Original Research Article

ISOLATION, CULTURE, SEMI-LARGE PRODUCTION OF SELECTED MICRO ALGAL SPECIES FROM FRESHWATER TRIBUTARIES OF CARIGARA BAY, LEYTE

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.

Keywords: freshwater tributaries, biodiversity, semi-large scale cultures

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 feed providing an initiative revenue for algae industry even microalgae cultivation is only a few decades old (Anemaet et al., 2010). However, adequate studies on the suitability of these microalgae as animal feed are limited (Olaizola 2003; Sathasivam et al., 2017). 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 (Feng et al., 2016). Thus this study on the identification and assessment of microalgae in Carigara Bay and freshwater resources in its vicinity, situated at the five (5) towns surrounding the bay would serve as baseline information for future studies.

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

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The island of Leyte where Carigara Bay is sited and connected with freshwater tributaries, is a sister island of Samar and one of the ten (10) biggest land masses in the country. Sampling stations in the area were determined in coordination with Dr. Paciente A. Cordero, Jr. with the supervision of the faculty members and staff of the College of Fisheries at Eastern Visayas State University, Carigara Campus. Fourteen (14) stations were established at the upper coastal towns adjoining the Carigara Bay. Station 1 was the Carigara Bay at Brgy. Libertad (Capoocan); Station 2 was a stream at Brgy. Libertad (Capoocan); Station 3 Carigara Bay at Brgy. Visoria (Carigara); Station 4 was the Lindog River at Brgy. Uyawan (Carigara); Station 5 was the Bislig River at Brgy Bislig (Carigara); Station 6 was the Carigara Bay at Brgy. Duka (Barugo); Station 7 was the Himanglos River at Brgy. Hilaba (Barugo); Station 8 was the Canomantag River at Brgy. Canomantag (Barugo); Station 9 was the Carigara Bay at Brgy. Mawod-pawod (San Miguel); Station 10 was the Caraycaray River at Brgy. Caraycaray (San Miguel); Station 11 was the Lipasan Falls at Brgy. Pinarigusan (San Miguel); Station 12 was the Carigara Bay at Brgy. Kalangawan Guti (Babatngon); Station 13 was the Tula-an Falls at Brgy. Tula-an (Babatngon) and Station 14 was the Busay Falls at Brgy. Busay (Babatngon). The water samples were collected from July 2-8, 2018 which was considered as the 1^{st} sampling and from November 23-26, 2018 which was the 2^{nd} sampling. Collection of water samples from the bay was done by towing 30 meters away from the shoreline. The water samples from the bay were gathered 30 meters away from the shoreline with a depth of 1-1.5 meters only. The water samples from the bay were used for phytoplankton quantification analysis only. For 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, therefore, ideal for integrated sampler. Depths were measured using the plexi glass sampler built in with improvised measurement in meters and/or the secchi disk. The three (3) L of water samples from the freshwater ecosystems were divided from which the 1 L was taken and fixed with Lugol's solution to preserve the cell wall; 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 for *ex situ* nutrient determination of the water samples such as nitrate and phosphates and the other one (1) L was used as a live sample for the isolation of microalgae. Furthermore, prior to collection physical parameters such as surface water temperature, column depth, light penetration or transparency, and chemical parameters such as water pH, conductivity and dissolved oxygen were recorded in situ. All of the parameters were measured using the Xplorer GLX (PAASCO) water quality sensor except for the light penetration or transparency which was determined using a Secchi disc. In addition analysis of nitrate and phosphorus were also done through the Hach DR/2010 phosphate data logging spectrophotometer at the Roque Laboratory, Old Graduate School, University of Santo Tomas.

In order to quantify, 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 (Martinez, 1976; Baldia, 1992; Lee et. al., 2014). For quantification, an appropriate amount was was loaded in a Neubauer Counting Chamber for density determination as viewed using the Olympus CH20 compound light microscope. (Martinez et. al., 1975). The species were identified using several references on micro algal taxonomy and biodiversity. For the isolation, Binangonan Research Station Pantastico medium (BRSP) was used for the multiple streaking technique. 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 ± 2^0 C, pH of 7.27 and light intensity of 3,000 lx. Six (6) isolates were selected for semi-mass production and large scale cultivation.

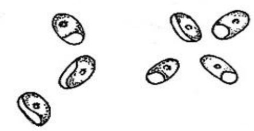
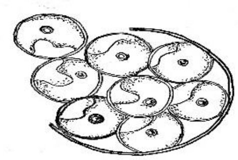
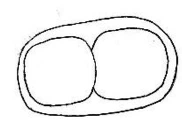
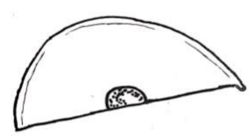
3. RESULTS AND DISCUSSION

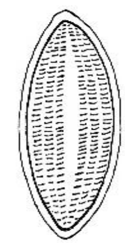
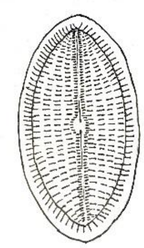
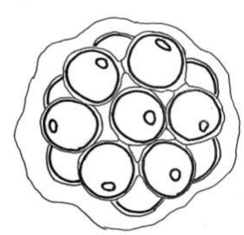
Biodiversity of Phytoplankton

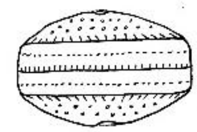
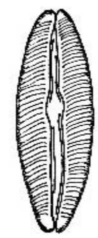
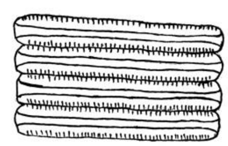
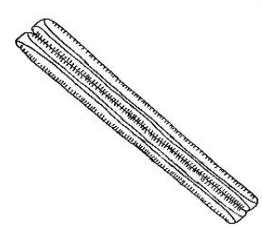
The variety of biological organisms is attributed by biotic and abiotic factors obtained in the study sites. These are the physico-chemical parameters and the type of ecological features which are bounded in the sampling areas in order to understand and explore the variety of living organisms present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

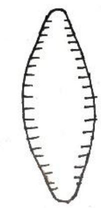
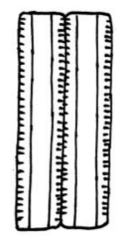
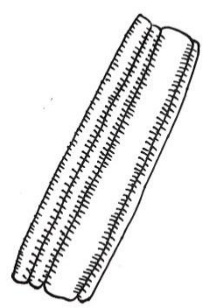
The following are the Genus identified and quantified in Carigara Bay and its freshwater tributaries. For Phylum Bacillariophyta, these are *Cocconeis* (Her.) Grun., 1868; *Coscinodiscus* Her., 1838 *Fragilaria* Lyng., 1819., emend, Rabenh., 1864; *Synedra* Ehrenberg, 1830; *Navicula* Bory, 1822 erend,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

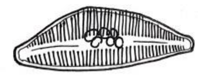
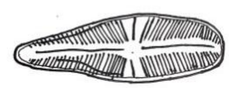
Table 1. List of all phytoplankton species with illustrations

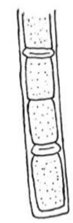
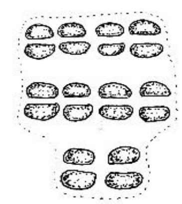
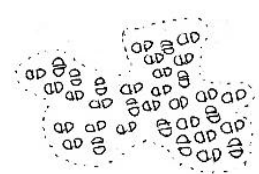
Species	Division	Size	Illustration
Chlorella ellipsoidea	**Chlorophyta**	Length: 9.2 um Width: 6.9 um	
Chlorella vulgaris	**Chlorophyta**	Length: 9.2 um Width: 9.2 um	
Chroococcus sp.	**Cyanophyta**	Length: 6-15 um Width: 9.6 um	
Closteridium sp.	**Chlorophyta**	Length: 96.6 um Width: 36.8 um	

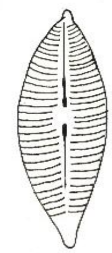
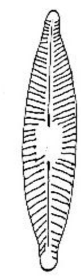
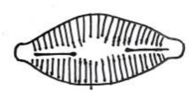
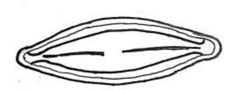
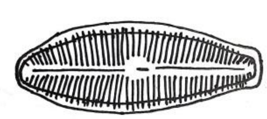
Closterium sp	**Chlorophyta**	Length: 48.3 um Width: 9.2 um	
Cocconeis placentula	**Bacillariophyta**	Length: 32.2 um Width: 13.8 um	
Cocconeis sp. (2)	**Bacillariophyta**	Length: 46 um Width: 27.6 um	
Coelastrum sp.	**Chlorophyta**	Length: 18.4 um Width: 11.5 um	
Coscinodiscus sp.	**Bacillariophyta**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

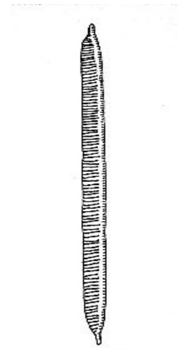
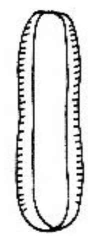
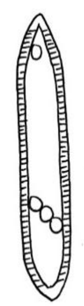
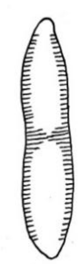
			
Cymbella sp.	**Bacillariophyta**	Length: 69 um Width: 25.3 um	
Diploneis sp. (1)	**Bacillariophyta**	Length: 39.1 um Width: 16.1 um	
Fragilaria sp.(1)	**Bacillariophyta**	Length: 41.4 um Width: 4.6 um	
Fragilaria sp. (2)	**Bacillariophyta**	Length: 59.9 um Width: 25.4 um	

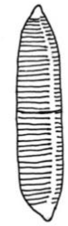
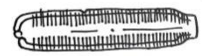
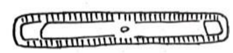
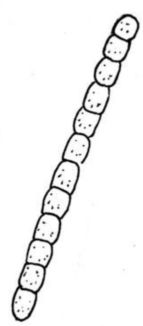
Fragilaria sp.(3)	**Bacillariophyta**	Length: 52.2 um Width: 18.4 um	
Fragilaria sp. (4)	**Bacillariophyta**	Length: 211.6 um Width: 9.2 um	
Fragilaria sp. (5)	**Bacillariophyta**	Length: 39.4 um Width: 3.9 um	
Fragilaria sp. (6)	**Bacillariophyta**	Length: 52.9 um Width: 11.5 um	

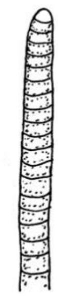
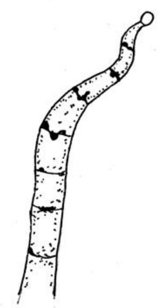
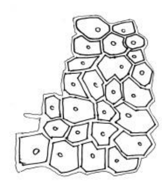
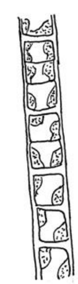
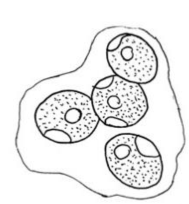
Gomphonema sp. (1)	**Bacillariophyta**	Length: 36.8 um Width: 11.5 um	
Gomphonema sp. (2)	**Bacillariophyta**	Length: 36.8 um Width: 11.5 um	
Gomphonema sp. (3)	**Bacillariophyta**	Length: 41.4 um Width: 13.8 um	
Lyngbya sp.(1)	**Cyanophyta**	Length: 32.2 um Width: 9.2 um	

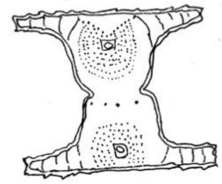
Lyngbya sp.(2)	**Cyanophyta**	Length: 87.4 um Width: 9.2 um	
Melosira sp.	**Bacillariophyta**	Length: 18.4 um Width: 9.2 um	
Merismopedia sp.(1)	**Cyanophyta**	Length: 103.5 um Width: 11.5	
Merismopedia sp.(2)	**Cyanophyta**	Length: 69 um Width: 13. 8 um	
Navicula sp.(1)	**Bacillariophyta**	Length: 55.2 um Width: 23 um	

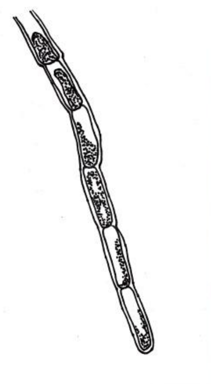
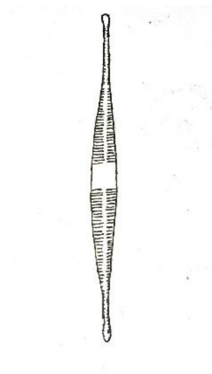
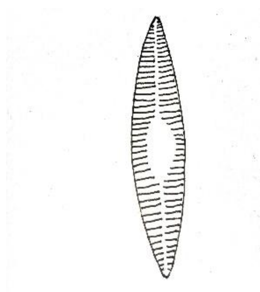
Navicula sp.(2)	**Bacillariophyta**	Length: 23 um Width: 18.4 um	
Navicula sp.(3)	**Bacillariophyta**	Length: 46 um Width: 9.2 um	
Navicula sp.(4)	**Bacillariophyta**	Length: 39.1 um Width: 11.5 um	
Navicula sp.(5)	**Bacillariophyta**	Length: 55.2 um Width: 16.1 um	
Neidium sp.	**Bacillariophyta**	Length: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

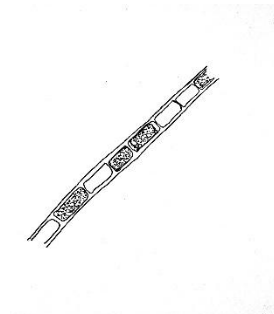
Nitszchia sp.(1)	**Bacillariophyta**	Length: 105.8 um Width: 9.2 um	
Nitszchia sp.(2)	**Bacillariophyta**	Length: 78.2 um Width: 20.7 um	
Nitszchia sp. (3)	**Bacillariophyta**	Length: 78.2 um Width: 13.8 um	
Nitszchia sp. (4)	**Bacillariophyta**	Length: 59.8 um Width: 13. 8 um	

Nitszchia sp. (5)	**Bacillariophyta**	Length: 46 um Width: 18.4 um	
Nitszchia sp. (6)	**Bacillariophyta**	Length: 59.8 um Width: 13.8 um	
Nitszchia sp. (7)	**Bacillariophyta**	Length: 115 um Width: 13.8 um	
Nostoc sp.	**Cyanophyta**	Length: 8 um Width: 6.7 um	
Oscillatoria sp.(1)	**Cyanophyta**	Length: 41.4 um Width: 2.3 um	

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

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

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

Ulothrix sp.	**Chlorophyta**	Length: 18.4 um Width: 9.2 um	

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.

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, ^{o}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

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

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

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

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	*Asterococcus limneticus* G.M. Smith	10.2 um	22.3 um
		Nostoc sp.	4.6 um	4.1 um
		Anabaena sp.	6.9 um	4.6 um
		Anabaena azollae Strasburger	6.1 um	4.6 um
4	Lindog River, Brgy. Uyawan, Carigara	*Chlorococcum humicola* (Nageli) Rabenhorst	11.5 um	6.9 um
		Stigeoclonium attenuatum (Hazen) Collins	22.3 um	5.7 um
		Synechococcus sp.	8.2 um	5.6 um
5	Bislig River, Brgy. Bislig, Carigara	*Chlorella ellipsoidea* Gerneck	8.1 um	6.3 um
		Chlorococcum humicola (Nageli) Rabenhorst	10.3 um	6.5 um
		Nostoc sp.	4.6 um	4.1 um
		Chroococcus limneticus Lemmermann	8.3 um	6.1 um
7	Himanglos River, Bgry. Hilaba, Barugo	*Chlorella vulgaris* Beyerinck	7.2 um	5.9 um
		Asterococcus limneticus G.M.	10.2 um	22.3 um

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

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

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, Capoocan	*Asterococcus limneticus* G.M. Smith	green	spherical	colonial	contains pyrenoids
		Nostoc sp.	blue-green	cells are globose	trichomes are chain-like	more akinetes
		Anabaena sp.	blue-green	cells are ellipsoid	trichomes are coiled	contents are granular
		Anabaena azollae Strasburger	blue-green	cells are subglobose	trichomes are coiled	contents are granular
4	Lindog River, Brgy. Uyawan, Carigara	*Chlorococcum humicola* (Nageli) Rabenhorst	Green	spherical	solitary	lateral notch and single pyrenoid
		Stigeoclonium attenuatum (Hazen) Collins	Green	cells are cylindrical	filaments are branched and elongated	prostrate portion of thallus little developed
		Synechococcus sp.	blue-green	oblong	solitary	no sheath

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

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

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 brevistrata, 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 envelopes for the foremost species and lammellated gelatinous envelope for the latter species.

4. CONCLUSION

Carigara Bay and its freshwater resources are bounded with different micro algal species. 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) and 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. Moreover, six (6) genera namely *Asterococcus, Anabaena, Nostoc, Chlorococcum, Synechococcus* and *Oscillatoria* were isolated, cultured and semi-mass produced for optimization procedure.

REFERENCES

Anderson, P & Alford, A.B. (2014). Ghost fishing activity in derelict blue crab traps in Louisiana. *Mar. Pollut. Bull., 79,* 261-267.

Anemaet, I., Bekker, M., Hellingwerf, K.J. (2010). Algal photosynthesis as the primary driver for a sustainable development in energy, feed and food production. *Mar Biotechnol., 12,* 619-629.

Becker, W. (2004). Microalgae in human and animal nutrition. In book: Handbook of Microalgal Culture: Biotechnology and Applied Phycology, 312 – 351. doi: 10.1002/9780470995280.ch18.

Chen, X., He, G., Deng, Z., Wang, N., Jiang, W., Chen, S. (2014). Screening Of Microalgae For Biodiesel Feedstock. Advances in Microbiology. Scientific Research. 4, 365-376.

Duong, V.T., Li, Y., Nowak, E., Schenk, P.M. (2012). Microalgae Isolation and Selection for Prospective Biodiesel Production. *Energies, 5*, 1835-1849. doi:10.3390/en5061835.

Feng, J., Guo, Y., Zhang, X., Wang, G., Lv, J., Liu, Q., Xie, S. (2016). Identification and Characterization of a symbiotic alga from soil bryophyte for lipid profiles. *The Company of Biologists, 5,* 1317-1323. doi: 10.1242/bio.019992.

Ferdowshi, Z. (2013). Screening of fresh water microalgae and Swedish pulp and paper mill waste waters with the focus on high algal biomass production. Master's Thesis. Department of Chemical and Biological Engineering. Industrial Biotechnology Research Group. Chalmers University of Technology. Gothenburg, Sweden.

Ibanez, E., Herrero, M., Mendiola, J.A., Castro-Puyana, M. (2012). Extraction and Characterization of Bioactive Compounds with Health Benefits from Marine Resources: Macro and Micro Algae, Cyanobacteria and Invertebrates. Marine Bioactive Compounds: Sources, Characterization and Applications. M. Hayes (ed). Springer Science. doi. 10.1007/978-1-4614-1247-2_2.

Karthikeyan, S. (2012). A critical review: microalgae as a renewable source for biofuel production. *International Journal of Engineering Research and Technology, 1*, 1-6.

Kaushik, P. & Chauhan, A. (2008). In vitro antibacterial activity of laboratory grown culture of *Spirulina platensis. Indian J Microbiol., 48,* 348-352.

Kovac, D.J., Simeunovic, J.B., Babic, O.B., Misan, A.C., Milovanovic, I.L. (2013). Algae in Food and Feed. Review Article. *Food and Feed Research, 40*(1), 21-31.

Makabenta, E.T. Jr. (1995). Carigara. Published by Carigara 400, Inc. ISBN 971-91575-0-X.

Meena, P. (2013). Natural bioactive compounds and their characterization isolated from terrestrial plants and marine algae. *Microbial pathogens and strategies for* *combating them: Science, Technology and Education.* A. Mendez-Vilas, Ed. Formatex. 1181-1187.

Nicoletti, M. (2016). Microalgae Nutraceuticals. A Review. *MDPI. Foods, 5*(54), 1-13. doi.10.3390/foods5030054.

Olaizola, M. (2003). Commercial Development of microalgal biotechnology: from the test tube to the marketplace. *Biomolecular Engineering, 20,* 459-466.

Park, J.H., Yoon, J.J., Park, H.D., Kim, Y.J., Lim, D.J. et. al. (2011). Feasibility of biohydrogen production from *Gelidium amansii. Int. J. Hydrogen Energ., 36,* 13997-14003.

Richmond, A. (2004). Basic Culturing Techniques and Downstream processing of cellmass and products. Handbook of microalgal culture: Biotechnology and Applied Phycology. Blackwell Science Ltd.

Raja, R., Hemaiswarya, S., Ashok, K.N., Sridhar, S., Rengasamy, R. (2008). A perspective on the biotechnological potential of microalgae. *Crit. Rev. Microbiol., 34*, 77-88.

Santos, R.A.V., Pabiling, R.R., Granili, J., Aguilon, N. (1999). Water quality assessment in Carigara Bay. University Library. University of the Philippines, Los Baños, Laguna.

Sathasivam, R., Radhakrishnan, R., Hashem, A., Abd_Allah, E. (2017). Microalgae metabolites: A rich source for food and medicine. *Saudi Journal of Biological Sources*. doi.org/10.1016/j.sjbs.2017.11.003.

Ugoala, E., Ndukwe, G.I., Mustapha, K.B., Ayo, R.I. (2012). Constraints to large scale algae biomass production and utilization. *Journal of Algal Biomass Utilization, 3*(2), 14-32.

Voort, M. de, Vulsteke, E. and Visser, C. de. (2014). Macro-economics of algae. EnAlgae report WP2A7.01. Public Output. 1-49.