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RESEARCH ARTICLE

Correlation of Chlorophyll-a and Dissolved Oxygen in the Phytoplankton Abundance in Leyte Gulf, Philippines

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ABSTRACT

Leyte Gulf is an important fishing ground in the Philippines that serves as a livelihood and food source for many coastal communities. This study aimed to determine the composition, abundance, and diversity of phytoplankton, examine the chlorophyll-a (chl-a) and dissolved oxygen (DO) concentration and distribution, and correlate the results of phytoplankton abundance to the examined water parameters in the Leyte Gulf. Samples were collected at 20 established oceanographic stations from April 24 to May 8, 2020, using 10 liters (L) of Niskin bottles at a depth of 10 meters (m), 25 m, 50 m, and 75 m. Phytoplankton were categorized into three classes, namely diatoms, dinoflagellates, and blue-green algae. Results of this study showed that diatoms were the most dominant class comprising about 89% abundance. Of all the identified phytoplankton taxa, *Leptocylindrus spp., Rhizosolenia spp.,* and *Coscinodiscus* spp. were the most abundant. *Leptocylindrus* spp. was found to have a higher density in all sampling stations. Based on the diversity index and density-depth analysis, the phytoplankton abundance has a similar distribution in Leyte Gulf. A high concentration of phytoplankton abundance in the Leyte group was observed, wherein a high concentration of chl-a and DO occurred. Additionally, the status of water parameters throughout the study area was tolerable. Results further revealed that the relationship between the chl-a and DO was strongly positively correlated to phytoplankton. Therefore, continued monitoring of phytoplankton and other important water parameters within the gulf must be done.

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1. INTRODUCTION

Phytoplankton serves as primary producers and primary source of food for pelagic herbivores, including fish larvae, and supports organisms at a higher trophic level in the marine food webs (Tweddle et al. 2018; Kim et al. 2020). Tweddle et al. (2018) and Dacayana et al. (2015) added that phytoplankton contributes about 90% of the primary production and supports commercial fisheries. Additionally, changes in fish production are strongly relatable to the changes in phytoplankton production (Blanchard et al. 2012). Therefore, the abundance, composition, biomass, distribution, and frequency of phytoplankton are vital in understanding primary and export production, food web dynamics, and the ecological status of water (Garmendia et al. 2013; Menden-Deuer et al. 2020). Phytoplankton is being used as an ecological indicator to provide information on fisheries' status in a particular area (Lacuna et al. 2012). Furthermore, using the physical and chemical parameters of water to assess water quality can determine the productivity and sustainability of waters (Brillante and Masagca 2018).

Chlorophyll-a (Chl-a) concentration is widely recognized as a substitute for measuring phytoplankton biomass to estimate the rate of primary production and is used in eutrophication studies (Huot et al. 2007; Carneiro et al. 2014; Davies et al. 2018). Chl-a concentration is the usual ecological tool for managing water quality in an aquatic ecosystem (Carneiro et al. 2014). It is an indicator of phytoplankton biomass and an index of biomass of primary producers (Boyer et al. 2009; Davies et al. 2018). On the other hand, water's dissolved oxygen (DO) concentration is an essential factor in the quality of habitat for fish (Franklin 2014). According to Zhao et al. (2009), DO is an attribute to measure water quality. Moreover, DO is indispensable as it controls biological productivity in an aquatic ecosystem (Prasad et al. 2014).

Various studies on phytoplankton assessment have been published globally; however, studies on phytoplankton and chemical parameters of water in the Philippines, particularly in the Leyte Gulf, are minimal and focused only on San Pedro Bay, Leyte (Yap-Dejeto et al. 2016). Thus, this study was conducted to assess the phytoplankton in the waters of Leyte Gulf. Specifically, it aimed to determine the composition, abundance, and diversity of phytoplankton in Leyte Gulf, examine the chl-a and DO concentration and distribution, and correlate the results of phytoplankton densities to the examined water parameters in the gulf. The study's results could be a reference to the phytoplankton status and productivity in the gulf and a basis for formulating policy in the region.

2. MATERIALS AND METHODS

2.1. Study area

An oceanographic survey was conducted at Leyte Gulf from April 24 to May 8, 2020, with the M/V DA-BFAR vessel as a platform. Twenty (20) oceanographic sampling stations were established in the study area located between the coordinates 10°32'055" to 11°03'541" N latitude and 125°03'182" to 125°39'007" E longitude. Sampling stations were divided into three groups: Leyte, Samar, and the Central part of the gulf (Figure 1; Table 1).

2.2. Sample collection and processing

2.2.1. Phytoplankton

Water samples were collected manually using 10 liters (L) Niskin bottles attached to Conductivity, Temperature, and Depth (CTD) deployed to 10 meters (m), 25 m, 50 m, and 75 m depth. Samples for phytoplankton analysis were taken using 1 L polyethylene bottles covered with dark film. Water samples were sieved through a 20 micrometer (µm) bolting silk, transferred into 20 milliliters (ml) vials, and preserved with 1 ml of 10% formaldehyde solution and borax solution for further analysis. One (1) ml of water sample was placed in a Sedgewick rafter for cell counting. Identification of samples was made in the laboratory using an inverted microscope with a 20x magnification objective of Nikon Eclipse TS100. Phytoplankton were identified at the lowest possible taxon whenever possible with the aid of the phytoplankton guidebook of Takuo et al. (2012). Phytoplankton was categorized into three classifications, namely diatoms (Class Bacillariophyceae), dinoflagellates (Class Dinophyceae), and blue-green algae (Class Cyanophyceae). All sampling procedure for phytoplankton was based on Furio and Gonzales (2002).

2.2.2. Chlorophyll-a and dissolved oxygen

Samples for chl-a and DO collected from different depths were transferred to 1 L polyethylene

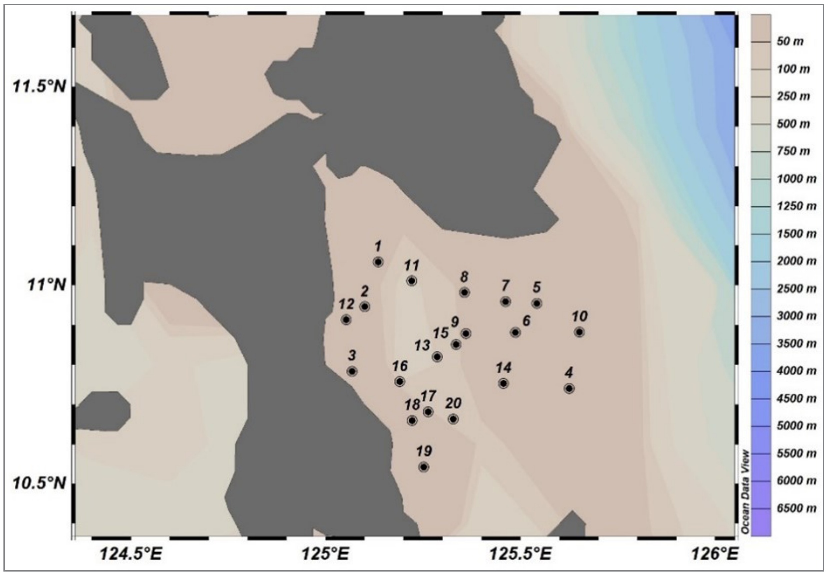


Figure 1. Map of Leyte Gulf showing the location of 20 established oceanographic sampling stations.

Table 1. Different groups of sampling stations in Leyte Gulf

Group	Vicinity (Station)
Leyte	Tolosa (1), Dulag (2 &12), Abuyog (3, 16, 17, 18 & 20), and Silago (19)
Samar	Manicani Island (5 & 10), Giporlos (7), Balangiga (8), and Marabut (11)
Center	Homonhon Island (4 &14) and central part (6, 9, 13 & 15)

bottles and BOD bottles, respectively. Samples for dissolved oxygen analysis have two replicates in each sampling depth. All collected water samples were brought to the laboratory for initial processing.

Chl-a samples were filtered with a 0.45 µm pore size filter membrane. While filtering, 3-5 drops of Magnesium Carbonate ($MgCO3$) were added to prevent acidity in the filter membrane. Filter membranes were then covered with foil to avoid direct light penetration and kept in the freezer for further analysis. Each filter membrane was placed inside a test tube, dissolved with 7 ml 90% acetone, and then stored in the refrigerator overnight. Initially, processed filter membranes were placed in the centrifuge for about 8 minutes to let the precipitate settle. A small amount of the sample was then transferred to a cuvette and placed inside a UV VIS spectrophotometer for wavelength readings. Five wavelengths (750 nanometers (nm), 665 nm, 664 nm, 647 nm, and 630 nm) were constantly used during analysis, and each wavelength had a corresponding blank (90% acetone). Readings of a blank were noted in every wavelength measured. The same procedure was done with the remaining samples.

Water samples for DO were processed using Winkler's Titration Method. One ml Manganous Chloride ($MnCl2$) and 1 ml Alkaline Iodide solution ($NaOH$ and NaI) were added to the samples and were mixed thoroughly. Another 1 ml Sulfuric Acid ($H2SO4$) was added to the sample and shaken well until the precipitate disappeared and the water turned yellow. Fixed samples were transferred to the Erlenmeyer flask for titration. During titration, samples were titrated with sodium thiosulfate ($Na2S2O3$) until their color turned light yellow and added with at least 0.5 ml starch as an indicator. Continued titration was performed until the water became clear and back to its original color. All the readings were recorded.

2.3. Data analysis

Phytoplankton density (cell/L) was calculated using the equation used by Furio and Gonzales 2002:

$$D = \frac{N \times V_1}{VS}$$

Where:

N = average number of cells in 2 trials of a sample (1 ml)
V_1 = volume of aliquot sample (20 ml)
V_S = total volume of sample (10 L).

All data gathered were consolidated using Microsoft Excel and analyzed using a correlation coefficient for the relationship between phytoplankton abundance, chl-a, and DO. The Kruskal-Wallis and Mann-Whitney was used to determine the significant differences in phytoplankton density and water parameters (chl-a and DO). The cluster analysis and Principal Component Analysis (PCA) were used to categorize the group (Leyte, Samar, and Center) and ordination plot of the water quality that influences the phytoplankton abundance by the use of Paleontological Statistics (PAST 4.03; Hammer et al. 2001). The diversity index was computed using the formula of the Shannon-Wiener Index (H') of each group for the phytoplankton diversity. The Ocean Data View software (ODV 5.1.7; Schlitzer 2019) was used for station mapping and surface profiling to perceive the gulf's phytoplankton density and concentration of water parameters.

3. RESULTS

3.1. Phytoplankton composition, abundance, and diversity

A total of 45 phytoplankton genera were identified, comprising 30 diatoms, 14 dinoflagellates, and only one blue-green alga (Table 2). In all study sites, diatoms were the most abundant among the three classifications. In terms of sampling groups, the Center recorded the highest (31) identified phytoplankton taxa, followed by Leyte (30) and Samar (29).

Table 2. Phytoplankton composition and species richness in sampling areas in Leyte Gulf.

Phytoplankton taxa	Leyte	Samar	Center
Diatoms (Class Bacillariophyceae)			
Acanthometron spp.	+	-	-
Acanthostomella spp.	-	-	+
Amphorellopsis spp.	+	+	-
Bacteriastrum spp.	+	-	-
Biddulphia spp.	+	-	-
Calocyclas spp.	+	-	+
Chaetoceros spp.	+	+	+
Codonellopsis spp.	+	-	+
Coscinodiscus spp.	+	+	+
Dactyliosolen spp.	+	+	+
Ditylum spp.	+	-	+
Eucampia spp.	+	+	-
Favella spp.	-	+	+

Phytoplankton taxa	Leyte	Samar	Center
Fragilidium spp.	-	-	+
Globigerina spp.	-	+	+
Gossleriella spp.	-	+	-
Guinardia spp.	+	+	+
Hemialus spp.	+	+	+
Lauderia spp.	-	-	+
Leptocylindrus spp.	+	+	+
Nitzchia spp.	-	+	+
Parafavella spp.	-	+	-
Planktoniella spp.	-	+	-
Pleurosigma spp.	+	+	+
Rhadonella spp.	+	-	+
Rhizosolenia spp.	+	+	+
Thalassionema spp.	+	+	+
Thalassiosira spp.	-	+	+
Thalassiothrix spp.	+	+	+
Triceratium spp.	+	-	-
Dinoflagellates (Class Dinophyceae)			
Ceratium spp.	+	+	+
Cladopyxis spp.	+	+	-
Diploneis spp.	-	+	-
Gambierdiscus spp.	+	+	+
Gonyaulax spp.	+	-	+
Ornithocercus spp.	+	-	+
Ostreopsis spp.	-	+	-
Oxytoxum spp.	+	-	+
Peridinium spp.	-	+	-
Podolampas spp.	+	-	+
Prorocentrum spp.	+	+	+
Protoperidinium spp.	+	+	+
Pyrocystis spp.	-	+	+
Pyrophacus spp.	-	-	+
Blue-green algae (Class Cyanophyceae)			
Trichodesmium spp.	+	+	+
Total	**30**	**29**	**31**

Note: (+) means "present"; (-) means "absent"

The total percentage of phytoplankton abundance in the gulf covers 89% diatoms, 9% dinoflagellates, and 2% blue-green algae (Figure 2). Among the diatoms, *Leptocylindrus* spp. (52%) was found to have the highest taxa, followed by *Rhizosolenia* spp. (11%), *Coscinodiscus* spp. (10%), and *Dactyliosolen* spp. (6%).

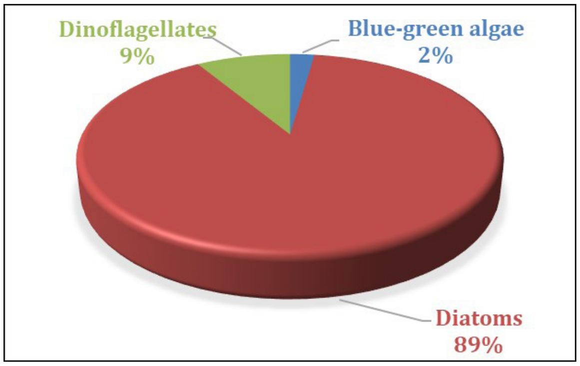


Figure 2. Total percentage (%) of phytoplankton abundance in Leyte Gulf.

The relative abundance of phytoplankton in Leyte Gulf varies in every sampling group, with Leyte having the highest percentage of diatoms (90%) and Center having the highest percentage of dinoflagellates (12%). Only 2% of blue-green algae were found in Leyte and Samar groups (Figure 3). The Leyte area and Samar area recorded the dominance of phytoplankton *Leptocylindrus* spp. (57% and 44%), *Rhizosolenia* spp. (12% and 13%), *Coscinodiscus* spp. (8% and 11%) and *Dactyliosolen* spp. (5% and 9%) while higher densities of *Leptocylindrus* spp. (47%), *Coscinodiscus* spp. (15%), *Ceratium* spp. (6%), and *Dactyliosolen* spp. (5%) were notable in the central part (Figure 4).

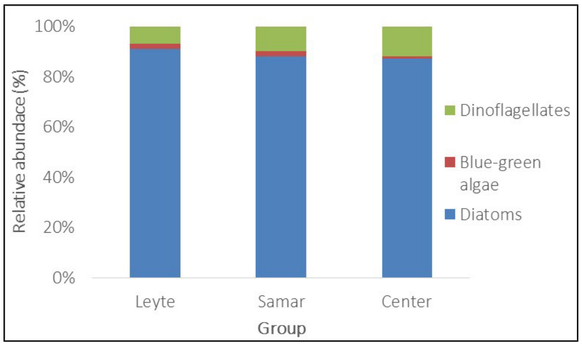


Figure 3. Relative abundance (%) of phytoplankton groups of sampling areas in Leyte Gulf.

Phytoplankton density within the gulf ranged from 20.0 cell/L to 860.0 cell/L with an average of 238.21 cell/L. The highest density (860.0 cell/L) was recorded in Dulag, Leyte (Station 2), while the lowest (20.0 cell/L) was noted in Manicani Island, Samar (Station 6) (Figure 6). The average phytoplankton density in each group (Leyte, Samar, and Center) varies in every sampling depth (10 m, 25 m, 50 m, and 75 m) with a density ranging from 30.0 cell/L to 382.5

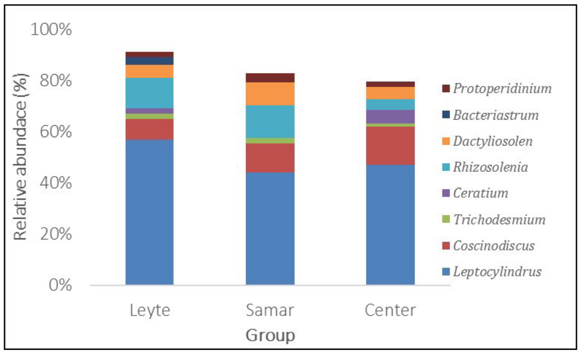


Figure 4. Relative abundance (%) of the top 5 phytoplankton taxa in each group of Leyte Gulf.

cell/L. The highest density (383.0 cell/L) was noted at 25 m (Leyte), while the lowest (30.0 cell/L) was at 50 m (Center). It is visible that the densities at Leyte were found to be higher at 10 m and 25 m depth, and as depth decreases, lower densities are observed at 50 m and 75 m. However, densities in the Center and Samar were highest at the surface (10 m and 25 m) but fluctuated at deeper depths (50 m and 75 m) (Figure 7). Mann-Whitney analysis was employed in this study to determine the difference between phytoplankton density and depth. The analysis proved that there was no significant difference (p>0.05) in phytoplankton density between 10 m, 25 m, 50 m, and 75 m depth.

This study used cluster analysis to categorize the group of Leyte, Samar, and Center in the gulf. Cluster analysis has resulted in two different clusters, cluster I and cluster II (Figure 5). Most stations in the 1st cluster were observed in Leyte, while the 2nd cluster was observed in both Samar and Center. These clusters observed that the similarity of phytoplankton density was found in Leyte, which is higher compared to Samar and Center group with lower phytoplankton density. However, the clustering showed that the groupings in this study were not within their distinct clusters.

The phytoplankton diversity in the three groups (Leyte, Samar, and Center) of Leyte Gulf is projected in Table 3. In Leyte, a higher abundance of phytoplankton is shown compared to the two groups but minimal difference in the diversity (H') and

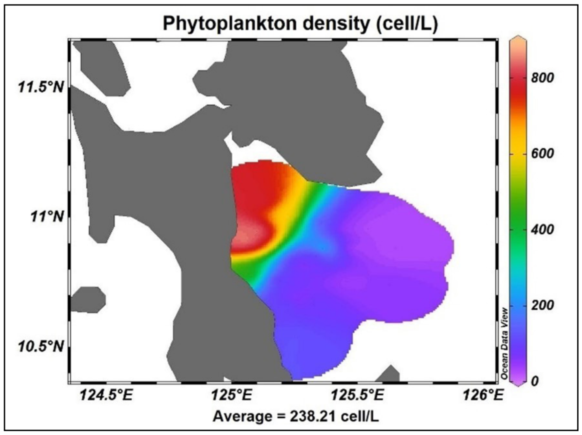


Figure 6. Surface profile of phytoplankton density in Leyte Gulf.

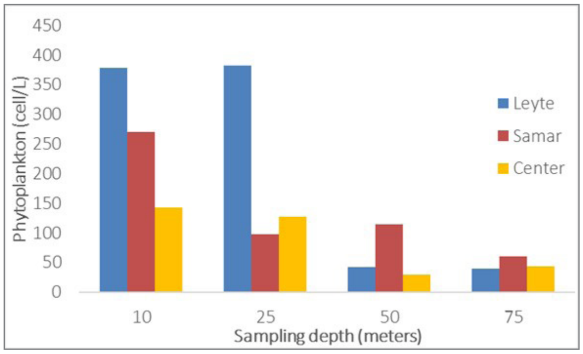


Figure 7. Phytoplankton density (cell/L) from different sampling depths in Leyte Gulf.

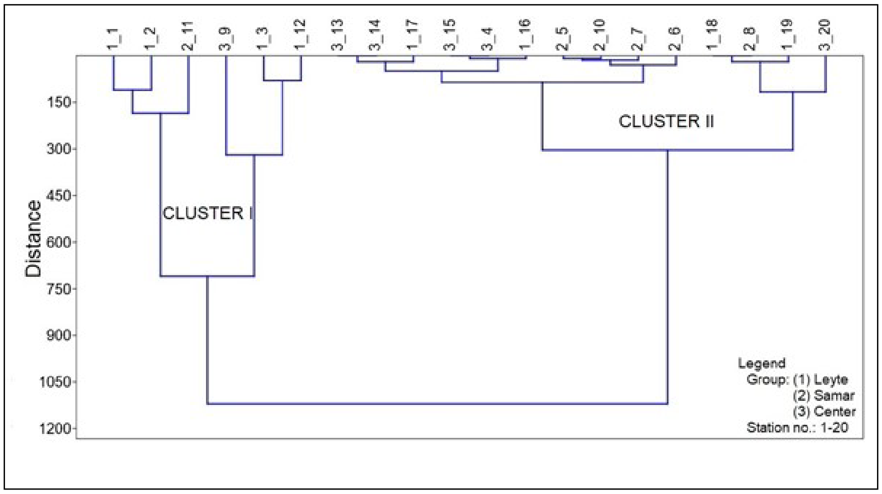


Figure 5. Cluster diagram showing similarities of phytoplankton of three sampling areas in Leyte Gulf.

evenness (E) of phytoplankton species. In this study, Kruskal-Wallis analysis was employed to determine the difference between species diversity and groups in Leyte Gulf. The analysis proved that there were no significant differences (p>0.05) in the phytoplankton diversity in each group of the gulf. This implies that the phytoplankton community in Leyte Gulf has a similar diversity between the groups.

Table 3. Diversity profiles of phytoplankton in three groups within Leyte Gulf.

Diversity index	**Leyte**	**Samar**	**Center**
Phytoplankton (cell/L)	6830.00	2650.00	2060.00
Evenness (E)	0.19	0.28	0.27
Shannon-Wiener (H')	1.74	2.09	2.14

3.2. Chlorophyll-a and Dissolved oxygen

Levels of chl-a in the study area ranged from 0.001 to 0.727 µg/L (0.33 µg/L on average), with the highest level recorded in Tolosa, Leyte near the mouth of San Pedro Bay and San Juanico Strait (northwestern part of the gulf). In contrast, the lowest level was noticeable offshore of Abuyog, Leyte. In terms of sampling depth, the chl-a level was observed to be highest at 75 m with 0.55 µg/L (Samar) while lowest at 50 m with 0.21 µg/L (Leyte).

The DO levels recorded in the gulf are about 6.10 mg/L on average, ranging from 5.44 mg/L to 6.51 mg/L, with its highest concentration (6.44 mg/L) at Dulag Leyte and lowest at Balangiga, Samar (5.95 mg/L). At 10 m depth, the highest level of DO (6.21 mg/L) was observed, while the lowest (5.92 mg/L) DO level was found at 75 m depth, where both are located in Samar.

The distribution of chl-a and DO was distinguishably high along the northwest portion of the gulf (Figure 8). Chl-a concentration was the highest level at 75 m depth, and DO was the highest level at 10 m depth (Figure 9). Mann-Whitney analysis was used to determine the differences between chl-a and DO with depth. Statistically, chl-a and DO were not significantly different (p>0.05) with depth.

3.3. Correlation of phytoplankton to chlorophyll-a and dissolved oxygen

Phytoplankton abundance and chl-a showed a significant positive correlation with an r-value of 0.72 and a p-value of 0.0023 (Figure 10a). Moreover, phytoplankton abundance and DO also showed a significant positive correlation (r = 0.78; p = 0.0028) (Figure 10b).

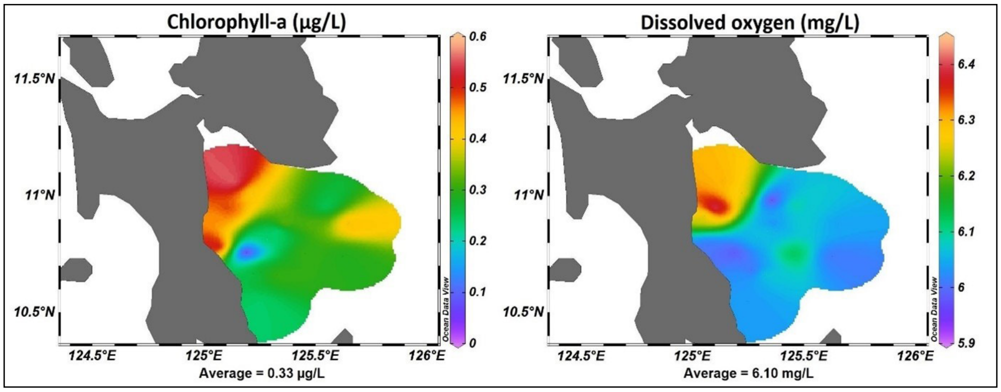
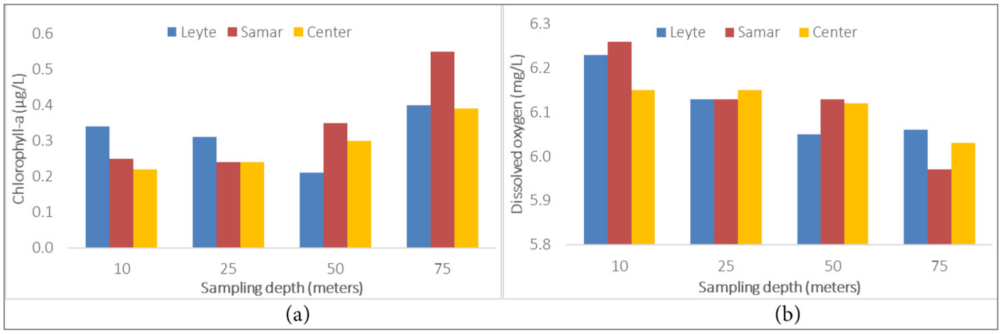
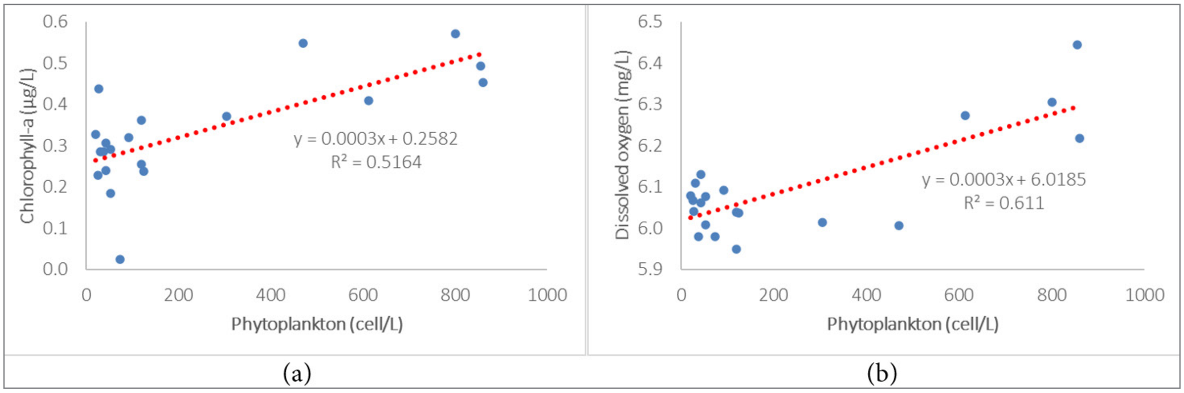


Figure 8. Chlorophyll-a and dissolved oxygen concentration and distribution in Leyte Gulf.



(a) (b)

Figure 9. Average chlorophyll-a (a) and dissolved oxygen (b) from different sampling depth in Leyte Gulf.



(a) (b)

Figure 10. Correlation of phytoplankton abundance with chlorophyll-a (a) and dissolved oxygen (b) in Leyte Gulf.

Principal component one (PC 1) has a 78.3% variance, while principal component two (PC 2) has a 16.0% variance. The values of both parameters in PC 1 increased together with phytoplankton abundance. Several of the stations in the Leyte group were observable within the positive values, while the other stations were in negative values. The data showed that stations with high phytoplankton abundance were coordinated with chl-a and DO concentration (Figure 11). The result of this Principal Component Analysis (PCA) supported the correlation analysis.

4. DISCUSSION

Results revealed that diatoms were the most dominant classification (89%) in Leyte, Gulf compared to the study of Asis et al. (2006) in Calamianes Island, Palawan, with 85% relative abundance. This result is much higher in the West Philippine Sea, where reported diatom assemblage of 73% (SEAFDEC 2000a). Furthermore, the study by Angara et al. (2013) in Casiguran waters showed the highest abundant (>80 %) among other phytoplankton groups. However, the relative abundance of diatom in the gulf was lower than in San Pedro Bay (96%) (Yap-Dejeto et al. 2016) and Panguil Bay, Mindanao (90%) (Lacuna et al. 2012). In Tubajon, Philippines, the occurrence of diatoms is abundant in the area and is a common phenomenon in coastal waters (Dacayana et al. 2015).

Leptocylindrus spp. showed a higher dominance in the study area, and more than half (52.0%) of this taxon was present in all sampling stations. The dominance of *Leptocylindrus* spp. was also observed in Subic Port, with a total population of 34.8% (Austero and Azanza 2018). According to Song et al. (2004), *Leptocylindru*s spp. was dominant during May in Daya Bay, South China Sea. This species typically peaked during spring (March to May) and autumn (September to November) around the South China Sea (Phyto'pedia 2012). In Malaysia, the dominant phytoplankton community is *Leptocylindrus* spp. and *Dactyliosolen* spp. during the

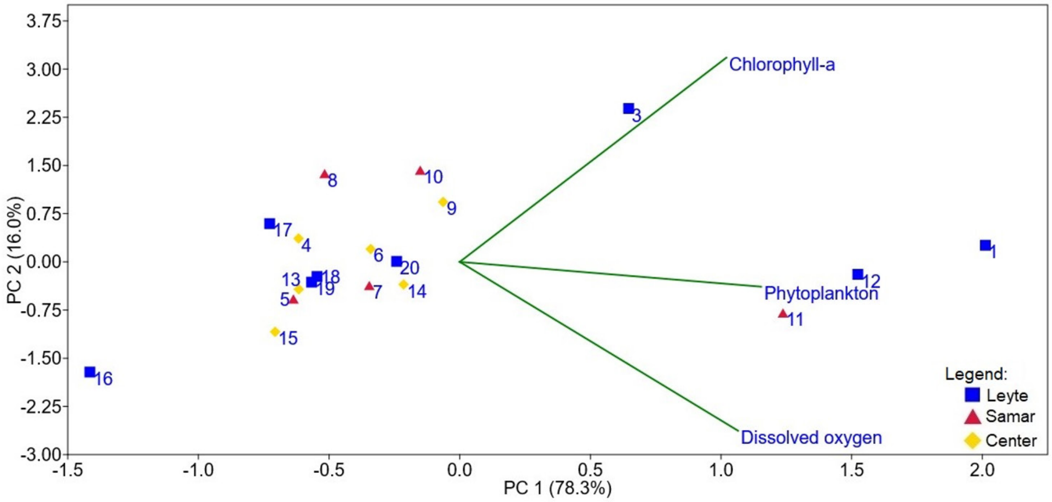


Figure 11. Ordination plot of phytoplankton abundance with chlorophyll-a and dissolved oxygen.

northeast monsoon (NEM) from November to March, which was preceded by a peak in silicate and nitrate (Chong et al. 2020). Further, in the coastal waters of Andaman Island, 95-99% of *Leptocylindrus* spp. was recorded (Karthik et al. 2017).

A higher abundance of phytoplankton was found in the northwest part of Leyte Gulf, close to San Pedro Bay, nearby the coastal areas. According to Masotti et al. (2018), there is high phytoplankton biomass in coastal areas and river mouths. Similar results were comparable in both studies of SEAFDEC (2000b) and Gatdula et al. (2017), which recorded phytoplankton abundance highest towards the coast, and phytoplankton densities typically flourished on the eastern coast of Manila Bay. Dacayana et al. (2015) added that the dominance of phytoplankton assemblage in coastal water is a common phenomenon. In addition, water temperature is a crucial factor affecting phytoplankton bloom dynamics in shallow, productive coastal waters (Trombetta et al. 2019). In relation, high nutrient availability for the rainy season must have influenced the behavior of the phytoplankton despite differences in temperature and light intensity (Yap-Dejeto and Batula 2016). This was supported by Zeitzschel (1978), who said that phytoplankton generally takes place in the euphotic zone (where light can penetrate), where physical factors are important for their quantitative development. The primary production of phytoplankton in an environment is caused by the variability of light, nutrients, and hydrographic conditions. Annisa et al. (2019) further agreed that phytoplankton abundance is influenced by the concentration of nutrients that affects the growth of phytoplankton. Conversely, results from the density-depth analysis of this study revealed that there were no significant differences in the abundance of phytoplankton in every depth.

There are two significant clusters found in this study. These clusters revealed that the three groups were not within their separate clusters. The implication of these clusters was connected to the results of the surface profile observed in the Leyte group, which had higher phytoplankton density and was near the coastal zone. Compared to the groups of Samar and Center, where most of the stations were in the central part or near the mouth of the gulf.

Based on the diversity profile, the phytoplankton diversity was uniformly distributed within the gulf. Leyte Gulf is mainly connected to the Philippine Sea, located in the Western Pacific Ocean (Yap-Dejeto and Batula 2016). Comparatively, phytoplankton diversity in Casiguran waters (facing the Eastern Pacific Ocean) has high species diversity values (Angara et al. 2013). In Manila Bay, the diversity indices show low to moderate species diversity, and this low diversity was caused by aquatic pollution in the bay (Gatdula et al. 2017). The other possible factors that affect phytoplankton diversity are physical and chemical variables of water, as well as species competition, particularly in the Pantanal wetland habitat (Cardoso et al. 2012).

The recorded chl-a and DO within the gulf have optimal range levels, and both parameters were related to the phytoplankton abundance. Based on the study of Carneiro et al. (2014), the chl-a showed a positive relationship between phosphorus, turbidity, and depth, indicating the high phytoplankton biomass production. This study observed lower chl-a compared to Yap-Dejeto et al. (2016) and Jacinto et al. (2011) because most of their sampling sites are situated in coastal areas where the depth is shallow. Most phytoplankton species occurred from the surface where a maximum chlorophyll concentration was observed (SEAFDEC 2000a). The increase in phytoplankton abundance is also followed by an increase in DO concentration in the waters caused by photosynthesis (Annisa et al. 2019). Similar to the result of Felip and Catalan (2000) that a strong positive relationship was found between phytoplankton and chl-a and DO. However, this relationship changed with the season and the taxonomic composition of the phytoplankton. In addition, this result supports the study of Limates et al. (2016), where the level of chl-a and DO concentration accorded with the increased phytoplankton abundance and high nutrients. Furthermore, phytoplankton abundance tends to increase when there are favorable conditions for light and nutrients (Cardoso et al. 2012). According to Asis et al. (2006), the nutrient supply is mainly responsible for the abundance of the different phytoplankton species in marine waters. In San Pedro Bay, Leyte, there is an occurrence of high nitrate concentration and more rainfall; hence, the highest phytoplankton density was recorded (Yap-Dejeto et al. 2016).

5. CONCLUSION

This study concludes that Leyte Gulf has a uniform distribution of phytoplankton abundance in terms of depth and diversity. The concentration of phytoplankton abundance was found high nearby the coast of Leyte. Diatoms are the most abundant phytoplankton group in the area, and *Leptocylindrus* spp. has the highest density in all sampling stations.

The chl-a and DO have a tolerable range in the gulf. There is a strong positive relationship between chlorophyll-a and dissolved oxygen correlated to the phytoplankton abundance.

This study recommends conducting other significant water nutrients studies (e.g., silicate, nitrate, and phosphate) in Leyte Gulf that directly affect phytoplankton abundance. Also, the temperature and salinity profile to determine the thermocline depth. The study needs to monitor the gulf two or three times a year to know the changes in phytoplankton composition, abundance, and diversity for each season.

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AUTHOR CONTRIBUTIONS

Salonga JS: Writing – Original Draft, Formal analysis, Investigation, Resources, Data Curation, and Writing – Review and Editing**. Gino CF:** Formal analysis, Investigation, Resources, Data Curation, and Writing – Review and Editing. **Galve DG:** Investigation, Resources. **Sapul ES**: Formal analysis, Resources, Data Curation. **Leyson JS**: Investigation, Resources. **Camu DGY:** Investigation, Resources. ***Fortaliza RT:*** Investigation, Resources. **Salazar CB**: Investigation, Writing – Review and Editing.

CONFLICTS OF INTEREST

There is no conflict of interest in this paper. All authors have no financial or personal relationship with other people or organizations that could inappropriately influence the paper's content.

ETHICS STATEMENT

No animal or human studies were carried out by the authors.

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