guti (Babatngon); Station 13 was the Tula-an Falls at Brgy. Tula-an
96 (Babatngon) and Station 14 was the Busay Falls at Brgy. Busay (Babatngon). The water
97 samples were collected from July 2-8, 2018 which was considered as the 1st sampling and
98 from November 23-26, 2018 which was the 2nd sampling. Collection of water samples from
99 the bay was done by towing 30 meters away from the shoreline. The water samples from the
100 bay were gathered 30 meters away from the shoreline with a depth of 1-1.5 meters only. The
101 water samples from the bay were used for phytoplankton quantification analysis only. For the
102 freshwater tributaries of the bay, the water samples were collected in integrated manner,
103 thus, from surface, middle and approximately 0.5 meter away from the bottom. Most have
104 depths ranging from 0.75- 2 meters, therefore, ideal for integrated sampler. Depths were
105 measured using the plexi glass sampler built in with improvised measurement in meters
106 and/or the secchi disk. The three (3) L of water samples from the freshwater ecosystems
107 were divided from which the 1 L was taken and fixed with Lugol's solution to preserve the
108 cell wall; one (1) L was kept cooled during the transport at University of Santo Tomas,
109 Manila and immediately stored in freezer or placed in a refrigerated condition for *ex situ*
110 nutrient determination of the water samples such as nitrate and phosphates and the other
111 one (1) L was used as a live sample for the isolation of microalgae. Furthermore, prior to
112 collection physical parameters such as surface water temperature, column depth, light
113 penetration or transparency, and chemical parameters such as water pH, conductivity and
114 dissolved oxygen were recorded in situ. All of the parameters were measured using the
115 Xplorer GLX (PAASCO) water quality sensor except for the light penetration or transparency
116 which was determined using a Secchi disc. In addition analysis of nitrate and phosphorus

117 were also done through the Hach DR/2010 phosphate data logging spectrophotometer at the
118 Roque Laboratory, Old Graduate School, University of Santo Tomas.

119 In order to quantify, identify, isolate and separate micro algal species, standard
120 protocol on washing and plating techniques were done to ensure the isolation of all the
121 microalgae components from water samples collected (Martinez, 1976; Baldia, 1992; Lee et.
122 al., 2014). For quantification, an appropriate amount was was loaded in a Neubauer
123 Counting Chamber for density determination as viewed using the Olympus CH20 compound
124 light microscope. (Martinez et. al., 1975). The species were identified using several
125 references on micro algal taxonomy and biodiversity. For the isolation, Binangonan
126 Research Station Pantastico medium (BRSP) was used for the multiple streaking technique.
127 After growth colonies and establishment of uni-algal cultures, the isolates were maintained in
128 each test tubes containing 10 ml of BRSP media at a temperature of $25 \pm 2^{\circ}$ C, pH of 7.27
129 and light intensity of 3,000 lx. Six (6) isolates were selected for semi-mass production and
130 large scale cultivation.

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132 **3. RESULTS AND DISCUSSION**

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134 **Biodiversity of Phytoplankton**


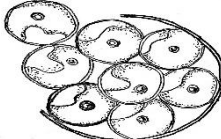
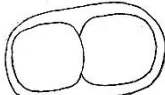
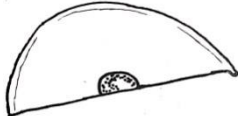
135 The variety of biological organisms is attributed by biotic and abiotic factors obtained
136 in the study sites. These are the physico-chemical parameters and the type of ecological
137 features which are bounded in the sampling areas in order to understand and explore the
138 variety of living organisms present in the said locations. Moreover, studies on phytoplankton
139 diversity can in turn control the physico-chemical and biological conditions in certain aquatic
140 ecosystem (Ariyadej et al., 2004; Ali et al., 2010). The species are identified taxonomically,
141 uni-isolated and mass-cultured for their biological potentials particularly in food industry,
142 pharmaceutical and biotechnology.



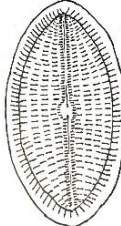
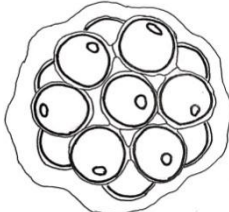
143 The following are the Genus identified and quantified in Carigara Bay and its
144 freshwater tributaries. For Phylum Bacillariophyta, these are *Cocconeis* (Her.) Grun., 1868;
145 *Coscinodiscus* Her., 1838 *Fragilaria* Lyng., 1819., emend, Rabenh., 1864; *Synedra*
146 Ehrenberg, 1830; *Navicula* Bory, 1822 erend, Cl., 1894; *Nitzschia* Hassal, 1845, emend,
147 Grun., 1880; *Stauroneis* Her., 1843; *Cymbella* Agardh, 1836; *Melosira* Agardh, 1824;
148 *Diploneis* Ehrenberg, 1894; *Gomphonema* Ehrenberg, 1832 and *Neidium* Pfitzer, 1871. For
149 Phylum Chlorophyta, these are *Chlorella ellipsoidea* Gerneck 1907 and *Chlorella vulgaris*
150 Beyerinck 1890; *Staurastrum* Meyen ex Ralfs, 1848; *Stigeoclonium* Kuetzing 1843;
151 *Schizomeris* Kuetzing 1843; *Sphaerocystis* Chodat 1897; *Ulothrix* Kuetzing, 1833;
152 *Closteridium* Reinsch, 1888; *Closterium* Brebisson, 1856; *Coelastrum* Naegeli in Kuetzing,
153 1849 and *Pediastrum* Meyen, 1829. For Phylum Cyanophyta, *Lyngbya* Ag., 1824;
154 *Oscillatoria* Vaucher, 1803; *Chroococcus* Naeg., 1848; *Merismopedia elegans* A. Braun in
155 Kuetzing 1849 and *Nostoc* Vaucher, 1803.

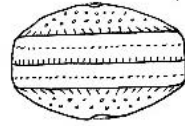
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Table 1. List of all phytoplankton species with illustrations

Species	Division	Size	Illustration
<i>Chlorella ellipsoidea</i>	Chlorophyta	Length: 9.2 μ m Width: 6.9 μ m	
<i>Chlorella vulgaris</i>	Chlorophyta	Length: 9.2 μ m Width: 9.2 μ m	
<i>Chroococcus</i> <i>sp.</i>	Cyanophyta	Length: 6-15 μ m Width: 9.6 μ m	
<i>Closteridium</i> <i>sp.</i>	Chlorophyta	Length: 96.6 μ m Width: 36.8 μ m	

<i>Closterium sp</i>	Chlorophyta	Length: 48.3 um Width: 9.2 um	
<i>Cocconeis placentula</i>	Bacillariophyta	Length: 32.2 um Width: 13.8 um	
<i>Cocconeis sp.</i> (2)	Bacillariophyta	Length: 46 um Width: 27.6 um	
<i>Coelastrum sp.</i>	Chlorophyta	Length: 18.4 um Width: 11.5 um	
<i>Coscinodiscus sp.</i>	Bacillariophyta	Length: 59.8 um Width: 13.8 um	



Cymbella sp.

Bacillariophyta

Length:
69 um
Width:
25.3 um



***Diploneis sp.*
(1)**

Bacillariophyta

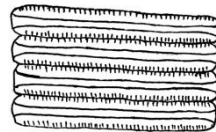
Length:
39.1 um
Width:
16.1 um



***Fragilaria sp.*(1)**

Bacillariophyta

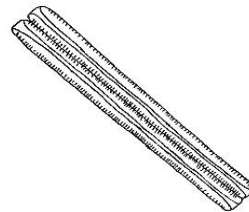
Length:
41.4 um
Width:
4.6 um



***Fragilaria sp.*
(2)**

Bacillariophyta

Length:
59.9 um
Width:
25.4 um



Fragilaria sp.(3)

Bacillariophyta

Length:
52.2 um
Width:
18.4 um



***Fragilaria sp.*
(4)**

Bacillariophyta

Length:
211.6
um
Width:
9.2 um



***Fragilaria sp.*
(5)**

Bacillariophyta

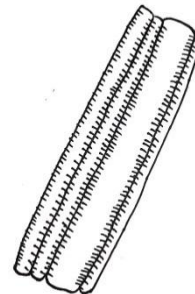
Length:
39.4 um
Width:
3.9 um

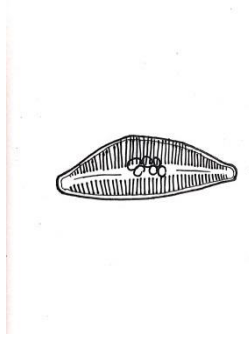



***Fragilaria sp.*
(6)**

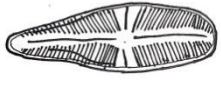
Bacillariophyta


Length:
52.9 um
Width:
11.5 um


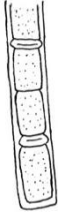
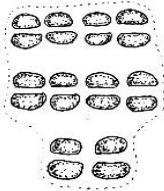
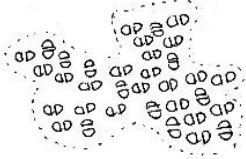








<i>Gomphonema</i> sp. (1)	Bacillariophyta	Length: 36.8 um Width: 11.5 um	
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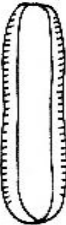


<i>Gomphonema</i> sp. (2)	Bacillariophyta	Length: 36.8 um Width: 11.5 um	
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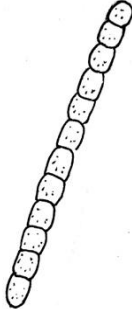
<i>Gomphonema</i> sp. (3)	Bacillariophyta	Length: 41.4 um Width: 13.8 um	
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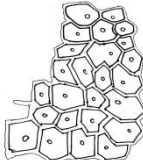

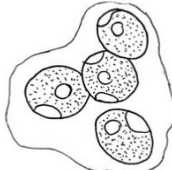
<i>Lyngbya</i> sp.(1)	Cyanophyta	Length: 32.2 um Width: 9.2 um	
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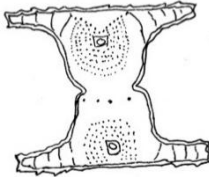




<i>Lyngbya sp.(2)</i>	Cyanophyta	Length: 87.4 um Width: 9.2 um	
<i>Melosira sp.</i>	Bacillariophyta	Length: 18.4 um Width: 9.2 um	
<i>Merismopedia sp.(1)</i>	Cyanophyta	Length: 103.5 um Width: 11.5	
<i>Merismopedia sp.(2)</i>	Cyanophyta	Length: 69 um Width: 13.8 um	
<i>Navicula sp.(1)</i>	Bacillariophyta	Length: 55.2 um Width: 23 um	


<i>Navicula sp.(2)</i>	Bacillariophyta	Length: 23 um Width: 18.4 um	
<i>Navicula sp.(3)</i>	Bacillariophyta	Length: 46 um Width: 9.2 um	
<i>Navicula sp.(4)</i>	Bacillariophyta	Length: 39.1 um Width: 11.5 um	
<i>Navicula sp.(5)</i>	Bacillariophyta	Length: 55.2 um Width: 16.1 um	
<i>Neidium sp.</i>	Bacillariophyta	Length: 46.0 um Width: 13.8 um	


<i>Nitzschia sp.(1)</i>	Bacillariophyta	Length: 105.8 um Width: 9.2 um	
<i>Nitzschia sp.(2)</i>	Bacillariophyta	Length: 78.2 um Width: 20.7 um	
<i>Nitzschia sp. (3)</i>	Bacillariophyta	Length: 78.2 um Width: 13.8 um	
<i>Nitzschia sp. (4)</i>	Bacillariophyta	Length: 59.8 um Width: 13.8 um	


<i>Nitzschia sp.</i> (5)	Bacillariophyta	Length: 46 um Width: 18.4 um	
<i>Nitzschia sp.</i> (6)	Bacillariophyta	Length: 59.8 um Width: 13.8 um	
<i>Nitzschia sp.</i> (7)	Bacillariophyta	Length: 115 um Width: 13.8 um	
<i>Nostoc sp.</i>	Cyanophyta	Length: 8 um Width: 6.7 um	
<i>Oscillatoria sp.</i> (1)	Cyanophyta	Length: 41.4 um Width: 2.3 um	


<i>Oscillatoria</i> sp.(2)	Cyanophyta	Length: 64.4 um Width: 2.3 um	
<i>Oscillatoria</i> sp.(3)	Cyanophyta	Length: 92 um Width: 2.3 um	
<i>Pediastrum</i> sp.	Chlorophyta	Length: 29.9 um Width: 16.1 um	
<i>Schizomeris</i> sp.	Chlorophyta	Length: 9.2 um Width: 9.2 um	
<i>Sphaerocystis</i> sp.	Chlorophyta	Length: 9.2 um Width: 9.2 um	

<i>Staurastrum</i> sp.	Chlorophyta	Length: 27.6 um Width: 13.8 um	
<i>Stauroneis</i> sp.(1)	Bacillariophyta	Length: 27.6 um Width: 13.8 um	
<i>Stauroneis</i> sp.(2)	Bacillariophyta	Length: 29.9 um Width: 13.8 um	
<i>Stigeoclonium</i> sp.(1)	Chlorophyta	Length: 18.4 um Width: 4.6 um	
<i>Stigeoclonium</i> sp.(2)	Chlorophyta	Length: 23 um Width: 9.2 um	

<i>Stigeoclonium</i> sp. (3)	Chlorophyta	Length: 18.4 um Width: 9.2 um	
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<i>Stigeoclonium</i> sp. (4)	Chlorophyta	Length: 35.1 um Width: 2.9 um	
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<i>Synedra</i> sp.(1)	Bacillariophyta	Length: 285.2 um Width: 2.3 um	
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<i>Synedra</i> sp.(2)	Bacillariophyta	Length: 62.1 um Width: 11.5 um	
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Ulothrix sp.

Chlorophyta

Length:
18.4 um
Width:
9.2 um



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These number of micro algal cells found in Carigara bay and its freshwater resources was further correlated on the physico-chemical parameters gathered in the area as shown in Table 2.

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Table 2. Correlation Analysis on the Mean of the Physico-chemical Parameters and the Total Number of Micro algal Cells found in Carigara Bay and its Freshwater Resources

Physico-chemical Parameters	Mean	Standard Deviation	Correlation Analysis (N=42)	Number of Micro algal cells (M=16.88; SD=15.86)
Surface Water Temperature, °C	29.10	3.20	Pearson correlation	0.136
			Sig (2-tailed)	0.390
Column Depth, meters	0.9464	0.50	Pearson correlation	-0.047
			Sig (2-tailed)	0.770
Light Penetration, meters	0.65	0.25	Pearson correlation	-0.091
			Sig (2-tailed)	0.566
Water pH	8.20	0.40	Pearson correlation	0.499**
			Sig (2-tailed)	0.001
Conductivity, Siemens per meter	264.14	295.75	Pearson correlation	0.519**
			Sig (2-tailed)	0.000
Dissolved Oxygen, milligrams per liter	7.76	1.82	Pearson correlation	-0.351*
			Sig (2-tailed)	0.023
Nitrates, milligrams per liter	3.22	2.83	Pearson correlation	0.244
			Sig (2-tailed)	0.119
Phosphates, milligrams per liter	5.22	3.83	Pearson correlation	0.446**
			Sig (2-tailed)	0.003

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****Correlation is significant at the 0.01 level (2-tailed)**

***Correlation is significant at the 0.05 level (2-tailed)**

The above table shows that there was a significant positive correlation between water pH (Mean = 8.20; SD = 0.40) and the total number of micro algal cells found in the water sample (M = 16.88; SD = 15.86), $r = 0.499$; $p = 0.001$. Same results were obtained between conductivity (Mean= 264.14; SD=295.75) and total number of micro algal cells, $r=0.519$; $p=0.001$. It was also found that there exists a significant positive correlation between the amount of phosphates (Mean= 5.22; SD= 3.83) and the total number of micro algal cells found in the water sample, $r = 0.446$; $p = 0.003$. This means that increasing the water pH, conductivity and amount of phosphates in the water sample will lead to an increase in the number of micro algal cells found in the water sample. On the other hand, there exists a negative relationship between the amount of dissolved oxygen (Mean= 7.76; SD= 1.82) and total number of micro algal cells, $r=-0.351$; $p=0.023$. This means that a higher amount of dissolved oxygen in water will result to a corresponding decrease in the total number of micro algal cells in the water sample. Finally, no significant relationships were observed between the total number of micro algal cells and the following parameters: water temperature (Mean= 29.10; SD= 3.20), column depth (Mean= 0.9464; SD= 0.50), light penetration (Mean= 0.65; SD= 0.25), and nitrate content (Mean= 3.22; SD= 2.83). This means that changes in these parameters do not affect the total amount of micro algal cells in the water samples.

Isolation of Phytoplankton

After quantification of phytoplankton, the water samples were subjected for the isolation, purification and identification of micro algal species. There were 18 isolates that were purified and grown in BRSP liquid medium but only few were chosen and at the same time screened for their suitability as microalgae for large-scale production. The following are the species of microalgae isolated and identified. These were arranged according to phylum regardless of the stations where these microalgae were found. Phylum Chlorophyta has six (6) genus; Phylum Cyanophyta has five (5) genus and Phylum Bacillariophyta has two (2)

200 genus. For Phylum Chlorophyta, these are *Genus Asterococcus Scherffel 1908*; *Genus*
 201 *Chlorella Beyerinck 1890*; *Genus Stigeoclonium Kuetzing 1843*; *Genus Chlorococcum Fries,*
 202 *1825 (incl. Cystococcus Naeg.)*; *Genus Hormidium Kuetzing 1843* and *Genus Gloeotheca*
 203 *Naegeli 1849*. For Phylum Cyanophyta, *Genus Anabaena Bory, 1822*; *Genus Nostoc*
 204 *Vaucher, 1803*; *Genus Chroococcus Naeg., 1848*; *Genus Synechococcus Naegeli 1849* and
 205 *Genus Oscillatoria Vaucher 1803* were isolated. For Phylum Bacillariophyta, we have *Genus*
 206 *Fragilaria Lyng., 1819., emend, Rabenh., 1864* and *Genus Navicula Bory, 1822 erend, Cl.,*
 207 *1894.*

208 Furthermore, the isolated microalgae were tabulated in terms of their quantitative
 209 and qualitative forms. Table 2 and 3 summarize the qualitative and quantitative data of the
 210 18 microalgae that were isolated. The microalgae were arranged according to stations. We
 211 can also deduce from these tables the list of microalgae that were found in various
 212 freshwater stations. There were nine (9) freshwater ecosystems that served as sampling
 213 stations. These freshwater stations are Stations 2, 4, 5, 7, 8, 10, 11, 13 and 14.

214 Table 2 presents the microalgae isolated from each station as well as the
 215 measurements of each of the cells of the various species of microalgae. The sizes of the
 216 micro algal cells are in average form.

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Table 3. Quantitative analysis on the microalgae isolated from the freshwater stations

Station No.	Name of the Freshwater Ecosystem	Name of Microalgae	Quantitative	
			Size (Length)	Size (Width/diameter)
2	Stream, Brgy Libertad, Capoocan	<i>Asterococcus limneticus</i> G.M. Smith	10.2 um	22.3 um
		<i>Nostoc</i> sp.	4.6 um	4.1 um
		<i>Anabaena</i> sp.	6.9 um	4.6 um
4	Lindog River, Brgy. Uyawan, Carigara	<i>Anabaena azollae</i> Strasburger	6.1 um	4.6 um
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	11.5 um	6.9 um
		<i>Stigeoclonium attenuatum</i> (Hazen) Collins	22.3 um	5.7 um
5	Bislig River, Brgy. Bislig, Carigara	<i>Synechococcus</i> sp.	8.2 um	5.6 um
		<i>Chlorella ellipsoidea</i> Gerneck	8.1 um	6.3 um
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	10.3 um	6.5 um
7	Himanglos River, Brgy. Hilaba, Barugo	<i>Nostoc</i> sp.	4.6 um	4.1 um
		<i>Chroococcus limneticus</i> Lemmermann	8.3 um	6.1 um
		<i>Chlorella vulgaris</i> Beyerinck	7.2 um	5.9 um
		<i>Asterococcus limneticus</i> G.M.	10.2 um	22.3 um

		Smith		
		<i>Synechococcus</i> sp.	8.2 um	5.6 um
8	Canomantag River, Brgy. Canomantag, Barugo	<i>Chlorella vulgaris</i> Beyerinck	7.2 um	5.9 um
		<i>Anabaena azollae</i>	6.1 um	4.6 um
		<i>Synechococcus</i> sp.	8.2 um	5.6 um
10	Caray-caray River, Brgy. Caray-caray, San Miguel	<i>Gloeocystis ampla</i> Kutzing	10.1 um	9.2 um
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	10.3 um	6.5 um
		<i>Oscillatoria</i> sp.	25.4 um	3.4 um
11	Lipasan Falls, Brgy. Pinarigusan, San Miguel	<i>Chroococcus dispersus</i> (Keissler) Lemmermann	4.3 um	3.5 um
		<i>Fragilaria brevisstrata</i> Grun	41.4 um	4.6 um
		<i>Navicula</i> sp.	10.3 um	6.5 um
13	Tula-an Falls, Brgy. Tula-an, Babatngon	<i>Gloeocystis ampla</i> (Kuetz) Lagerheim	10.1 um	9.2 um
		<i>Chlorococcum infusionum</i> (Schrank) Meneghini	12.8 um	6.8 um
		<i>Navicula cincta</i> (Ehrenberg) Ralfs	9.8 um	7.1 um
14	Busay Falls, Brgy. Busay, Babatngon	<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	10.3 um	6.5 um
		<i>Fragilaria brevisstrata</i> Grun	41.4 um	4.6 um
		<i>Hormidium klebsii</i> G.M Smith	9.2 um	23 um
		<i>Oscillatoria</i> sp.	25.4 um	3.4 um

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For Station 2 which is the stream located at Brgy. Libertad, Capoocan, there were 4 microalgae isolated namely *Asterococcus limneticus* with length of 10.2 um and width of 22.3 um, *Nostoc* sp. with length of 4.6 um and 4.1 um and two (2) species of Genus *Anabaena* with length ranged from 6.1-6.9 um and both width of 4.6 um. With regards to Station 4, new species of microalgae were observed and isolated. These are *Chlorococcum humicola* with length and width of 11.5 and 6.9 um; *Stigeoclonium attenuatum* with length and width of 22.3 and 5.7 um and lastly *Synechococcus* sp. with length and width of 8.2 and 5.6 um. In Station 5, there are four (4) micro algal species where two (2) of them are new isolates in the said station namely *Chlorella ellipsoidea* (length=8.1; width= 6.3um) and *Chroococcus limneticus* (length=8.3; width=6.1um) and two (2) were already found at Station 2 and 4 namely *Nostoc* sp. and *Chlorococcum humicola* respectively. Furthermore, at Station 7, three (3) microalgae were examined such as *Chlorella vulgaris* (length=7.2; width=5.9 um) which is first time to be isolated in the said station, *Asterococcus limneticus*

233 and *Synechococcus* sp. *A. limneticus* and *Synechococcus* sp. were already isolated at
 234 Station 2 and 4. Moreover, no new isolates were observed at Station 8. The three (3)
 235 isolates found were the following: *Chlorella vulgaris*; *Anabaena azollae* and *Synechococcus*
 236 sp. In addition at Station 10, three (3) isolates were surveyed, two (2) of them are new
 237 isolates namely *Gloeocystis ampla* (length=10.1; width=9.2 μ m) and *Oscillatoria* sp.
 238 (length=25.4; width= 3.4 μ m) and one (1) microalgae, *Chlorococcum humicola* was already
 239 isolated at Stations 4 and 5. Uniquely, in Station 11, the three (3) microalgae observed were
 240 new isolates namely *Chroococcus disperses* (length=3.5; width=4.3 μ m), *Fragilaria*
 241 *brevistrata* (length=41.1; width=4.6 μ m) and *Navicula* sp. (length=10.3; width=6.5 μ m). In
 242 another scenario, three (3) species of phytoplankton were also isolated at Station 13 where
 243 in two (2) of them were first time to be studies and one (1) microalgae, *Gloeocystis ampla*
 244 was already observed at the previous stations discussed. The two (2) microalgae are
 245 *Chlorococcum infusionum* (length=12.8; width=6.8 μ m) and *Navicula cincta* (length=9.1;
 246 width=7.1 μ m). Lastly, Station 14 has four (4) isolates but three (3) of the microalgae were
 247 already examined from the previous stations and one (1) is a new isolate namely, *Hormidium*
 248 *klebsii* (length=25.4; width=3.4 μ m). From the above discussion, most of the stations have
 249 one (1) new micro algal isolates scrutinized. The most observed phytoplankton were
 250 *Chlorococcum humicola* and *Synechococcus* sp. found in 3-4 stations. These microalgae
 251 belong to Phylum Chlorophyta and Cyanophyta respectively. Species of Phylum
 252 Bacillariophyta were also noted but most of them are found in only one (1) station.
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Table 4. Qualitative Analysis on the Microalgae isolated from the freshwater stations.

Station No.	Name of the Freshwater Ecosystem	Name of Microalgae	Qualitative			
			Color/Pigmentation	Shape	Arrangement	Unique Trait/s
2	Stream, Brgy Libertad, Capocan	<i>Asterococcus limneticus</i> G.M. Smith	green	spherical	colonial	contains pyrenoids
		<i>Nostoc</i> sp.	blue-green	cells are globose	trichomes are chain-like	more akinetes
		<i>Anabaena</i> sp.	blue-green	cells are ellipsoid	trichomes are coiled	contents are granular
4	Lindog River, Brgy. Uyawan, Carigara	<i>Anabaena azollae</i> Strasburger	blue-green	cells are subglobose	trichomes are coiled	contents are granular
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	Green	spherical	solitary	lateral notch and single pyrenoid
		<i>Stigeoclonium attenuatum</i> (Hazen) Collins	Green	cells are cylindrical	filaments are branched and elongated	prostrate portion of thallus little developed
		<i>Synechococcus</i> sp.	blue-green	oblong	solitary	no sheath

5	Bislig River, Brgy. Bislig, Carigara	<i>Chlorella ellipsoidea</i> Gerneck	Green	ellipsoidal	solitary	chloroplast folded over the cells
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	Green	spherical	solitary	lateral notch and single pyrenoid
		<i>Nostoc</i> sp.	blue-green	cells are globose	trichomes are chain-like	more akinetes
		<i>Chroococcus limneticus</i> Lemmermann	Green	ovate	colonial	with mucilaginous envelopes
7	Himanglos River, Brgy. Hilaba, Barugo	<i>Chlorella vulgaris</i> Beyerinck	Green	spherical	solitary	chloroplast like a parietal cup
		<i>Asterococcus limneticus</i> G.M. Smith	Green	spherical	colonial	contains pyrenoids
		<i>Synechococcus</i> sp.	blue-green	oblong	solitary	no sheath
8	Canomantag River, Brgy. Canomantag, Barugo	<i>Chlorella vulgaris</i> Beyerinck	Green	spherical	solitary	chloroplast like a parietal cup
		<i>Anabaena azollae</i>	blue-green	cells are subglobose	trichomes are coiled	contents are granular
		<i>Synechococcus</i> sp.	blue-green	oblong	solitary	no sheath
10	Caray-caray River, Brgy. Caray-caray, San Miguel	<i>Gloeocystis ampla</i> Kutzing	Green	ovoid	colonial	embedded in unlamellated gelatinous envelopes
		<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	Green	spherical	solitary	single pyrenoid
		<i>Oscillatoria</i> sp.	blue-green	elongated	filamentous	distinct sheath-like called calyptra
11	Lipasan Falls, Brgy. Pinarigusan, San Miguel	<i>Chroococcus dispersus</i> (Keissler) Lemmermann	green	ovate	colonial	with mucilaginous envelopes
		<i>Fragilaria brevivtrata</i>	brown	linear	solitary	striae are short

		Grun				
		<i>Navicula</i> sp.	brown	linear to elliptic	solitary	striations are transverse
13	Tula-an Falls, Brgy. Tula-an, Babatngon	<i>Gleocystis ampla</i> (Kuetz) Lagerheim	green	ovoid	colonial	embedded in unlamellated gelatinous envelopes
		<i>Chlorococcum infusionum</i> (Schrank) Meneghini	green	spherical	colonial	notch on one side and single pyrenoid
		<i>Navicula cincta</i> (Ehrenberg) Ralfs	brown	linear	solitary	striations are transverse
14	Busay Falls, Brgy. Busay, Babatngon	<i>Chlorococcum humicola</i> (Nageli) Rabenhorst	green	spherical	solitary	single pyrenoid
		<i>Fragilaria brevisstrata</i> Grun	brown	linear	solitary	striae are short
		<i>Hormidium klebsii</i> G.M Smith	green	cells are cylindrical	unbranched filaments	Chloroplast a parietal plate
		<i>Oscillatoria</i> sp.	blue-green	elongated	filamentous	distinct sheath-like called calyptra

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For Table 3, a thorough discussion on the morphological characteristics of the 18 micro algal isolates that were studied in the 9 freshwater tributaries of Carigara bay. Most of the isolates are chlorophytes (green) and cyanophytes (blue-green) and few are bacillariophytes (brown algae). The brown algae are the following: *Fragilaria brevisstrata*, *Navicula cincta* and *Navicula* sp. These microalgae are linear to oblong, solitary in nature and have striations which are short and transverse. Furthermore, the blue-green algae are *Nostoc* sp., *Synechococcus* sp., *Oscillatoria* sp. and two (2) species of Genus *Anabaena*. The *Nostoc* sp. and *Anabaena* spp. are ellipsoidal to globose, with trichomes which are chain-like and coiled and with akinetes. On the other hand, *Synechococcus* sp. are solitary and oblong and *Oscillatoria* sp. are filamentous and elongated in nature. The difference of this 2 species is the absence and presence of sheath respectively. Lastly, the green algae has the highest in number in which seven (7) genera were isolated and studied. These are *Asterococcus limneticus*, *Chlorococcum* spp. and *Chlorella* spp. which are all spherical and the first species is colonial and the two (2) latter species are solitary in nature. In addition, *Stigeoclonium attenuatum* and *Hormidium klebsii* are also observed in which their cells are cylindrical and filaments are elongated but differ in terms of the unbranching and branching of their filaments respectively. Finally, *Chroococcus* spp. and *Gleocystis ampla* which are ovate and colonial but different in the contents of their envelopes which are mucilaginous

274 envelopes for the foremost species and lammellated gelatinous envelope for the latter
275 species.

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278 4. CONCLUSION

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280 Carigara Bay and its freshwater resources are bounded with different micro algal
281 species. Phyla Bacillariophyta, Chlorophyta and Cyanophyta are 44, 37 and 19% of the total
282 phytoplankton identified. Furthermore, there was a significant positive correlation between
283 water pH ($r = 0.499$; $p = 0.001$), conductivity ($r=0.519$; $p=0.001$) and amount of phosphates
284 ($r = 0.446$; $p = 0.003$) and a negative relationship between the amount of dissolved oxygen
285 (Mean= 7.76; SD= 1.82) and total number of micro algal cells, $r=-0.351$; $p=0.023$. Moreover,
286 six (6) genera namely *Asterococcus*, *Anabaena*, *Nostoc*, *Chlorococcum*, *Synechococcus*
287 and *Oscillatoria* were isolated, cultured and semi-mass produced for optimization procedure.

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300 REFERENCES

301

- 302 Anderson, P & Alford, A.B. (2014). Ghost fishing activity in derelict blue crab traps in
303 Louisiana. *Mar. Pollut. Bull.*, 79, 261-267.
- 304 Anemaet, I., Bekker, M., Hellingwerf, K.J. (2010). Algal photosynthesis as the primary driver
305 for a sustainable development in energy, feed and food production. *Mar Biotechnol.*,
306 12, 619-629.
- 307 Becker, W. (2004). Microalgae in human and animal nutrition. In book: Handbook of
308 Microalgal Culture: Biotechnology and Applied Phycology, 312 – 351. doi:
309 [10.1002/9780470995280.ch18](https://doi.org/10.1002/9780470995280.ch18).
- 310 Chen, X., He, G., Deng, Z., Wang, N., Jiang, W., Chen, S. (2014). Screening Of Microalgae
311 For Biodiesel Feedstock. *Advances in Microbiology. Scientific Research.* 4, 365-376.
- 312 Duong, V.T., Li, Y., Nowak, E., Schenk, P.M. (2012). Microalgae Isolation and Selection for
313 Prospective Biodiesel Production. *Energies*, 5, 1835-1849. doi:10.3390/en5061835.
- 314 Feng, J., Guo, Y., Zhang, X., Wang, G., Lv, J., Liu, Q., Xie, S. (2016). Identification and
315 Characterization of a symbiotic alga from soil bryophyte for lipid profiles. *The*
316 *Company of Biologists*, 5, 1317-1323. doi: 10.1242/bio.019992.
- 317 Ferdowshi, Z. (2013). Screening of fresh water microalgae and Swedish pulp and paper mill
318 waste waters with the focus on high algal biomass production. Master's Thesis.
319 Department of Chemical and Biological Engineering. Industrial Biotechnology
320 Research Group. Chalmers University of Technology. Gothenburg, Sweden.
- 321 Ibanez, E., Herrero, M., Mendiola, J.A., Castro-Puyana, M. (2012). Extraction and
322 Characterization of Bioactive Compounds with Health Benefits from Marine
323 Resources: Macro and Micro Algae, Cyanobacteria and Invertebrates. *Marine*
324 *Bioactive Compounds: Sources, Characterization and Applications.* M. Hayes (ed).
325 Springer Science. doi. 10.1007/978-1-4614-1247-2_2.

326 Karthikeyan, S. (2012). A critical review: microalgae as a renewable source for biofuel
327 production. *International Journal of Engineering Research and Technology*, 1, 1-6.
328 Kaushik, P. & Chauhan, A. (2008). In vitro antibacterial activity of laboratory grown culture of
329 *Spirulina platensis*. *Indian J Microbiol.*, 48, 348-352.
330 Kovac, D.J., Simeunovic, J.B., Babic, O.B., Misan, A.C., Milovanovic, I.L. (2013). Algae in
331 Food and Feed. Review Article. *Food and Feed Research*, 40(1), 21-31.
332 Makabenta, E.T. Jr. (1995). Carigara. Published by Carigara 400, Inc. ISBN 971-91575-0-X.
333 Meena, P. (2013). Natural bioactive compounds and their characterization isolated from
334 terrestrial plants and marine algae. *Microbial pathogens and strategies for*
335 *combating them: Science, Technology and Education*. A. Mendez-Vilas, Ed.
336 Formatex. 1181-1187.
337 Nicoletti, M. (2016). Microalgae Nutraceuticals. A Review. *MDPI. Foods*, 5(54), 1-13.
338 doi.10.3390/foods5030054.
339 Olaizola, M. (2003). Commercial Development of microalgal biotechnology: from the test
340 tube to the marketplace. *Biomolecular Engineering*, 20, 459-466.
341 Park, J.H., Yoon, J.J., Park, H.D., Kim, Y.J., Lim, D.J. et. al. (2011). Feasibility of
342 biohydrogen production from *Gelidium amansii*. *Int. J. Hydrogen Energ.*, 36, 13997-
343 14003.
344 Richmond, A. (2004). Basic Culturing Techniques and Downstream processing of cellmass
345 and products. Handbook of microalgal culture: Biotechnology and Applied
346 Phycology. Blackwell Science Ltd.
347 Raja, R., Hemaiswarya, S., Ashok, K.N., Sridhar, S., Rengasamy, R. (2008). A perspective
348 on the biotechnological potential of microalgae. *Crit. Rev. Microbiol.*, 34, 77-88.
349 Santos, R.A.V., Pabiling, R.R., Granili, J., Aguilon, N. (1999). Water quality assessment in
350 Carigara Bay. University Library. University of the Philippines, Los Baños, Laguna.
351 Sathasivam, R., Radhakrishnan, R., Hashem, A., Abd_Allah, E. (2017). Microalgae
352 metabolites: A rich source for food and medicine. *Saudi Journal of Biological*
353 *Sources*. doi.org/10.1016/j.sjbs.2017.11.003.
354 Ugoala, E., Ndukwe, G.I., Mustapha, K.B., Ayo, R.I. (2012). Constraints to large scale algae
355 biomass production and utilization. *Journal of Algal Biomass Utilization*, 3(2), 14-32.
356 Voort, M. de, Vulsteke, E. and Visser, C. de. (2014). Macro-economics of algae. EnAlgae
357 report WP2A7.01. Public Output. 1-49.