

Density Account of Cyanobacteria (Genus: *Trichodesmium*) in Selected Cebu Provinces, Philippines

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Abstract: This study investigated the density of genus Trichodesmium on the coastal water of Carmen, Daan-Bantayan, Balamban, and Dalaguete, Cebu Philippines. Major objective was to account the density of Trichodesmium species in selected sampling sites. Field collection was performed in 4 sampling sites from July to September 2017. There were only 2 identified cyanobacteria species belonging to genus Trichodesmium.T. erythraeum were the dominant in Carmen, with total number of 629 (89.86%), while highest density of T. thiebautii was 3959 (99.27%) in Daan-Bantayan. The relative density per species was also computed. The present study indicated that Trichodesmium thiebautii was also comparable to Dalaguete and Balamban. It was suggested to perform field collection during dry season and summer for comparison.

Keywords: cyanobacteria, nitrogen fixation, trichomes, density, characterization.

1. INTRODUCTION

Cyanobacteria of the genus Trichodesmium contribute significant amounts of nitrogen (N) to tropical and subtropical oligotrophic oceans through N_2 fixation (Capone et al., 1997). The spatial and temporal distributions of Trichodesmium sp. must be understood, to know the mechanics of the nitrogen inputs in the marine ecosystem. Cell counts, specifically trichome (filament) and colony counts, can be effective (Letelier and Karl, 1996; Post et al., 2002; Tyrrell et al., 2003). There is little study on Trichodesmium species identification in Central Visayas, Philippines. Although the size is usually microscopic, but when conditions are ideal for them both can undergo a phenomenon known as "bloom". Cyanobacteria are not always visible on the surface of water. Generally, they are largely in one particular area. Heavy blooms can overtake water bodies and even check out portions of stream or rivers. But it is difficult to predict when a bloom will occur. Trichomes and colonies vary in size and their counts may not represent true Trichodesmium biomass. Cell counts can be laborious, and identifying trichomes to species based on morphology can be confounding. Trichodesmium biomass was long been considered to be concentrated in the upper 50 m of the water column (Letelier and Karl, 1996; Carpenter et al., 2004). The collection of *Trichodesmium* species in this study via net tows is limited to surface and subsurface regions of at least 10m deep along the coastal margins, because long hauls may damage the fragile trichomes.

Classical identification of *Trichodesmium* was based on cell width and length, sheath characteristics, distribution of gas vesicles, and colony morphology. There are six well-described species of *Trichodesmium: Trichodesmium contortum, Trichodesmium erythraeum, Trichodesmium hildebrandtii, Trichodesmium pelagicum* (formerly *Katagnymenespiralis* and *Katagnymenepelagica*), *Trichodesmiumtenue*, and *Trichodesmium thiebautii*. Generally, two species *T. erythraeum* and *T. thiebautii* were common in the Philippine coastal marine environment. These species of *Trichodesmium* are genetically similar, but morphologically distinct. Classification by cell and colony morphologies can be confusing and misleading due to variable and overlapping characteristics (Anagnostidis and Komárek, 1988; Janson et al., 1995). *Trichodesmium* colonies come in a variety of morphologies including spherical puffs, fusiform tufts, and bowties. Some species can have more than one morphology, for example, *T. thiebautii* forms both puff and tuft colonies, and different species can have the same colony morphology: *T. thiebautii, T. hildebrandtii,* and *T. erythraeum* all form tufts. Single colonies may also contain a variety of filaments, indicating that they are not clonal and suggesting that colonies may coalesce from single trichomes of several species (Hynes et al., 2009).

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Many species originally identified as *Trichodesmium* were brought under the umbrella of the genus Oscillatoria and then later were separated back to *Trichodesmium* (Geitler, 1932; Rippka et al., 1979; Anagnostidis and Komárek, 1988). Species of the genus *Katagnymene* were found to be genetically similar to *T. thiebautii* with respect to the nitrogenase gene nifH, the 16S-23S internal transcribed spacer (ITS), and the heterocyst differentiation gene hetR, so Katagnymene spp. have been included in the genus *Trichodesmium* (Lundgren et al., 2001; Orcutt et al., 2002; Lundgren et al., 2005). A non-invasive study of *Trichodesmium* distributions using a video plankton recorder (VPR) reported high concentrations of *Trichodesmium* colonies as deep as 130 m (Davis and McGillicuddy, 2006). *Trichodesmium* thrives in iron-rich oligotrophic marine environment (Moore et al., 2009; Shiozaki et al., 2010, 2014).Phytoplankton are among the primary producers in shallow coastal areas and factors that affect them will most likely affect overall productivity of a given site. Hence, characterization of phytoplankton assemblages contributes to the determination of productive potential. In this study, the researcher was addressing the abundance and distribution of the genus *Trichodesmium* in Daan-Bantayan, Carmen, Balamban, and Dalaguete Cebu, Philippines.

2. MATERIALS AND METHODS

2.1. Sampling Site

Collection of *Trichodesmium* was performed in the coastal waters of Dalaguete, Daan-bantayan, Carmen, and Balambanto represent the sampling sites (**Figure 1**). Themajor surrounding seas includes; Visayan Sea in the Northern part of Cebu and Bohol Sea in the southern part of Cebu, whereas; Camotes Sea in the eastern side and Tañon Strait in the western side of Cebu province. Actual fieldwork was conducted within last days of July to last days of September 2017 with a working timeframe of 10:00 in the morning until 2:00 in the afternoon.



Figure1. Study sites for distribution and species identification of Trichodesmium

2.2. Sample Collection and Preservation

The phytoplankton was collected by towing a plankton net (mesh size: $20 \ \mu m$, diameter: 0.5 m, cod end volume: 100 ml) in the subsurface water at low running speed of motorized banca. After every 5-minute tow, the filtered sample at the cod end was transferred directly to a pre-labeled 500 ml container and fixed in Lugol's solution (Okolodkov, 2011). The next towed was started after a 5 minutes traveling interval to create a distance gap between tows. The plankton is left to settle then the

top 250 ml of the water will be carefully siphoned out with opening covered with plankton net to concentrate following the procedure of Yap-Dejeto, et al., 2013. The upper layer of water was then sucked out, leaving 200 mL of concentrated plankton sample, which was then transferred to 250 mL graduated cylinders. Sampling bottles were then rinsed. The plankton in the graduated cylinder to the bottom and transferred into a small bottle with cap was then allowed to settle for at least another 48 h. The supernatant was sucked out once again, using a hooked capillary tube and pump, to leave about 20-50 mL of seawater and all collected plankton. The water samples were stored in a cooler under a temperature of at least 4°C.For the first few samples, a series of 1ml aliquots were examined with the use of a Sedgwick-Rafter counting cell until the sampled water were empty.

2.3. Isolation, Identification and Analysis

After every field collection, a homogenized mixture of preserved sample was counted. Individual *Trichodesmium* cells were recorded per station, isolated for identification with photo documentation at 10-x and 40-x magnification in a lighted compound microscope. Species level identification of *Trichodesmium* with the published guide of Tomas (1997),was used Okolodkov (2010), Gómez (2009)with the help of online resources. The identified species was taken a photo micrograph using phase contrast and differential inference contrast with a screen shot camera connected to a laptop computer as to the morphological profiling of the species. Mean total cell density and relative density of each of the genera identified were computed to determine percent contribution of the different genera to the phytoplankton community.

Total counts of species per surveyed area were presented in bar graphs. The work of Limates, et al., 2006 on the computation of relative cell density was used in the present study. In understanding the structure of the community, the relative abundance of the species was determined using the formula and was presented in the pie-chart:

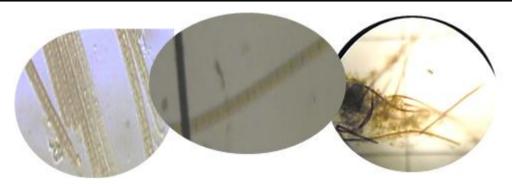
Relative density =<u>Total # of cells per species</u> x 100 Total # of cells for all species

3. RESULTS AND DISCUSSION

3.1. Density

Two phytoplankton species have been identified under genus *Trichodesmium* using for at least 20 ml standardized samples per sampled area (see Figure 2). As shown, the *Trichodesmium thiebautii* dominated comparably in three areas of Daanbantayan (3959 individuals), Balamban (3170), and Dalaguete (2948); whereas, very lowden sities observed in Carmen, Cebu. Dominated by *Trichodesmium erythraeum* from all sampled areas was in Carmen, Cebu having 629 individuals. The cell counts of *Trichodesmium thiebautii* was highest in Daanbantayanmarine waters which might correlates the good condition of the area and high nutrients. As reflected further in Figure 4 that the pattern of relative density among Balamban, Dalaguete and Daanbantayan seems comparable for *T. erythraeum* and for *T. thiebautii*. Following further onto this trend, Carmen composition and density of these species were very few (Figure 3). *Trichodesmium* can either live in a colonial habitat dominated by extensive physical interactions with both sister cells and other taxa and/or as free trichomes that can constitute a significant fraction of the *Trichodesmium* water column biomass (Orcutt et al., 2001).Field populations of *Trichodesmium* have previously been enumerated using cell counts (Letelier and Karl, 1996; Post et al., 2002; Tyrrell et al., 2003).

The surface *Trichodesmium* sp. relative density (Figure 4) were possibly correlated to depleted nitrogen in the area. Cyano bacteria of the genus *Trichodesmium* are often considered to be the principal N₂-fixing diazotrophs in tropical and subtropical regions (Carpenter and Romans, 1991; Capone et al., 1997; Karl et al., 1997; Langlois et al., 2008; Rijkenberg et al., 2011). Thus, microscopic counts performed at USC Marine Station were showing evidences forthe abundance and pattern of distribution among *Trichodesmium* species mainly; *Trichodesmium thiebautii T. erythraeum* within the randomly selected stations (Figure 2 and 3). Their total counts at the surface were widely varied as reflected in Figures 3 and 4. Zooplankton being the first heterotroph consumer of phytoplankton *T. erythraeum* and *T. thiebautii* were observed lower in three stations as compared to Carmen station with 629 individuals. But it was also in Carmen site which the presence of *T. thiebautii*, has its lowest total counts for only 71 individuals among the four sites.



a) Trichodesmiumerythraeum



b) Trichodesmiumthiebautii

Figure2. Micrograph of Trichodesmium sp. at the surface of the study areas

3.2. Trichodesmiumcounts and Composition

The distribution of *Trichodesmium* is influenced by temperature (Heynes, 2009). Research on *Trichodesmium* sp. abundance and distribution is important for two reasons. First, they contribute a significant portion (at least 40%) to global oceanic nitrogen fixation, and affects the biogeochemical carbon flux in tropical oceans, having connection with the climate condition. Second, massive coastal *Trichodesmium* blooms have toxic effects on invertebrates eating them and of other organisms that can potentially be harmful to humans (Karl et al., 2002; Devassy, 1979; Capone et al., 1997; Guo et al., 1994; Hawser et al., 1992; Lenes et al., 2001). The percentage composition of genus *Trichodesmium* was presented in Figure 4.

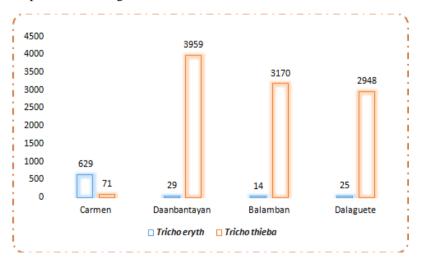


Figure 3. Total count of major species of genus: Trichodesmium in selected sites in Cebu, Philippines (2017)

The collection of cyano bacteria was conducted from last days of July and ended during the last days of September 2017, where it falls to a wet season time. A study by Calompong et al., 2013 in Ticao Pass, Masbate, Philippines showed that blue-green algae, particularly *Trichodesmium* was abundant during this wet season. *T. thiebautii* and *T. erythraeum* are the main cyanobacteria forming large blooms if under favored conditions (Orcutt, 2002), and will cause harm to fishes (fishkills) and may

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kill humans. Abundance of these species in the Philippine Sea can be attributed to the high iron requirement of *Trichodesmium* sp. for their growth compared to other diazotrophs and non-diazotrophs (Kustka et al., 2003; Saito et al., 2011). And *Trichodesmium* species is a major nitrogen fixer in the Kuroshio (Chen et al., 2014; Shiozaki et al., 2015), which are often found in low-nutrient waters (Lin et al., 1998), during periods of low wind stress and warm temperatures (Lenes et al., 2005), not so exposed to wind influence.

In this study, areas being randomly selected as sampling sites were part of the existing major seas might affect with the distribution of the phytoplankton such as; Bohol sea in the South portion of Cebu and Visayan sea in the north, and Camotes Sea in South portion and Tañon Straitin the west (Figure 1). To consider the cyano bacteria in areas having business factory establishments as observed in Balamban and Carmen, Cebu and business beach resorts as were observed in coastal shorelines of Dalaguete. Whereas, freshwater discharge from the big river in Daanbantayan with anthropogenic activities and the presence of other coastal and marine ecosystems (i.e. mangrove ecosystem, sea grass ecosystem) might contributed to the highest number of *Trichodesium thiebautii* in this area.

Rough weather and strong currents with strong waves was experienced during the sampling days of July, 2017 in Balamban, Cebu, that might affect the abundance of *Trichodesium erythraeum* but not on *T. thiebautii* in contrast to the findings of Matsuno et al., 2006 that turbulence near the sea floor influences the surface water in the shallower bottom region, and Zhang et al. (2012) that the physical disturbance reduces diazotrophy since *Trichodesmium* favor calm seas. Usually the mangrove stands lined across the shorelines of the sampled coastal area, may enhanced nutrient inputs (Odum and Heald, 1975) that favors higher phytoplankton biomass in these areas with mangroves. Hence, the high cell counts of *Trichodesmium* assemblage in Daanbantayan, Dalaguete, and Balamban may be attributed to the high productivity of waters bordering it, and factors such as current and nutrient inputs which act on a more local scale as noted during the towing of the plankton net might be the reason for the relatively high overall concentrations of *Trichodesmium thiebautii* (10,148 individuals) accumulated among the four provinces.

Further, houses and other business establishments were observed and estimated within 250 meters from the sampling site in Balamban and Dalaguete, such that the high nutrient concentrations and loading resulted in changes in the species composition of phytoplankton (Colijn et al., 2002; Burford and Pearson 1998). Furthermore, the cyanophycean represented by genus *Trichodesmium* has shown uneven distribution across sampling periods and study sites. It was also possible that nutrients were one responsible for the observed spatial pattern within the towed sites as supported by various studies. Single colonies may also contain a variety of filaments, indicating that they are not clonal and suggesting that colonies may coalesce from single trichomes of several species (Hynes et al., 2009).Low presence of phosphorus and iron in marine environment will restrict the growth and N₂ fixation capacity of *Trichodesmium* sp. (Moore et al., 2009) and their abundance (Shiozaki et al., 2015).

One of the emergent aspects in this present effort is to provide baseline data of the site, which might be determined by combinations of biological and physicochemical factors, diversity and abundance of primary producer and consumer assemblages, nutrient dynamics, water movement and overall habitat structure and complexity.

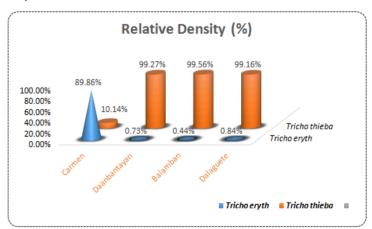


Figure4. Relative density of genus Trichodesmium from the four sampling site

4. CONCLUSION AND RECOMMENDATIONS

Trichodesmium thiebautii was highest in Daanbantayan with 3959 individuals and and lowest in Carmen with 71 individuals. Similarly, *Trichodesmium erythraeum* was highest (629 ind.) in Carmen and lowest (14 ind.) in Balamban coastal area. Researcher hypothesized that the high abundance of *Trichodesmium thiebautii* were as crib able not only to the load of nutrient in the marine environment, favored temperature, but also onto the supply of *Trichodesmium* species and other phytoplankton and zooplankton from the selected areas; Balamban, Dalaguete, and Daanbantayan. In contrast, *Trichodesmium erythraeum* was observed lowest in these areas where *T. thiebautii* was abundant. Phytoplankton monitoring can be used as biological indicator and can give feedback on the quality of water as influenced by land use and nutrient inputs contributed by anthropogenic activities. Uneven abundance and distribution was observed in the four sites. Therefore, the abundance of *Trichodesmium* sp. Would likely be influenced by the nutrient availability, temperature and physical factors such as wet season (rainy season) and strong water currents, in the sampling site in favor of the specific species of *Trichodesmium*.

As such, monitoring should be conducted regularly and institutionalized to determine the impact of nutrient loading to the coastal water, and can provide feedback on the ecological status of the coastal ecosystem particularly the quality of the coastal waters within the Cebu provinces. Implementation of strong multi-sectoral monitoring (from private NGOs to concerned bureau such as DOST and academe such as CTU) is deemed necessary. Lessons learned from genus *Trichodesmium* can be applied to other species of phytoplankton which are not as well studied. It is further suggested to study their abundance and distribution during dry season and summer months.

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