Species Richness of Cyanobacteria, Diatoms, and Ciliates in Microbial Mats of the Cabo Rojo Salterns, Puerto Rico

by

Lourdes Colón Ortiz

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Approved by:

Dimaris Acosta Mercado, Ph.D. Member, Graduate Committee	Date
Rafael Montalvo Rodríguez, Ph.D. Member, Graduate Committee	Date
Carlos Ríos Velázquez, Ph.D. Member, Graduate Committee	Date
Carlos J. Santos Flores, Ph.D. President, Graduate Committee	Date
Dallas E. Alston, Ph.D. Representative of Graduate Studies	Date
Nanette Diffoot Carlo, Ph.D. Chairperson of the Department	Date

ABSTRACT

Seasonal rains and the inflowof seawater into the Cabo Rojo salterns, Puerto Rico, induce changes in the concentration of dissolved salts. These environmental variations should have an effect in the composition and species richness of the microbial mats in these salterns. The purpose of this study was to analyze the changes in the richness of cyanobacteria when a decrease in salinity occurs. Three stations were selected and three aquaria without bottoms were set per station. Treatments of sterilized seawater, distilled water, and a control with ambient water from the same station were maintained *in situ*. Samples were analyzed after 24 and 72 hours and the procedure was repeated at different hydroperiods. The treatments showed no differences in the composition of the mat. Nevertheless, for the hydroperiod that comprised the dry season with high levels of seawater, there was a shift from the dominant filamentous cyanobacteria to the coccoid ones when the salinity decreased below 20 ppt. In general, 12 species of cyanobacteria were identified with *Microcoleus chtonoplastes* the most abundant, followed by the coccoid, *Aphanothece granulosa*. Diatoms and ciliates were also characterized. Nineteen species of diatoms were identified, where the most abundant were *Nitzschia lanceolata*, *Navicula* spp., and *Mastogloia braunii*. The most frequent ciliates were *Fabrea* (*F. salina)* and *Nassula* sp.

RESUMEN

Las lluvias estacionales y la entrada de agua de mar en las salinas de Cabo Rojo, Puerto Rico, inducen cambios en la concentración de sales disueltas. Estas variaciones ambientales pueden tener un efecto en la composición y en la riqueza de especies del tapete microbiano en dichas salinas. El propósito de este estudio fue analizar los cambios en la riqueza de especies de cianobacterias al disminuir la salinidad. Se establecieron tres estaciones y se colocaron tres acuarios sin fondo en cada estación. Se añadieron tratamientos *in situ* de agua de mar esterilizada y agua destilada, además de mantener un tratamiento control con agua de la misma estación. Las muestras fueron analizadas luego de 24 y 72 horas y el procedimiento fue repetido durante distintos hidroperiodos. Los tratamientos no mostraron diferencias significativas en la composición de cianobacterias en el tapete. Sin embargo, durante el hidroperiodo que comprende la temporada de sequía y altos niveles de agua de mar, ocurrió un cambio en el tipo de cianobacteria dominante, de las filamentosas a las cocoides, cuando la salinidad fue menos de 20 ppmil. En general, se identificaron 12 especies de cianobacterias, donde la filamentosa *Microcoleus chtonopastes* fue la más abundante, seguida por la cocoide *Aphanothece granulosa*. También se caracterizaron las diatomeas y los ciliados en el tapete microbiano. Se identificaron 19 especies de diatomeas, de las cuales *Nitzschia lanceolata*, *Navicula* spp. y *Mastogloia braunii* fueron las más abundantes. Los ciliados más frecuentes fueron *Fabrea* (*F. salina)* y *Nassula* sp.

DEDICATION

To my parents, Luz E. Ortiz Rodríguez and Ramón L. Colón Ortiz, who have been my models of responsibility and dedication to reach every goal proposed. They are the ones that taught me that by the hand of God everything is possible. Their love for the nature and their constant desire to contribute to the well-being of the Society has been my inspiration.

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INTRODUCTION

The Cabo Rojo Salterns are part of the Cabo Rojo National Wildlife Refuge, administered by the US Fish and Wildlife Services. In addition to its importance for the extraction and commercialization of salts, it functions as a place in the Caribbean for many migratory birds to rest and obtain food. The hypersaline lagoons in this Refuge (Candelaria and Fraternidad) have microbial mats in their benthos. The main components of these mats are primary producers, being the base of the trophic chain in this ecosystem.

The environmental factors (such as seawater entrance, rainfall or salinity gradients) play a major role determining the diversity of the species and, thus, the composition of the microbial mat. “The biodiversity is a measure of important ecological processes such as resource partitioning, competition, succession, and community productivity, and is also an indicator of community stability”[1]. This is because there are species that can tolerate disturbances better than others, and the ecosystem can keep functioning continuously.

In order to observe if cyanobacterial species (main components of the mat) that exist in Laguna Candelaria change with reductions in the salt content, we applied treatments of distilled water, seawater, and ambient water from the site and analyzed samples from each one. Also we took samples along the salinity gradient and over time. The rationale of the study was that if dilution treatments

[1] Morris, Bardin, Berge, Frey-Klett, Fromin, Girardin, Guinebretièrre, Lebaron, Thièry, and Troussellier. 2002. Microbial biodiversity: Approaches to experimental design and hypothesis testing in primary scientific literature from 1975 to 1999.

were applied, then cyanobacterial species richness would increase and their abundance would decrease, therefore changing the composition of the mat. This was based on the intermediate disturbance hypothesis which states that intermediate levels of disturbances increase the species richness by allowing rapid colonizers to co-exist with the competitive species[2][3].

In addition, some protists in the microbial mats (i.e. diatoms and ciliates) were identified and characterized. For each group, an illustrated guide and a taxonomic key are provided and a brief analysis on the species richness was done. The data obtained are reported for future studies in the ecology of the site, and physiological or molecular studies of the organisms.

[2] Dial, R., and J. Roughgarden. 1998. Theory of marine communities: the intermediate disturbance hypothesis. Ecology. 79: 1412-1424.
[3] Townsend, C. R., and M. R. Scarsbrook. 1997. The intermediate disturbance hypothesis, refugia, and biodiversity in streams. Limnol. Oceanogr. 42: 938-949.

LITERATURE REVIEW

Microbial mats are communities of mostly prokaryotic microorganisms that may represent the first ecosystem created on Earth 3,500 million years ago (Urmeneta *et al.*, 2003). In those times, the Earth surface was dominated by organisms in these mats, which, by means of photosynthesis, released oxygen and changed the atmosphere. They usually consist of several layers, arranged vertically, where the organisms distribute themselves according to their physiological requirements (i.e. amount of light, oxygen, nutrients or temperature). Within these organisms, we can find photosynthetic bacteria like the cyanobacteria, green and purple bacteria, as well as non-phototrophic prokaryotes with capacity to reduce sulfate. In addition, we can observe eukaryotes such as diatoms, green algae, ciliates, copepods, nematodes, larvae of insects, and micro-crustaceans. The photosynthetic organisms (bacteria or eukaryotes) usually dominate in the mat, representing the base of the trophic chain.

Microbial mats are recognized for their multiple applications. In addition to producing large quantities of oxygen, they are studied because of their ability to reduce erosion from the coastlines (Sarkar *et al*., 2005) and to serve as a bioremediation tool in the pollution with petroleum and oil (Abed *et al*., 2002; Martínez-Alonso and Gaju, 2005; Abed *et al*., 2007). Another application is to compare the modern microbial mats with the data obtained for the stromatolites to understand the origin of life (Margulis and Sagan, 1992; Walter *et al*., 1992;

Shopf, 2000 and Stal, 2000). Stromatolites are organosedimentary-rocky-structures that date from the Precambrian (Javor and Castenholz, 1981). Microbial mats dominated all types of environment during this period; cyanobacteria, in addition to other prokaryotes, had the capability to precipitate minerals such as calcite, thus forming the stromatolites (Dupraz and Visscher, 2005). However, over time, the stromatolites stopped forming, and the modern microbial mats seem not to have the capability of lithification (i.e. to form rocks) (Stal, 2000). Some scientists think that the reason is because of the grazers that, when feeding on other microorganisms in the mat, do not allow the mineral deposition (Whitton and Potts, 2000; Stal, 2000). Because of the same reason, modern microbial mats are restricted to extreme environments, where the population of grazers is low. Distribution of stromatolites includes Lake Eyre and Shark Bay in Australia, the coast of Baja California and Guerrero Negro Lagoon in México, Abu Dhabi in the Persian Gulf, the coast of Ebro Delta in Spain, the Cenotes Lagoon in the Yucatán Peninsula, Mexico, and the Yellowstone National Park (Javor and Castenholz, 1981; Blinn, 1991; Golubic, 1992a and 1992b; Rocha *et al*., 1998; Ward *et al*., 1998; Navarrete *et al*., 2000; Urmeneta *et al*., 2003). Researchers have determined that species from the stromatolites include cyanobacteria (e.g. *Aphanothece halophytica*, *Microcoleus chtonoplastes*, *Lyngbya aestuarii*, *Arthrospira* sp., *Phormidium* sp., *Oscillatoria limnetica*), diatoms (e.g. *Nitzschia* sp., *Navicula* sp. *Pleurosigma* sp), and grazers, such as ciliates (e.g. *Fabrea salina*, *Blepharisma* sp.), copepods (e.g. *Paramisophria variabilis*, *Halicyclops cenoticola*), nematodes and ostracods.

In Puerto Rico, there are microbial mats in most of the coastline of the southwestern part of the island, from Cabo Rojo to Guánica, where hypersaline environments are found. However, there are few studies in such environments, mainly for the Cabo Rojo salterns (Navarro, 1988; Grear, 1992; Montalvo-Rodríguez *et al*., 1998; Montalvo-Rodríguez *et al*., 2000; Mercado-Álvarez, 2003; Casillas-Martinez, *et al*., 2005; Díaz-Muñoz and Montalvo-Rodríguez, 2005; Cantrell and Molina, 2006; Díaz-Muñoz, 2006; and Broche, 2006). Most emphasis of the species composition of stromatolites has focused on the archaea rather than than bacteria such as the cyanobacteria. These bacteria produce oxygen from photosynthetic processes and are dominant primary producers in microbial mats. Other studies include some data on diatoms, halophilic fungi, and microcrustaceans.

Cabo Rojo salterns: site description and hydrology

The Cabo Rojo salterns are part of the Cabo Rojo National Wildlife Refuge, administrated by the U.S. Fish and Wildlife Service (USFWS) (Figure 1). The refuge is composed by several ecosystems, such as dry and mangrove forests, hypersaline and marine lagoons, prairies of marine grass, and coral reefs. The hypersaline lagoons in this area are Fraternidad and Candelaria. Both are considered as artificial, thalassohaline lagoons because of the entry of seawater which is controlled by the USFWS by means of a dam. Laguna Candelaria receives seawater from Bahía Salinas, while Fraternidad receives it from Bahía Sucia.

The control of the seawater entrance has two main purposes. The first is to manipulate the salinity at the site. By closing the dam, the concentration of salts increases from evaporation of the seawater. When the water reaches a specific salinity, it is transported to the pools, where the evaporation and the rainfall of salts are enhanced. Then, the salts can be extracted and used for commercial purposes. The second purpose to control the seawater entrance is to provide a suitable environment for endemic and migratory birds that visit the place (Grear, 1992; Collazo *et al*., 1995; Broche, 2006). Collazo *et al*. (1995) studied the population of birds from 1985 to 1992. They found 28 different species and described the Salterns as “numerically, the most important site for shorebirds in Puerto Rico”. According to them, the shorebirds counted in the Cabo Rojo salterns compose 29% of all the birds that visit 22 different sites in the Caribbean. They think that the factors influencing their distribution include food availability and hydrological conditions, which are largely the result of salt extraction operations. Meanwhile, Grear (1992) and Broche (2006) suggest that the distribution of birds in the Cabo Rojo salterns depends on the salinity and water depth.

The USFWS established the best freshwater levels for the restoration of the Cartagena Lagoon (the only natural freshwater lagoon of Puerto Rico, in Lajas, at the eastern part of Cabo Rojo) and its optimization as a place for the endemic and migratory birds (Figure 2). USFWS uses these levels for the control of the seawater entrance into the Cabo Rojo salterns (personal communication with James Padilla, USFWS) to enhance the growth of organisms that function

as food for the birds. The highest levels (up to 50% of seawater, where 2 m of depth represents 100% seawater) corresponded to the months of January, February, October and December. During this time, the water levels were between 1-2 m. The water can reach these levels because of the entrance of seawater, but also by means of the groundwater. According to the US Geological Survey (USGS) (1999), the Cabo Rojo Salterns are classified as minor aquifers of volcanic origin – igneous and sedimentary rocks – and can generate from 5-10 gallons of water per minute. The water in them contains high concentrations of sodium chloride, bicarbonates, iron, and manganesum.

Water levels also depend on rainfall. The rainfall data from 1980 to 2006 are shown in Appendix I. Analyzing the averages, we can see a seasonal pattern of rainfall (Figure 2). The first dry season corresponds to the months from January to March, followed by a rainy season from April to May. Then, there is a second dry season, from June to July, followed by another rainy season from August to December. In this way, we can combine the seawater levels with the rainfall data and classify the entrance of water in four hydroperiods (Table 1).

Each hydroperiod could have an impact in the composition of species in the microbial mat. The environmental dilutions, caused by the seawater entrance and the rainfall, can be considered as disturbances which will change salinity and affect the dynamics of the community. According to Wooton (1998), a disturbance can increase the intensity of predation, which can increase the mortality in any trophic level. Thus, the composition in the microbial mat that

exists in the bottom of the Candelaria or Fraternidad lagoons could be affected by the increase, decrease, or disappearance of a group of microorganisms.

Figure 1. Cabo Rojo National Wildlife Refuge. It includes forests, salterns, mangroves, coral reefs, among other ecosystems. Laguna Candelaria receives seawater from Bahía Salinas, while Laguna Fraternidad receives it from Bahía Fraternidad. Figure provided by William Santiago, from the USFWS.

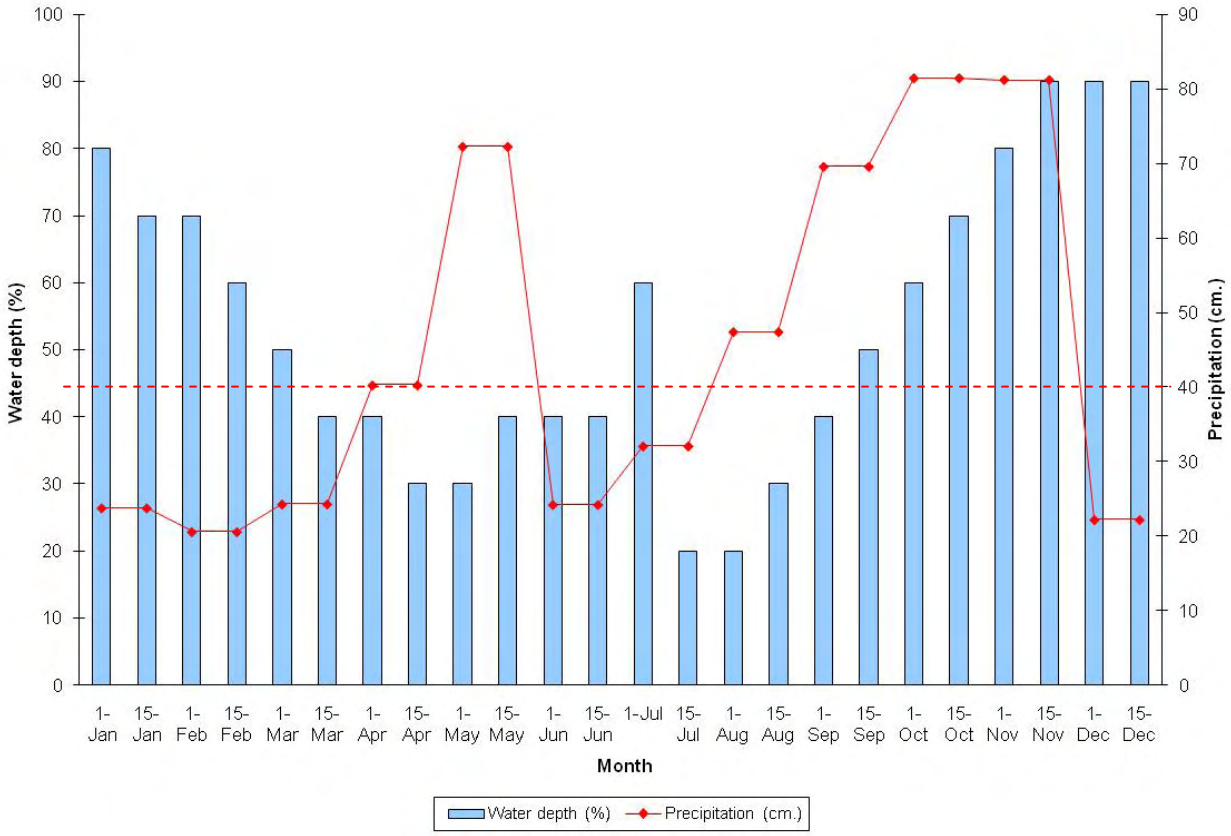

Figure 2. Seawater levels and rainfall in the Cabo Rojo salterns. The data of seawater are already established for the site, while the rainfall data are an average, from January 1980 to December 2006 (See Appendix I). The red line indicates the 50% of rainfall and seawater levels.

Table 1. Hydroperiods for the Cabo Rojo salterns, considering the data from seawater levels and rainfall (1980-2006). Months with less than 50% of rainfall belong to the dry season, and above that percent to the rainy season.

Hydroperiod	Season	Seawater Entrance	Months
I	Dry	>50%	January, February, July (from days 1 to 15), and December
II	Dry	≤50%	March, April, June, July (from days 15-30)
III	Rainy	>50%	October, November
IV	Rainy	≤50%	May, August, September

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CHAPTER I: Cyanobacteria: identification, characterization and responses to dilution treatments.

INTRODUCTION

Cyanobacteria are prokaryotic organisms, also called cyanophyta, or blue-green algae, which produce oxygen during photosynthesis (Komárek, 2003). Cyanobacteria include one of the oldest fossil records (Barghoorn, 1971; Schopf, 1993, 2006), suggesting that this group of organisms originated 3.5 billion years ago and contributed to the aerobic atmosphere that we have today. This event provided selection pressure for organisms capable of adapting to the new environment and provided a suitable habitat for new species.

Cyanobacteria probably have existed for so many years because they live in most habitats, including on rocks and in damp soil, freshwater, or seawater. Also, they can live in extreme environments, such as deserts (Friedmann, 1980; Friedmann and Kibler, 1980; Palme and Friedmann, 1990; Belnap, 1996; García-Pichel *et al.*, 2001), hypersaline waters (Por, 1980; Cornee, 1989; Golubic, 1991; Zavarzin, 1993; Davis *et al*., 1996; Kruschel and Castenholz, 1998; Montoya and Nubel *et al*., 2002), and thermal springs (Castenholz, 1969; Anagnostidis and Pantazidou, 1988). Komárek (2003) emphasized their importance in many aquatic and terrestrial communities “for their substantial biomass and primary production, nitrogen fixation, production of toxic components, creation of stromatolites, boring in limestone substrates, and their roles in symbioses”.

Symbioses involving cyanobacteria are typically associated with a myriad of organisms (Adams, 2000). Cyanobacteria promote nitrogen fixation plants (Obukowicz *et al*., 1981; Okoronkwo *et al*., 1989; Bergman *et al*., 1992; Osborne

et al., 1992; Obreht *et al*., 1993; Ow *et al*., 1999) and with fungi form lichens (Dick and Stewart, 1980; Lallemant, 1986; Canaani, 1988; Jensen and Siebke, 1997). Also, they can associate with protists, including algae (Kies, 1974; Floener and Bothe, 1980; Buck and Bentham, 1998; Janson *et al*., 1999;), and animals like tunicates (De Leo and Patricolo, 1980; Olson, 1983, 1986; Alberte *et al*., 1987; Duclaux *et al.*, 1988), sponges (Gillan *et al*., 1988; Garson *et al.*, 1994; Lee *et al*., 2001), and insects (Kleinhaus, 1989).

Cyanobacteria usually are dominant organisms within microbial mats, particularly in the superior layers. Their gliding ability allows them to move upwards after being covered by sand (Gemerden, 1993) and to position themselves according to their photosynthetic necessities. Furthermore, some cyanobacteria (e.g. *Entophysalis* and *Lyngbya*) can protect themselves against the damages of the sun by means of that motility or by the production of scytonemin, a yellow-brownish pigment that absorbs the excess of the ultraviolet rays (Golubic, 1992; Graham and Wilcox, 2000).

Within the mats, they have various ecological roles. For example, they produce a sticky layer of polysaccharides that traps sediments, which promotes the production of mats (Stal, 2000). Cyanobacteria also deposit calcium carbonate in their sheaths, which induce the formation of layers in the microbial mat, and eventually, can form stromatolites (Graham and Wilcox, 2000; Komárek *et al.*, 2003). Stal (2000) summarizes the importance of the cyanobacteria in the microbial mats by: 1) having a wide range of metabolic capacities that can adapt to changes and fluctuations in the environment; 2) using the light as the energy

source, water as the electron donor, and CO_2 as the carbon source; 3) having the capacity to use N_2 as the nitrogen source; 4) performing oxygenic photosynthesis or anoxygenic photosynthesis (some of them) depending on sulfur availability; 5) adapting to anoxic conditions, and 6) employing fermentation. Gemerden (1993) emphasized that cyanobacteria are one of the most important groups within the microbial mats because they provide growth substrates for other organisms as well as physical strength to the integrity of the mat. Growth and survival of the other organisms depend on excretion and lysis products of cyanobacteria.

The classification of cyanobacteria is still under discussion. Bacteriologists consider the physiology and phylogenetic relationships of the organisms that can be isolated in pure cultures, while the phycologists consider morphological forms, the environment in which they develop, and their ecological role (Komárek, 2003). The integration of both descriptions will give us a better understanding of cyanobacterial systematics and may induce changes in their classification. Nevertheless, “changes in the classification and identification of cyanobacteria can be expected in the future, but presently the traditional approach is still necessary, especially for field studies” (Komárek, 2003).

The traditional approach includes the morphological classification which divides cyanobacteria into five groups: Chroococales, Oscillatoriales, Nostocales, Stigonematales, and Pleurocapsales. The Chroococcales include all the unicellular or colonial cyanoprokaryotes, which do not form true filaments with the direct physiological interference between the cells (Komárek, 1998). The

Oscillatoriales, Stigonematales and Nostocales form the filamentous cyanobacteria. Komárek and Anagnostidis (2005) describe the genera under the Oscillatoriales as organisms that do not have heterocytes or akinetes (specialized cells which fix nitrogen, and resist extreme conditions, respectively), and do not have true branches (i.e. one or various cells that have two planes of division to change the direction of the filament within a common sheath). However, some genera within the Oscillatoriales can form false branches, which consist in a deviation of the filament (not in the plane of division) and each "branch" is on a different sheath. The Stigonematales can form filaments with true branches. Also, they can develop heterocytes and akinetes under some conditions, as well as the Nostocales (Komárek, 2003). Nostocales do not form true branches and have no variation in the size of the cells. The Pleurocapsales are characterized for the production of internal spores, baeocytes (Komárek, 2003).

Studies of cyanobacteria in Puerto Rico

Few studies exist about cyanobacteria in Puerto Rico. Some members of the New York Botanical Garden collected soil, freshwater, and seawater samples from 1890 to 1925. Gardner (1927) analyzed these samples, writing the first report of cyanobacteria for Puerto Rico, which included the saline water species *Anacystis minutissima* and *Lyngbya scytonematoides*. In 1932, Gardner confirmed these species and also described *Oscillatoria salinarum* and *Spirulina labyrinthiformis* as species that tolerate saline environments in some areas of

western Puerto Rico. Later, Almodóvar (1963) studied the terrestrial and freshwater cyanobacteria of Puerto Rico, some of which are euryhaline. Recently, Chacón *et al.* (2006) analyzed the biogeological signatures of cyanobacterial communities in carbonated substrates from the sandy beaches at Cabo Rojo. They found the presence of *Microcoleus* and *Halomicronema* through molecular tests; *Calothrix*, *Fischerella* (or *Matteia*), *Plectonema* and *Chroococcus* through cultivation of the samples; and *Leptolyngbya* and *Phormidium* through both, molecular and cultivation techniques.

Cyanobacteria in Cabo Rojo Salterns

For the Cabo Rojo Salterns, there is one published work (Casillas-Martínez *et al*., 2005), which reports the presence of *Lyngbya* sp., *Gloeocapsa* sp., *Johannesbaptistia* sp., and *Microcoleus chtonoplastes* within the microbial mats. There is also a masters thesis (Mercado-Álvarez, 2003) to measure the populations of the cyanobacteria *Gomphosphaeria aponina* and the crustacean *Artemia salina* in the Candelaria and Fraternidad lagoons. Nevertheless, there is no detailed study describing the cyanobacterial community in the microbial mats in this region.

The main objective of this investigation was to characterize the cyanobacterial biodiversity in the superficial layer of the microbial mat in Laguna Candelaria, as well as to provide a taxonomic key and an illustrated guide to these cyanobacteria. It was also our purpose to analyze changes of this community after a reduction in the salt content of the water and to compare the

dilution treatments along different hydroperiods of the Cabo Rojo Salterns. Our main hypothesis was that if dilution treatments (addition of seawater or distilled water) were applied, then grazing upon algae by salinity-intolerant predators would increase, thus, decreasing the biovolume of cyanobacteria in the microbial mats.

MATERIALS AND METHODS

Sampling

The salinity gradient may vary with rainfall and the levels of seawater. For that reason, we obtained samples during three different months: a) January: little rainfall and high levels of seawater; b) May: little rainfall and low levels of water; and c) November: high rainfall and high levels of seawater.

Figure 3 shows the sampling stations. We set three aquaria (50 cm by 25.5 cm by 28 cm, opened at each end) in each station (Figure 4) and added 15 L to each one of either distilled water, seawater, or water from the same station (our control). From each aquarium, three sub-samples of 1 cm^2 from the microbial mat were taken just before either of the water was added (labeled as day -1 in the graphs), and 1 and 3 days after treatments. The samples were fixed in formaldehyde at a final concentration of 4% and transported to the laboratory at room temperature. Also, physical and chemical parameters were taken. The temperature was measured with an alcohol thermometer and a refractometer was used to measure the salinity. Samples of water were taken in bottles and analyzed *in situ* for dissolved oxygen by the Winkler titration method, using a LaMotte® kit; and the water depth was measured with a ruler.

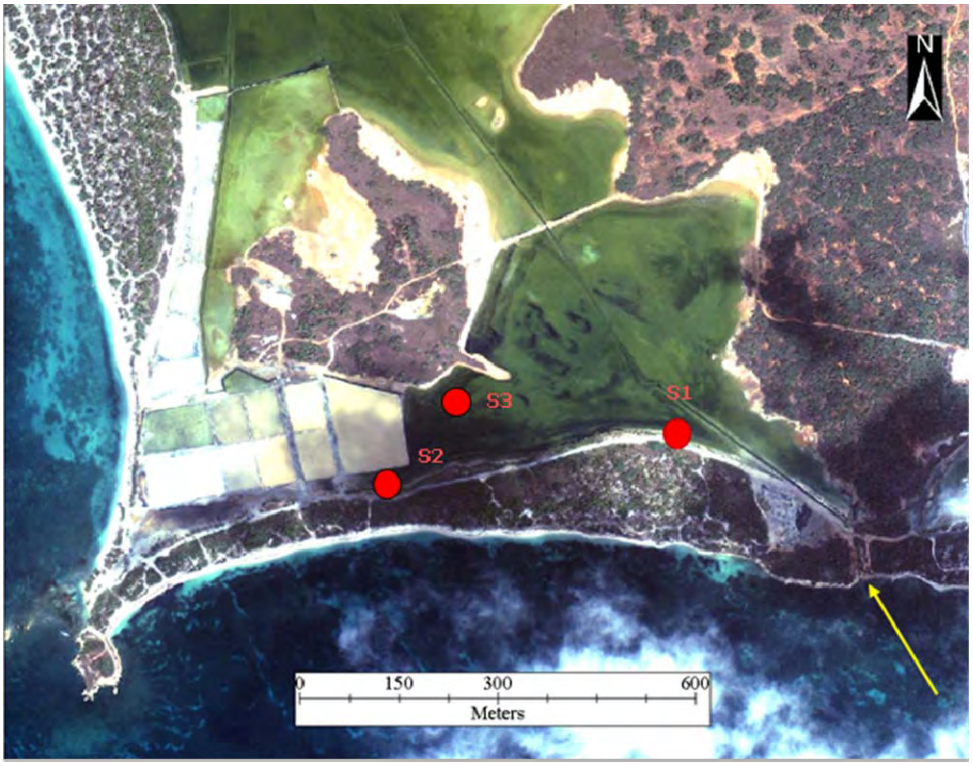

Figure 3. Area of study in the Cabo Rojo Salterns, Puerto Rico. The red dots indicate the sampling stations and the yellow arrow indicates the dam that controls the seawater level. This image of IKONOS was provided by the PaSCoR laboratory, University of Puerto Rico at Mayagüez.

Figure 4. Aquaria at a station. They were labeled as A, B and C for each treatment of either distilled water, seawater, or water from the station, respectively. Salt extraction pools and a salt mound can be observed behind the aquaria.

Slide preparation

The mounting media was prepared by adding 1 g of phenol to 100 ml of Karo® syrup (Reid, 1978; Skinner and Entwisle, 2001; Cambridge *et al*., 2007). Two drops of this solution and the sample were added to a slide and homogenized with a needle. A coverslip was placed over the sample. The slides were placed on a slide-warmer at 37°C for 48 hours. The excess of solution was cleaned out and the slides were sealed with fingernail enamel.

Morphological Analyses

To differentiate between cyanobacteria and green algae, we prepared slides and stained the samples with an iodine starch test solution to stain the starch granules of the green algae. The cyanobacteria do not accumulate polysaccharides in form of starch granules, so they remained blue-green (or some times brown, because the polysaccharides of the mucilage stained with the solution; but no granules were seen).

The slides mounted in phenol and Karo®syrup were observed through the compound light microscope, and sometimes with a confocal microscope. The species identification was made following Komárek (1998, 2003), Komárek *et al*. (2003), and Komárek and Anagnostidis (2005). These keys include characteristics such as form, measurements, differentiated cells, planes of division, and the environment in which they develop.

Ecological Analyses

In order to measure the cyanobacteria on each slide we first studied the slides of the preliminary study. This included the observation of 1 cm^2 samples of all the samples that were taken before addition of water to each aquaria, and subsequent 1 cm^2 samples at 24, 48, 72, 96, and 120 hours after them for each treatment at each station. All the visual fields were observed and a cumulative species curve was prepared. The highest point on the graph was determined at 15 visual fields.

The biovolume of the cyanobacteria was measured to determine the distribution of each genera in the microbial mat, since measuring abundance would not be sufficient to determine the specific space they are occupying (i.e. abundant species can be small, covering a small portion of the microbial mat and rare species can have the opposite effect). Thus, based on the highest point in the cumulative species curve, we took digital photos of 15 visual fields from each slide following an imaginary diagonal line across the coverslip, to avoid the effects of the margin. The photos were calibrated and the biovolume (μm^3) of each genus were obtained with the computer program SigmaScan Pro® ver. 5 (see Appendix 2). We did a summation of the biovolume of the genera for each slide and recorded it as the total biovolume of cyanobacteria for each sample. Mean biovolume was also estimated among sub-samples within treatments, resulting in a single number for each treatment. In adition, the species richness of cyanobacteria was recorded for each station and hydroperiod.

Statistical Analyses

The data from the total biovolume of cyanobacteria were analyzed using SAS v.9 to determine if distributions among stations and treatments were normal (Shapiro-Wilk), and a split block was performed to see if there were differences among stations, treatments, or samples within days. To know which station or hydroperiod was different from the others (depending on the split block), a Tukey test was performed. The species richness mean and standard deviaton (S.D.) for stations and hydroperiods were obtained through the program InfoStat v. 2.

RESULTS

The purpose of this study was to analyze the possible changes in the biovolume and richness of cyanobacterial genera during three different hydroperiods in Laguna Candelaria. Nevertheless, seawater levels that were supposed to be high during January were low. Thus, instead of having a third hydroperiod with little rainfall and high levels of seawater, the results shown will present only the changes that occurred during two periods of dry season, with little rainfall and low levels of seawater (January and April) and a rainy season in the month of November (high rainfall and high levels of sea water).

A total of 15 different species of cyanobacteria, belonging to 13 genera have been identified for Laguna Candelaria, but also worldwide (Table 2). Five species within these genera were observed only in the preliminary studies, while the others were seen during both the preliminary studies and the sampling periods. The species composition of these populations is described below.

Ecological Analyses

I. Species richness

Considering a Shapiro Wilk test, the richness data were not normal ($p = 0.0115$) (Appendix 5A1) even when it was transformed using $\log_{10}$, x-1 and the square root. This means that the distribution of the species in the microbial mat is not uniform among the stations, time, or treatments. Meanwhile, other observations about species richness can be done (Appendix 5A2, A3 and A4).

An overall species richness of 3.51 ± 1.25 cyanobacteria was found. The higher species richness was seen during the dry hydroperiods (Figure 5), especially during April (4.15 ± 1.30). During this hydroperiod, Stations 1 and 2 had the highest species richness (4.67 ± 1.18 and 4.67 ± 1.07, respectively). Nevertheless, during the dry hydroperiod of January, Station 2 had the lowest species richness (2.96 ± 0.76) and Station 1 the highest one (4.26 ± 1.16). Thus, considering both hydroperiods, Station 1 had the highest species richness (3.96 ± 1.33). All these changes in diversity are linked to the biovolume of species, as well as the salinity and water depth (see next section).

II. Species biovolume

A. Statistical results

The biovolume of each genus is shown in Appendix 4. The Shapiro Wilk test (Appendix 5B1) showed that the data were normal, ($W = 0.96$, $p = 0.24$). Thus, a split block test was performed to determine if there were significant differences among hydroperiods, stations, treatments or days (Appendix 5B2). The results showed significant differences in biovolume among hydroperiods ($p = 0.0115$) and also when this variable (hydroperiod) interacts with the stations ($p = 0.0496$) and among days ($p = 0.0336$). A Tukey test grouped the rainy hydroperiod and the dry one from April as the most similar in cyanobacterial biovolume, whereas the dry hydroperiod from January was different (Appendix 5B3). To determine the specific differences in biovolume between hydroperiods,

as well as the differences among stations and among days, the physicochemical parameters as well as the distribution of the cyanobacteria should be considered.

Table 2. Cyanobacterial species identified in Laguna Candelaria, Puerto Rico, and reports of their presence worldwide.

Cyanobacteria	Observed only in preliminary studies	Observed during the sampling periods	Previously recorded for the Cabo Rojo Salterns	Previously recorded elsewhere in Puerto Rico	Previously recorded for brackish water in the Caribbean	Previously recorded in other salterns	References
Aphanocapsa cf. *holsatica*	+						
Aphanocapsa cf. *litoralis*		+				+	Elrich and Dor (1985), Komárek and Anagnostidis (1998), Komárek and Komárkova-Legnerova (2007)
Aphanothece granulosa		+		+			Gardner (1927), Komárek (2007)
Arthrospira sp.	+						
Chlorogloea gessnerii		+				+	Schiller (1956)
Chroococcus pulcherrimus		+					Komárek et al. (2005), Komárek and Komárkova-Legnerova (2007)
Cyanosarcina cf thalassia		+					
cf. *Hydrococcus*	+						
Johannesbaptistia pellucida	+			+	+	+	Almodóvar (1963), Elrich and Dor (1985), Komárek and Anagnostidis (1998), Komárek and Komárkova-Legnerova (2007), Koster (1963)
Lyngbya aestuarii		+		+	+	+	Elrich and Dor (1985), Gardner (1927, 1932), Javor and Castenholz (1988), Koster (1963), Urmeneta *et al*. (2003)
Merismopedia cf. *affixa*	+						
Microcoleus chtonoplastes		+	+		+	+	Casillas-Martínez *et al*. (2005), Elrich and Dor (1985), Javor and Castenholz (1988), Koster (1963), Urmeneta *et al*. (2003)
Oscillatoria lloydiana		+				+	Javor and Castenholz (1988)
Oscillatoria nigro-viridis		+		+		+	Elrich and Dor (1985), Gardner (1932)
Spirulina subsalsa		+		+	+	+	Elrich and Dor (1985), Gardner (1927), Koster (1963)

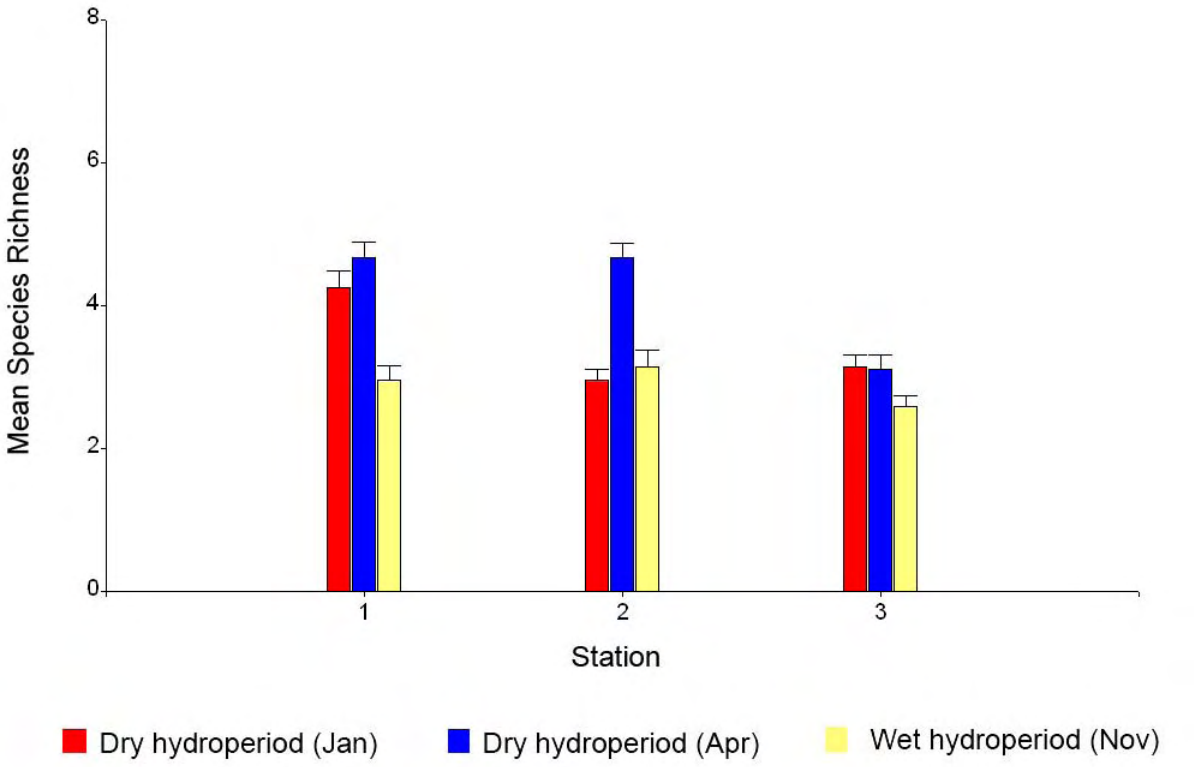

Figure 5. Mean species richness of cyanobacteria per station. The dry hydroperiod of April (blue) and Station 1 had the highest species richness. Observe that Station 2 had highest values of species richness during the dry hydroperiod of April and the rainy hydroperiod in November, but during the dry hydroperiod of January it was the station with the lowest value for this variable. Lines above the bars = S.D.

B. Physicochemical parameters

The physicochemical parameters are shown in Appendix 3. The temperature ranged from 21-33 ºC and the dissolved oxygen was not significantly correlated with cyanobacterial biovolume (r = -0.15). The water depth for each station for each hydroperiod is shown in Figure 6. As expected, during the rainy hydroperiod the water depth was higher in the majority of the stations and days and lower during the dry hydroperiod. In April, most depths were less than 6 cm. Moreover, in January all depths before treatments were less than 1.5 cm (0 cm in Station 2). After treatments, depths were not deeper than 7 cm in the stations far from the dam, but increased in Station 1, which is closer to the seawater inlet.

The salinity graph (Figure 7) showed to have a negative relation with the water depth one. During dry conditions in April, where the water depth was low, high values of salinities could be observed (81-97 ppt in the majority of the cases). Intermediate salinities were observed for the rainy season (63-78 ppt), where the water depth was higher. Meanwhile, a combination of salinities during the dry season of January ocurred. At that moment, the salinity before adding the treatments was high, exceeding 90 ppt (data from Station 2:-1 could not be collected, because the water depth was 0 cm), decreased after 24 hr of treatments (< 50 ppt), and increased again after 72 hr of treatment. This increase was higher in the station near the dam, and did not exceed 60 ppt at the other stations.

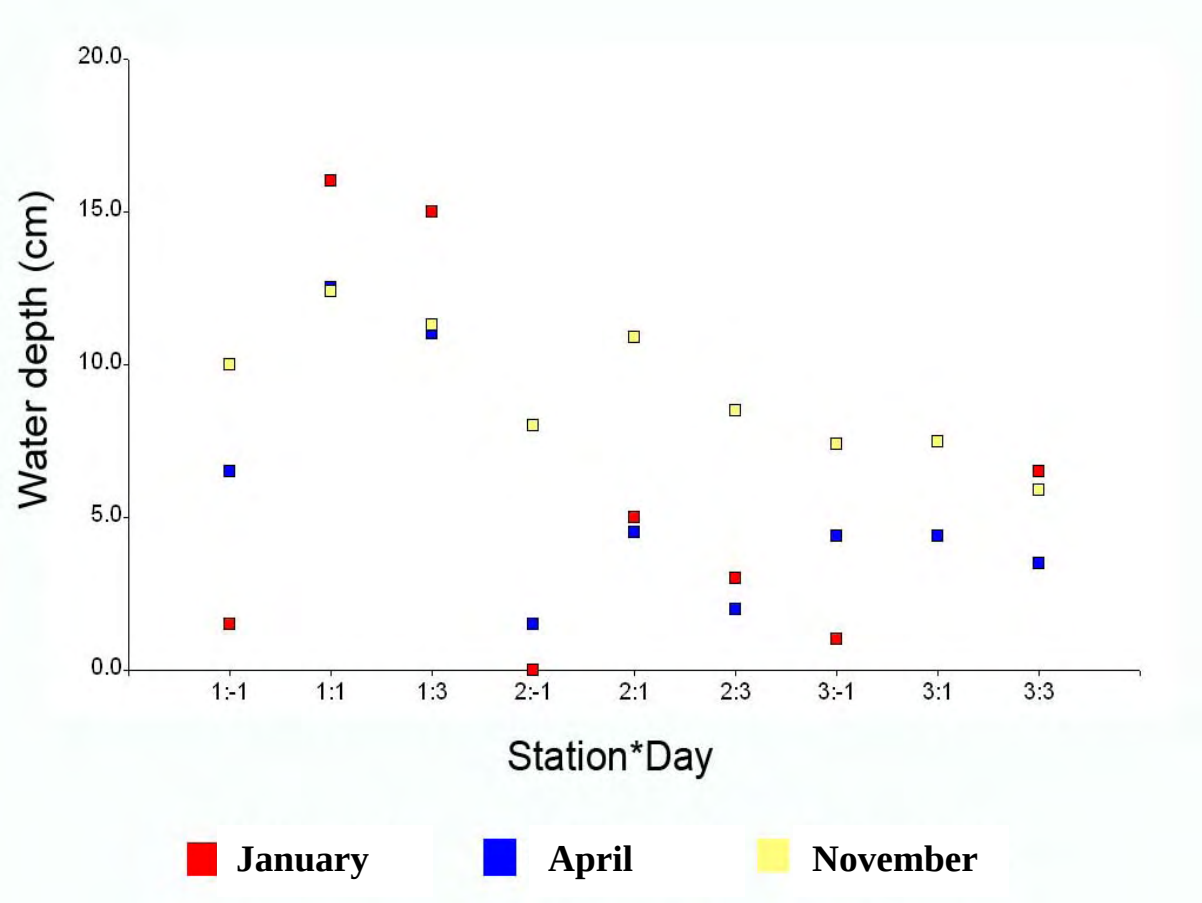

Figure 6. Water depth across sampling stations before treatments addition (-1), after 24 h (1), and after 72 h (3), for each hydroperiod. During the dry season of January the data were variable, having the highest value in Station 1 (closer to the seawater entrance) after 24 hrs of treatment, but also the lowest value for Station 2, before treatment (2:-1). The rainy season in November had the highest values, as expected (>6 cm). The dry hydroperiod in April presented low values (<5 cm), except for Station 1, that have values >5 cm. This was also expected, because that station is closer to the seawater entrance. n = 27.

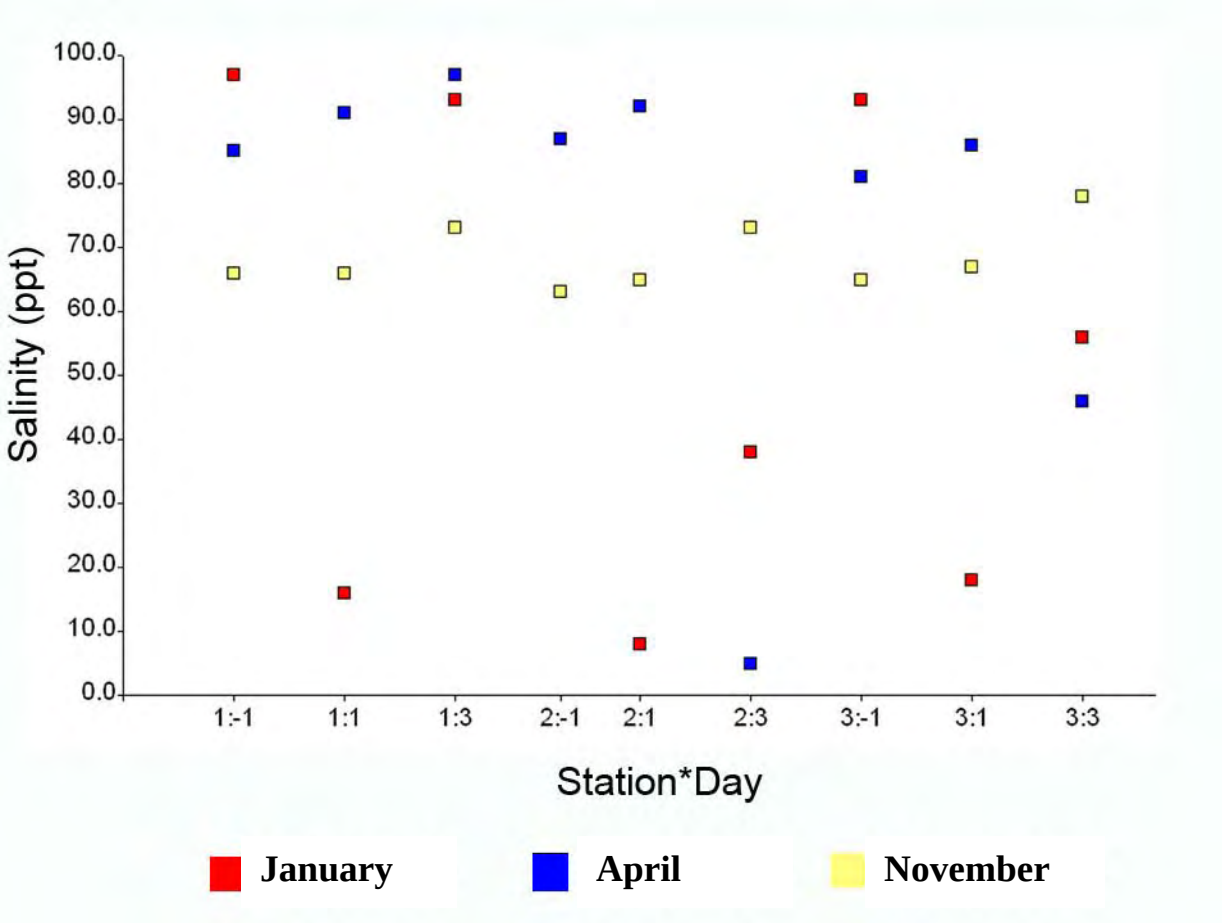

Figure 7. Salinity for each station (1, 2 and 3) before and after the treatments (-1, 1, and 3) during for each hydroperiod. Observe that during the dry season in January, there is not a salinity measurement in Station 2, before treatments (2:-1). At that moment, the water depth (in the previous figure) was 0 cm, and the salinity could not be measured. The data from this hydroperiod was variable, but most of the lower registrations (< 40 ppt) were present during this time. Most of the data obtained during the dry season in April indicated highest salinities (> 80 ppt), followed by the rainy season (November), with measurements from 60 to 78 ppt. n = 26.

C. Cyanobacterial composition in the microbial mat

A scale of occurrence of each genus was established (Table 3) following Locky and Bayley (2006). The scale was distributed as follows: all genera that contribute with ≤ 1% of the total biovolume were considered rare; those found a few times (2-10% of the biovolume) were considered few; genera found regularly, but did not dominate the community (11-74%) were considered common, and the dominant ones (75%-100%) were considered abundant. This table is complemented with Figure 8, which shows the relative abundance of each genus among hydroperiods, treatments, and stations. Generally, the filamentous cyanobacteria *Microcoleus* predominated in the microbial mats, but during the dry season in January, there was a shift in Station 2, where the dominant genera changed to the coccoid *Aphanothece* (Figure 8A). The overgrowth of *Aphanothece* in this station coincides with the decrease of species richness shown in Figure 5.

Finally, a community species composition according to the salinities for each hydroperiod is shown in Figure 9. *Microcoleus* appeared to dominate at all salinities during the dry season of April and the rainy season of November, but it varied during the dry season of January. During the latter, *Aphanothece* became dominant when the salinities ranged from 0-10 ppt or from 31-40 ppt. It is also important to observe that *Oscillatoria* was present in a wide range of salinities during the dry season in April, but it was more frequent at the highest range (91-

100 ppt). Meanwhile, *Chlorogloea* was present during all hydroperiods, but occurred more frequently in April from 91-100 ppt.

	Hydroperiod:Station								
Cyanobacteria	**I :1**	**I:2**	**I:3**	**II:1**	**II:2**	**II:3**	**III:1**	**III:2**	**III:3**
Aphanocapsa	+	+	+	+	+	+	+	+	
Aphanothece	+++	++++	++	+++	++	++	++	+++	++
Chlorogloea	+	+	+	+	+	+	+	+	
Chroococcus	+			+	+	+	+	+	+
Cyanosarcina	+	+	+	+	+	+	+	+	
Lyngbya	+		+				+	+	+
Microcoleus	++++	++	++++	++++	++++	++++	++++	+++	++++
Oscillatoria	+	+			+			+++	
Spirulina	+				+		+		

Table 3. Occurence of cyanobacterial genera among stations for each hydroperiod.

I = January, dry season; II = April, dry season, and III = November, rainy season. The scale is showed using the following symbols: rare (+), few (++), common (+++) and abundant (++++). Observe that *Microcoleus* was the most abundant genus, except for station 2 in hydroperiod I (dry season, January), where *Aphanothece* became the dominant one.

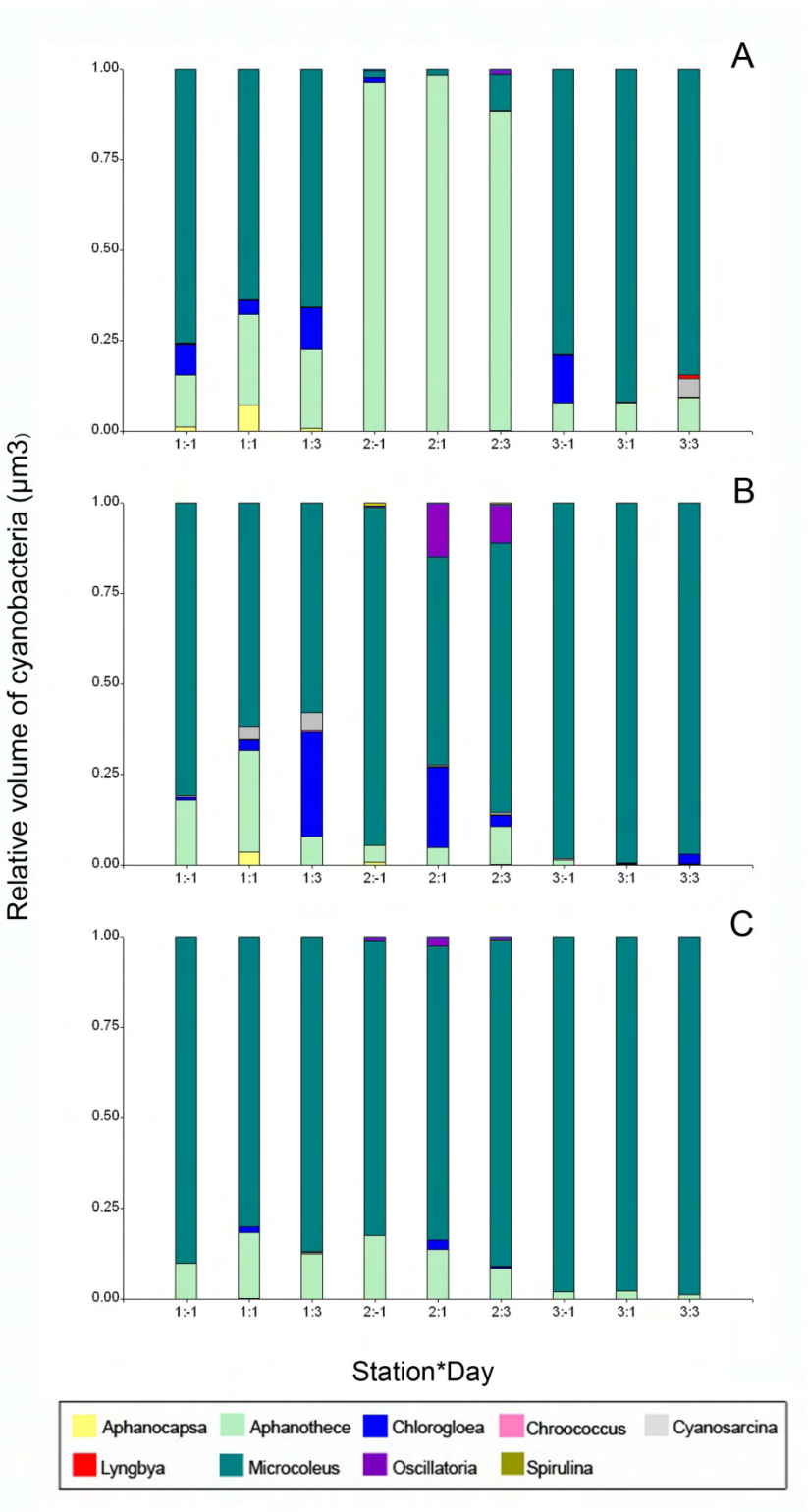

Figure 8. Relative biovolume of cyanobacteria (µm˘) for each station (1, 2, and 3) and days (-1 = before treatment, 1 = 24 hrs after the treatments, and 3 = 72 hrs. after the treatments. (A) Dry season in January; (B) Dry season in April; (C) Rainy season in November.

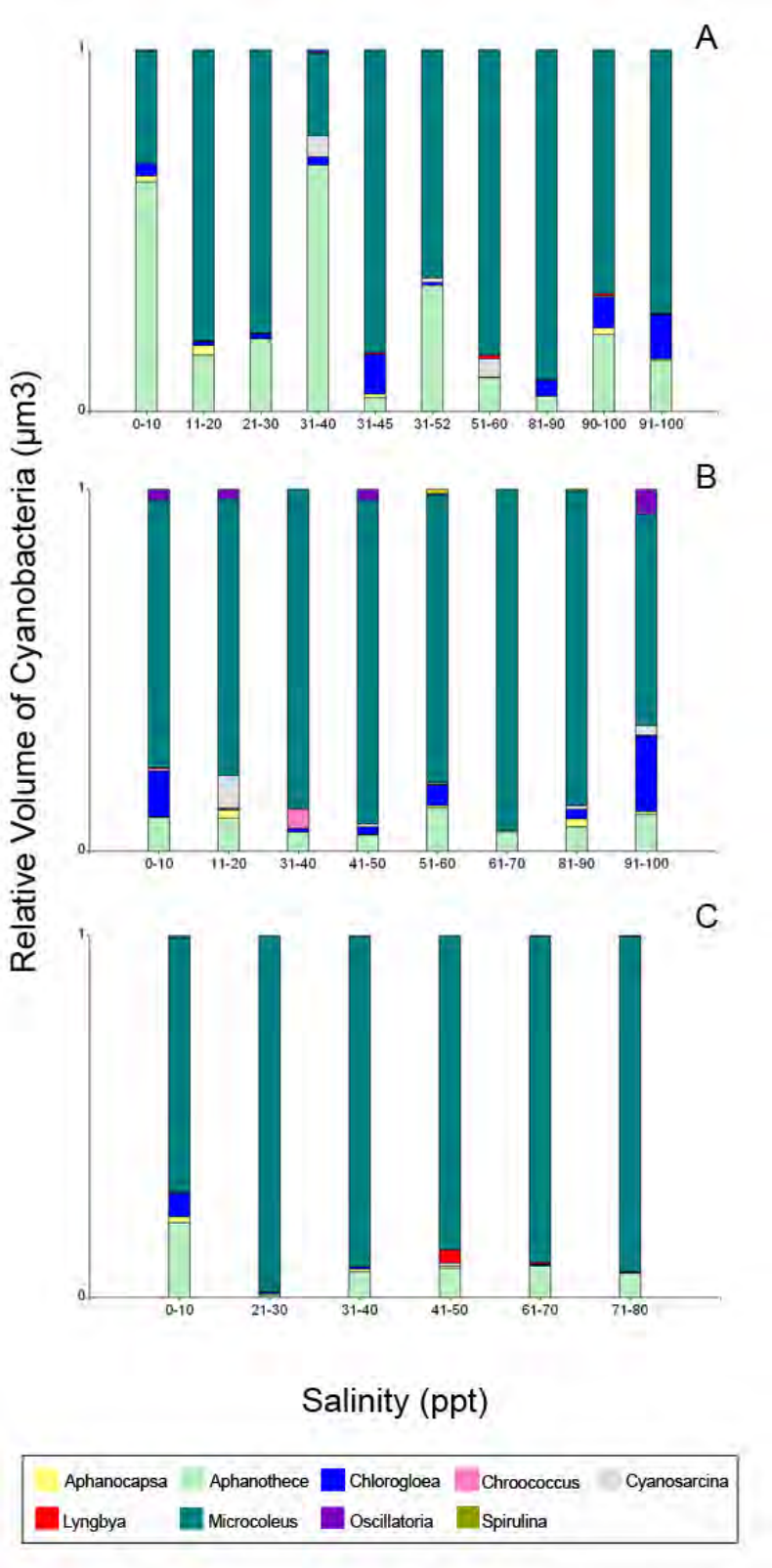

Figure 9. Relative biovolume of cyanobacteria (μm^3) in all the stations according to the salinity (in 10 ppt intervals). (A) Dry season, January; (B) Dry season, April; (C) Rainy season, November.

Identification and Characterization

I. Taxonomic Key

The following key was designed using the morphological features of the cyanobacterial species that were found in Laguna Candelaria. It is followed by a description of each species.

1a. Cells with different shapes. Can be solitary or forming colonies 2
1b. Cylindrical cells forming tricomes..... 10

2a. Cells of 1 µm or less 3
2b. Cells greater than 1 µm 4

3a. Groups of 4 cells arranged perpendicularly in flat colonies, ...
..... *Merismopedia cf. affixa*
3b. Cells densely aggregated in colonies of irregular shape.....
..... *Aphanocapsa cf. holsatica*

4a. Hemispherical cells arranged in pairs 5
4b. Oval or polygonal cells 7

5a. A single pair of cells in a common sheath ..... *Chroococcus pulcherrimus*
5b. More than a pair of cells in a common sheath 6

6a. Cells forming uniseriate rows in a common sheath (pseudofilament) .....
..... *Johannesbaptistia pellucida*
6b. Multiple pairs of cells forming a spherical colony in a common thick sheath .
.....*Aphanocapsa cf. litoralis*

7a. Oval to cylindrical cells arranged irregularly in a common sheath.....
.....*Aphanothece granulosa*
7b. Colonies of rounded of polygonal cells 8

8a. Solitary colonies with hexagonal cells ≥ 4 µm ..... cf. *Hydrococcus*
8b. Aggregated colonies, forming macroscopic aggregates 9

9a. Cells arranged in rows at the margin and irregularly at the center of the colony; groups of colonies forming a parenchymatous structure ..... *Chlorogloea gessnerii*
9b. Sarcinoid colonies formed by rounded to squared cells tightly packed within a common sheath; colonies near each other *Cyanosarcina cf. thalassia*

10a. Helicoidal tricomes 11
10b. Not helicoidal tricomes 12

11a. Helix with tight turns (approximately 5 turns in 10 µm) .... *Spirulina subsalsa*
11b. Helix with less than two turns in 10 µm ..... *Arthrospira* sp.

12a. Cells longer than wider, with multiple tricomes in the same sheath .... *Microcoleus chtonoplastes*
12b. Cells wider than longer 13

13a. Tricomes in a thick brownish/yellowish sheath ..... *Lyngbya aestuarii*
13b. Tricomes in a thin, colorless sheath (if visible) 14

14a. Apical cells rounded ..... *Oscillatoria nigro-viridis*
14b. Apical cells pointed (hook-like)..... *Oscillatoria lloydiana*

II. Cyanobacterial species

A. Coccoid and colonial cyanobacteria

1. *Aphanocapsa* cf. *holsatica* (Lemmermann) Cronberg et Komárek 1994

Fig. 10c

This species is characterized for having spherical cells about 1 µm in diameter, which are densely arranged in irregular colonies. This was a rare species, probably because it is planktonic. It was only seen in Station 1, during the preliminary studies.

2. *Aphanocapsa* cf. *litoralis* (Hansgirg) Komárek and Anagnostidis 1995

Fig. 12

The cells are rounded, with an olive green color and an approximate diameter of 3-4.5 µm. They can show granules and can be seen as pairs, forming a spherical colony. The colonies can be solitary or in groups. The sheath is firm, about 1 µm wide. Sometimes the cells can be seen individually, due to a breakage of this sheath.

This species was observed in all stations and hydroperiods (although it was rare). Komárek and Komárková-Legnerová (2007) have described a similar species from marshes with high salinity.

3. *Aphanothece granulosa* Gardner 1927 Fig. 11

The cells are oval to cylindrical. They have a length of 8 µm and a width of 4 µm, approximately. All the cells are arranged irregularly in the colony, having a common colorless sheath. The sheath is sometimes not visible. The color of the cells is almost always blue-green, but can vary from green-yellow to brown. Granulation is always visible. This species was described from Puerto Rico by Gardner (1927), and later by Komárek (2007) from saline salterns in Belize.

I have seen different morphotypes of *Aphanothece* for the Cabo Rojo salterns (Figure 5b). Nevertheless, for the sampling period, *A. granulosa* (Figure 6) was common. It usually dominated, after *M. chtonoplastes*, in all

the stations and under all the treatments. Thus, *A. granulosa* is considered one of the principal components of this microbial mat.

The identification of the species in this genus is difficult. Actually, Dr. Komárek considers this genus as heterogeneous and thinks that its delimitation will be helpful (Komárek, pers. comm., 2007). The most described species for hypersaline environments is *A. halophytica* Fremy in Hof et Fremy 1933. Nevertheless, “it does not exist a good original characteristics of Fremy, and the name occurs in literature in usually different concepts” (Komárek, pers. comm., 2007). Gardner (1927) also reported *A. baccilloidea*, *A. cylindracea*, and *A. opalescens* for Puerto Rico, but these are too small or too big to be the species that I describe in my samples.

4. *Chlorogloea gessnerii* Schiller 1956 Fig. 13

The cells are blue-green (sometimes grayish or pale olive green), about 3-3.5 µm long and 2-2.5 µm wide. They are polygonal, arranged in rows at the margin and in an irregular form (sometimes radially) at the center of the colony. It seems as if small colonies are grouped to form a larger one with a single common sheath. The arrangement of the colonies can also form a characteristic pseudo-parenchymal structure.

Chlorogloea gessnerii was usually present (although it was rare) in all the stations and treatments. The colonies were large, sometimes reaching 1 to 2 mm length. Komárek and Anagnostidis (1998) include in their work a report of this species from Schiller (1956) from salty waters in Isla de los Aves,

Venezuela. Nevertheless, because of its locality, Komárek and Anagnostidis (1998) suggest revision for that identification.

5. *Chroococcus pulcherrimus* Welsh 1965 Fig. 14

The cells are hemispherical, arranged in pairs within a common, colorless sheath. The diameter is about 31-34 µm. The color may vary from blue-green, grayish to pale olive-green. Also, they present homogeneous granules throughout the entire cell.

In this study, *C. pulcherrimus* was more abundant in Hydroperiod II (dry season and low levels of seawater). This species was reported from marshes with medium or high salinity in Belize (Komárek *et al*., 2005; Komárek and Komárková-Legnerová, 2007). It is often confused with *C. turgidus* (i.e. described from Puerto Rico by Gardner, 1932, and the Antilles by Koster, 1963). Nevertheless, they differ ecologically. The former is described from tropical regions, while the latter is described for temperate Nordic regions, and these data have been corroborated with molecular tests (Komárek *et al*., 2005; Komárek and Komárková-Legnerová, 2007).

6. *Cyanosarcina* cf. *thalassia* Anagnostidis and Pentazidou 1991 Fig. 15

Cells are rounded to squared, but when they become irregular can vary from 2-3 µm wide to 3-4 µm long. Their color varies from blue green to pale olive green. The cells are arranged within a sheath. The colonies are near each other, forming aggregates that, sometimes, can be seen

macroscopically. This species was seen at each station and during each hydroperiod (although not abundant).

7. cf. *Hydrococcus* Kützing 1833 Fig. 10a

It has oval to polygonal cells, arranged in irregular vertical rows, within a common sheath. Cells are about 4 µm wide and 6 µm long. This species is described for freshwater, but it is still not well known (Komárek, 1998). There are some species under revision, including some from marine habitats. In this work, it was seen once, in Station 1, during the preliminary studies.

8. *Johannesbaptistia pellucida* (Dickie) Taylor and Drouet in Drouet 1938 Fig. 16

Even though this organism forms uniseriate pseudo-filaments. it is a coccoid, colonial cyanobacteria. The cells are discoid, arranged in pairs, with an approximate width of 5 µm and a length of 1 µm. The end cells are wider (3µm).

This species was observed rarely in the preliminary studies, and was rare in the investigation samples. It was also described from freshwater in Puerto Rico by Almodóvar (1963), by Komárek and Komárková-Legnerová (2007) from marshes with high conductivity in Belize, and from the Antilles by Koster (1963).

9. *Merismopedia* cf. *affixa* Richter 1895 Fig.17

Cells are less than 1 µm in diameter. Usually arranged perpendicularly in flat colonies of 4 cells. Their color is almost always pale blue-green. This was a rare species that was observed in the preliminary studies, but not in the investigation samples.

B. Filamentous cyanobacteria

1. *Arthrospira* sp. Fig. 18

The cells are cylindrical, characterized by forming helicoidal filaments. No sheath is evident. This species was rare and appeared once in Station 1, during preliminary studies. No measurements could be taken.

2. *Lyngbya aestuarii* Liebman ex Gomont 1892 Fig. 19

The cells are cylindrical, of approximately 9.6 µm of diameter and 2.4 µm high (three times wider than high). These cells are organized vertically to form solitary tricomes without ramifications. The apical cells are more or less rounded. The mucilage sheath that surrounds to the tricome is thicker than in the other filamentous species in the samples, with an approximated thickness of 3.44 µm. The color of this mucilage goes from yellow to brown, possibly due to the presence of scytonemin, a pigment which function is to protect the cell against the ultraviolet radiation. Therefore, although its tricome is of blue-

green color, the filament that is observed by means of the light microscope appears to be brown.

In this study, *Lyngbya aestuarii* was considered a rare species, found only in Station 2. Nevertheless, when the microbial mats is forming in the shore of Laguna Candelaria with the highest salinity (between the second and third station), we can observe filamentous aggregates dominated by this species. This species was previously described for Puerto Rico by Gardner (1927, 1932). Koster (1963) reported this species from the Antilles, and indicated that it lives in brackish and marine waters, brines, sea-shores, thermal waters (some times freshwater), and on stones logs and soils.

3**.** *Microcoleus chthonoplastes* Thuret ex Gomont 1892 Fig. 20

The cells are cylindrical, with an approximate diameter of 3 µm and a high of 5 µm. They show a blue-green color, olive green, or dark green. In addition, constrictions in the cell wall are observed. Their apical cells are conical. The tricomes are not branched and are joined together in a common sheath of colorless mucilage.

This species is the one that dominates in the microbial mat for each station, among treatments, and over time. Casillas-Martínez *et al.* (2005) reported it previously from the Cabo Rojo salterns. Also, Koster (1963) described it from the Antilles, in brackish and marine waters, brines, and sea-shores. In 1927, Gardner described to *M. amplus* for Puerto Rico. Nevertheless, this species develops only in terrestrial habitats. Also, it does

not have constrictions, the opposite from the species in our samples, *M. chthonoplastes*.

4. *Oscillatoria lloydiana* Gomont 1899 Fig. 21
Phormidium lloydianum (Gomont) Anagnostidis et Komárek 1988

It has cylindrical cells of approximately 7.5-8 µm of diameter and 2.5-2.8 µm of high. The terminal cells are higher than the rest of the cells in the tricome. The apical cell is pointed and arched, forming a hook. The tricomes are not branched and the mucilage sheath that surrounds them cannot be appreciated. Necritic cells can be observed in most cases.

O. lloydiana does not dominate in all the stations at Laguna Candelaria, but it was common in station 2 and appeared in a single event of sampling in station 3.

5. *Oscillatoria nigro-viridis* Thwaites ex Gomont, 1892 Fig. 22

This species has cylindrical cells of 9.2-11.9 µm of diameter and 2.5 – 4.5 µm of high. When tricomes are formed, the constrictions in the cell wall are visible. Compared to *O. lloydiana*, the cells are wider (over 1.2 µm). In addition, their apical cells are more or less rounded (non-pointed) and the terminal cells are not higher than the central ones (although they are a little bit narrowed). Like *O. lloydiana*, the tricomes are not branched.

O. nigro-viridis was considered a rare species in this study. Nevertheless, it was present when the color of the microbial mat was dark green, almost

black, which occurred only at station 2. This species was previously described from Puerto Rico by Gardner (1932).

6. *Spirulina subsalsa* Oersted ex Gomont, 1892 Fig. 23

The genus *Spirulina* forms tricomes with a helical arrangement and almost never the division between the cells can be appreciated. The width of the tricomes of *S. subsalsa* varies from 1 to 2 µm and the helix formed by it can have a width from 2.13 to 3 µm. The turns in the helix almost never show divisions (cells generally are tightly united). The apical cells are rounded.

This species was described from Puerto Rico by Gardner (1927) and from the Antilles by Koster (1963) from quiet, brackish waters, waters with high content of salt, floating among other algae, or in lower littoral beds. In our study, *S. subsalsa* appeared mainly during hydroperiod II, for the days with lower disturbances (i.e. before treatments and in the third day after treatment).

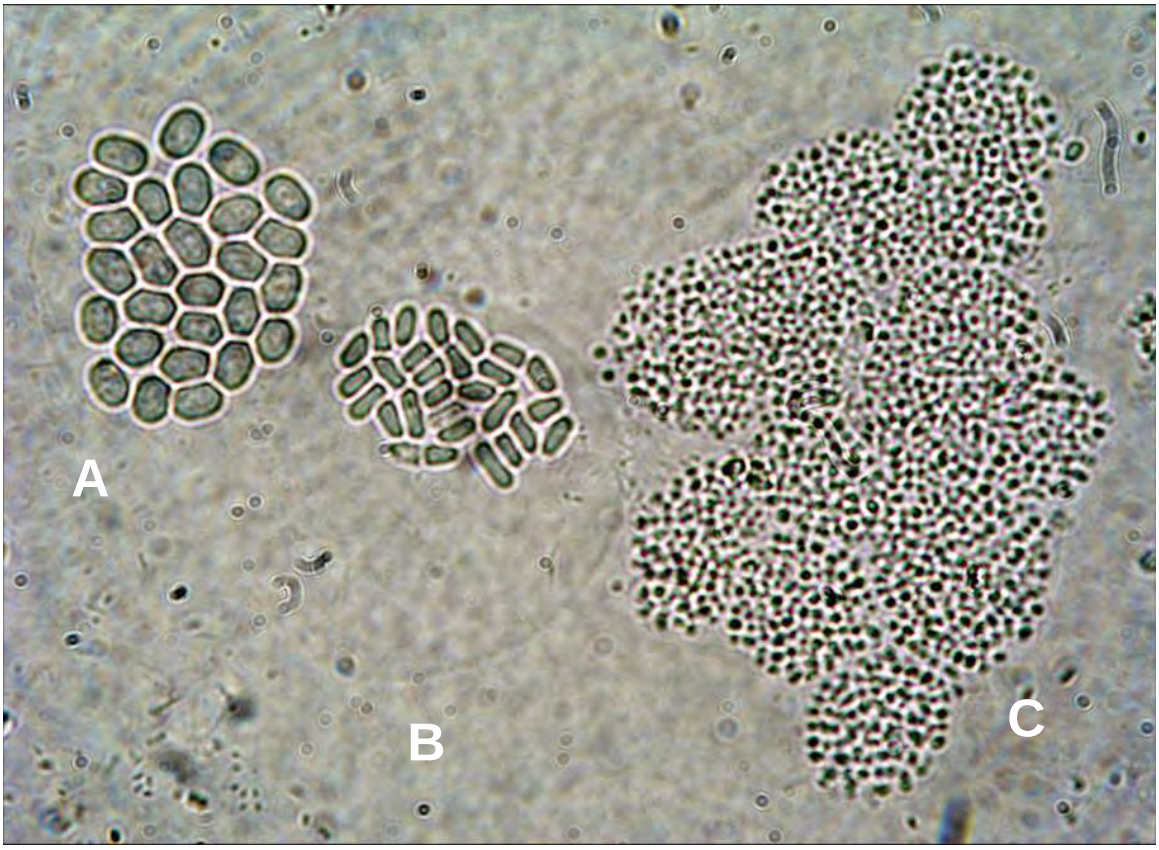

Figure 10. Coccoid cyanobacteria. (A) cf. *Hydrococcus*, (B) *Aphanothece* sp., and (C) *Aphanocapsa* cf. *holsatica*.

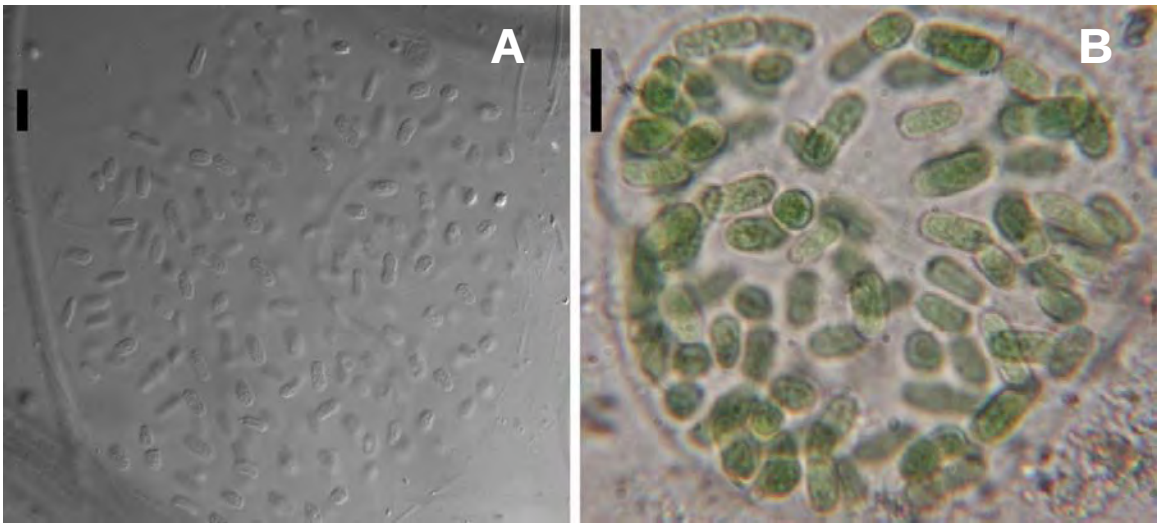

Figure 11. *Aphanothece granulosa*. Note the granules through the entire cell. (A) Phase contrast with Nomarski optics; (B) Light microscopy. Scale bars = 10 µm.

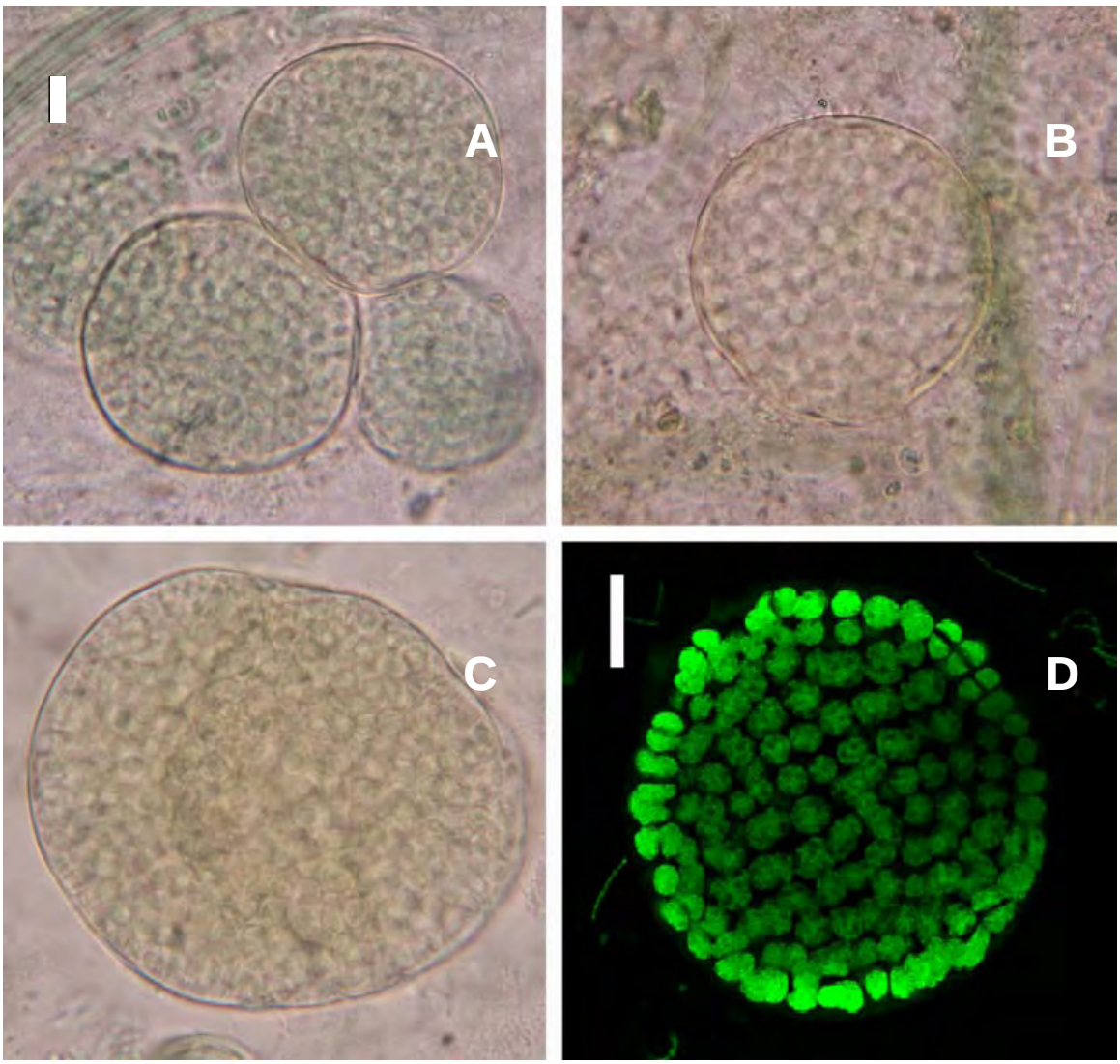

Figure 12. *Aphanocapsa* cf *litoralis*. Note the arrangement in pairs of the cells. (A-C) Light microscopy, same scale bar; (D) confocal microscopy. Scale bars = 10 µm.

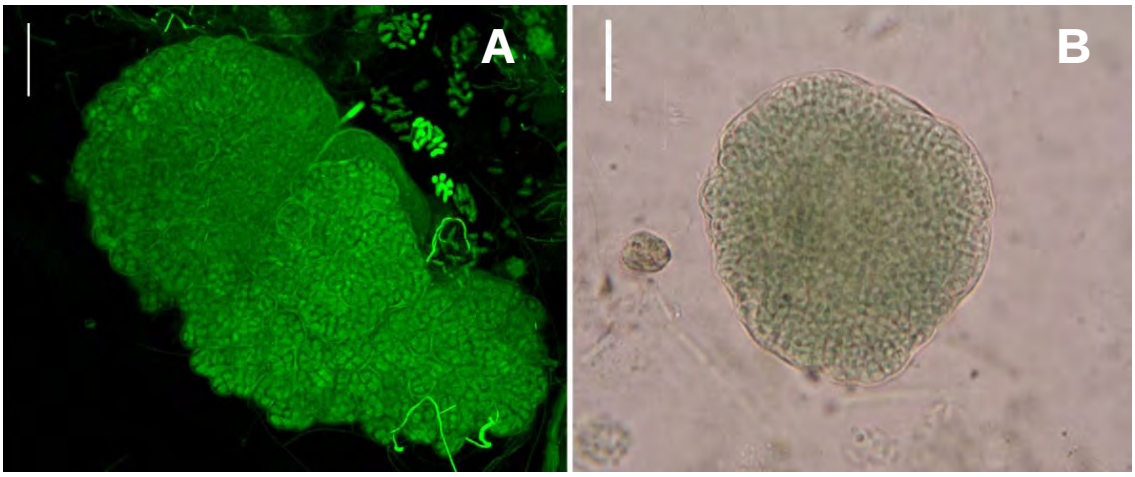

Figure 13. *Chlorogloea gessnerii.* Note the pseudo-parenchymatous form. (A) Confocal microscopy; (B) Light microscopy. Scale bars = 10 µm.

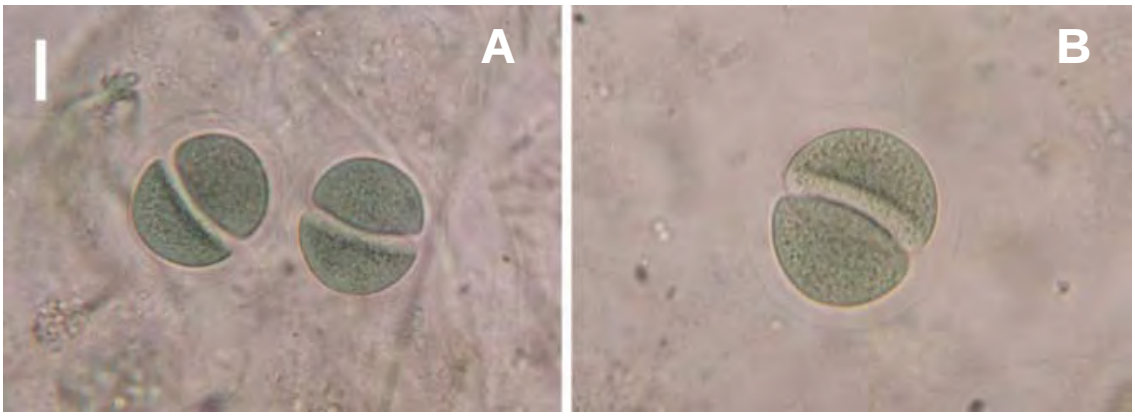

Figure 14. *Chroococcus pulcherrimus* using light microscopy. Scale bar = 10 µm for each picture.

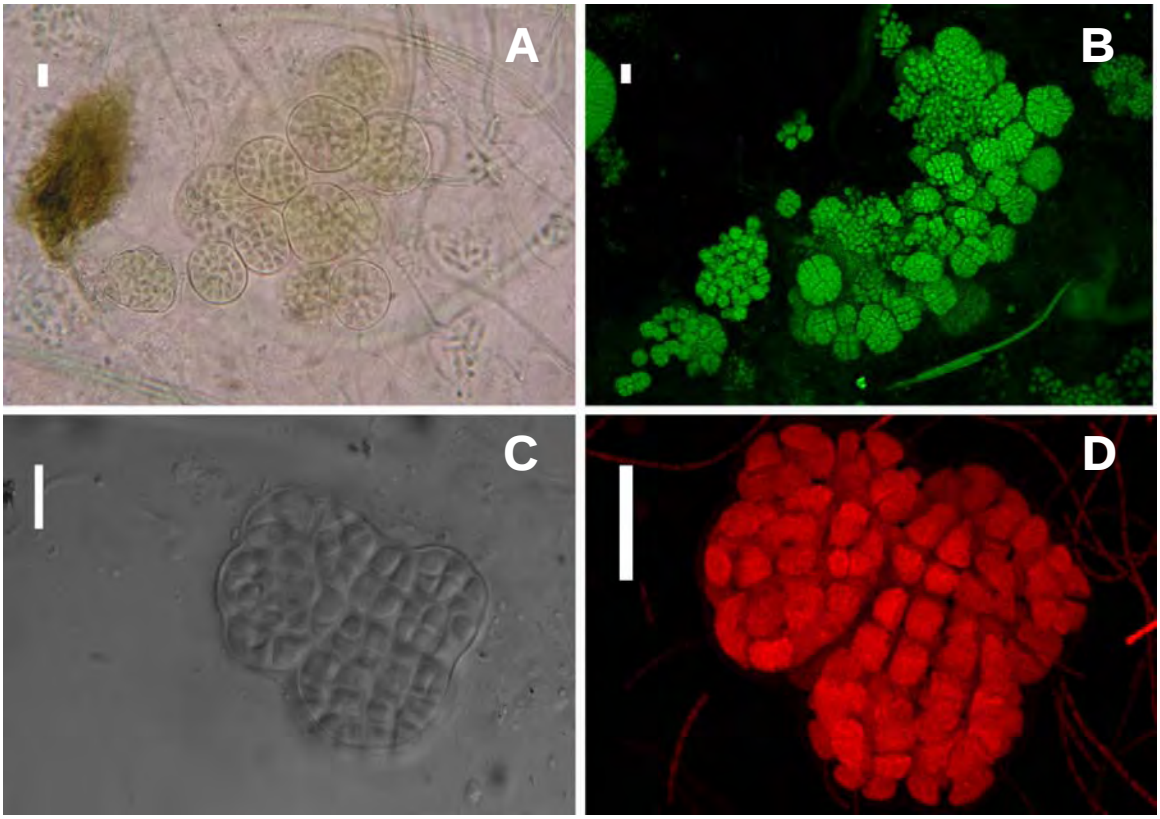

Figure 15. *Cyanosarcina* cf. *thalassia.* (A) Light microscopy; (B and D) confocal microscopy, and (C) phase contrast and Nomarski optics. Scale bars = 10 µm.

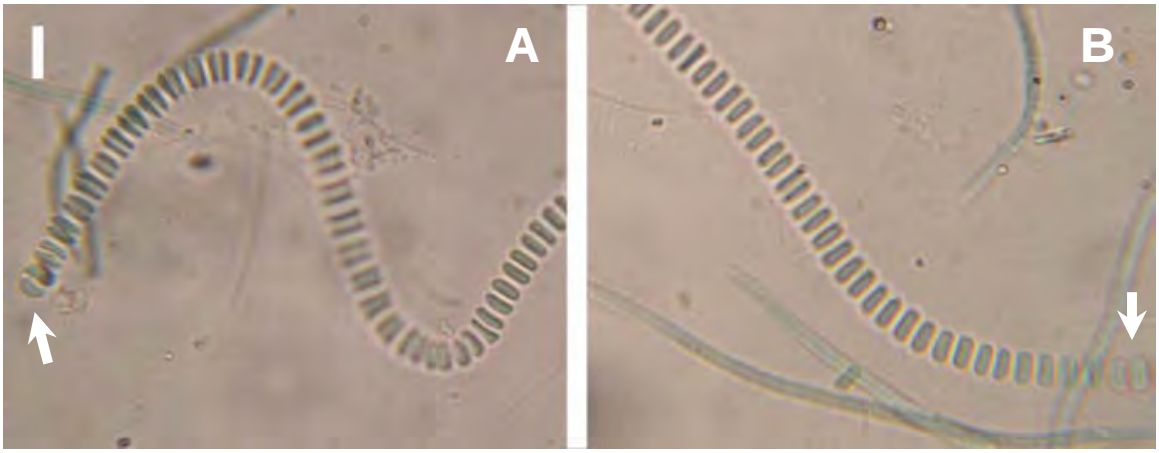

Figure 16. *Johannesbaptistia pellucida*. Note that the end cells are wider (white arrow). Both pictures were taken under light microscopy. Scale bar = 10 µm for each picture.

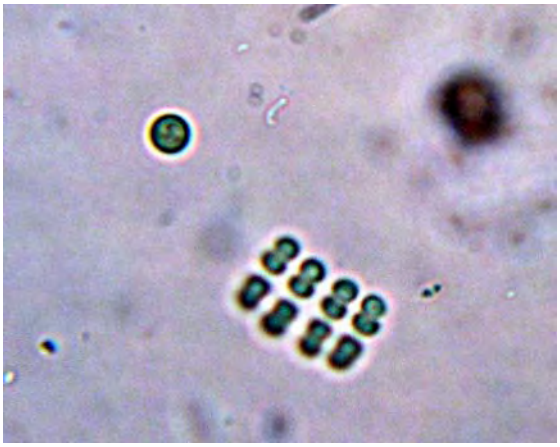

Figure 17. *Merismopedia* sp. Observe the arrangement of cells in groups of 4.

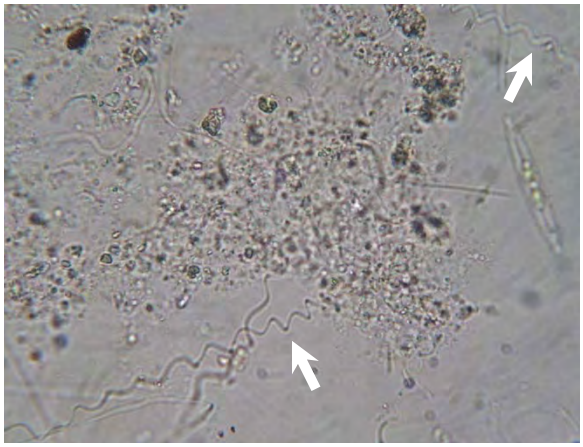

Figure 18. *Arthrospira* sp. (white arrows), characterized for the formation of helicoidal filaments.

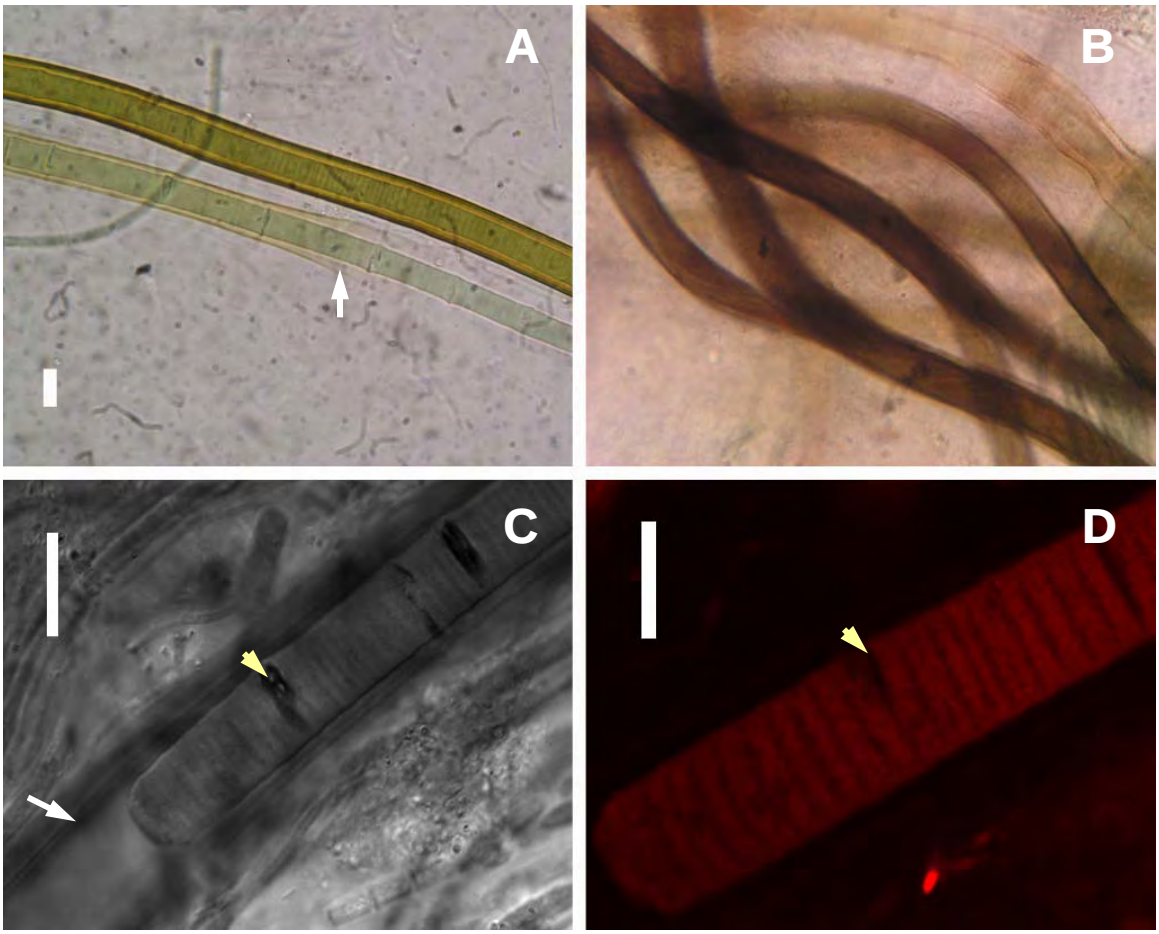

Figure 19. *Lyngbya aestuarii.* (A-B) Light microscopy, same scale bar for each picture; (C) phase contrast and Nomarski optics; (D) confocal microscopy. White arrows show the mucilage sheath, while yellow arrowheads show the necritic cells. Scale bars = 10 µm.

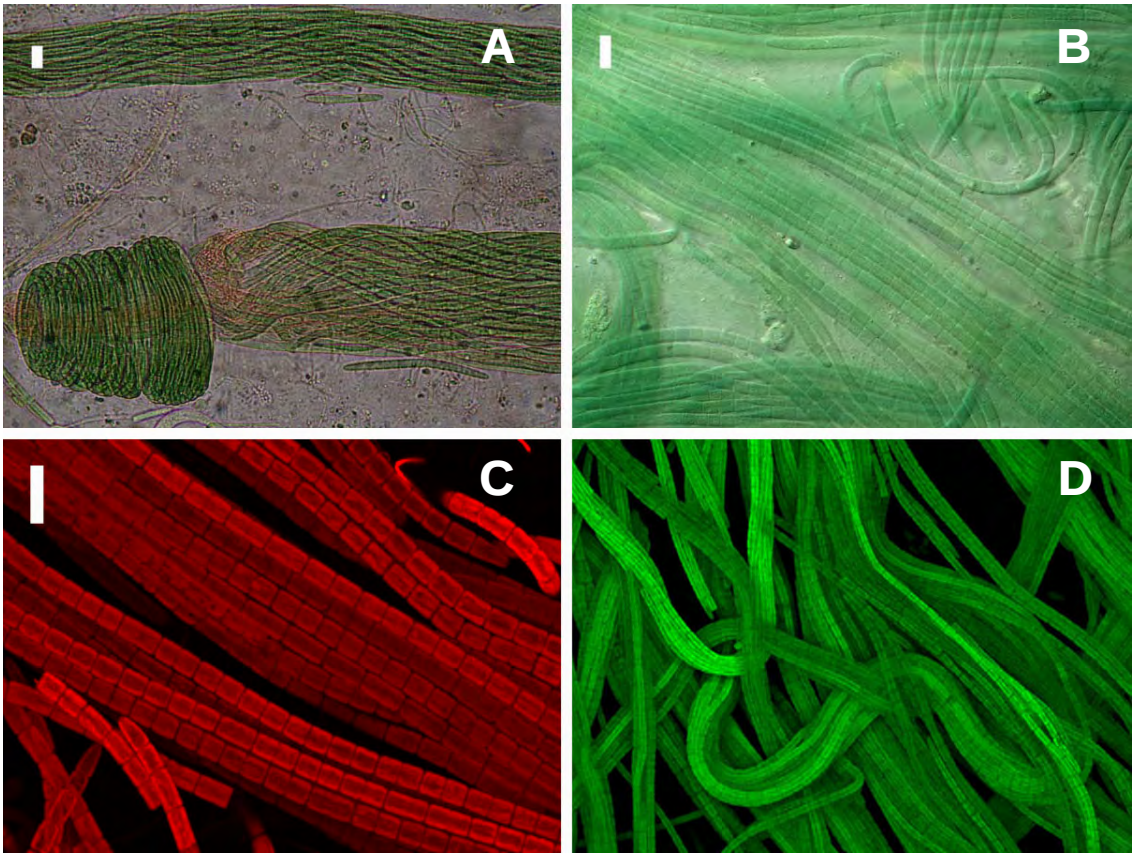

Figure 20. *Microcoleus chtonoplastes*. (A) light microscopy; (B) light microscopy with phase contrast and Nomarski optics; (C-D) confocal microscopy. Scale bars = 10 µm for A-C.

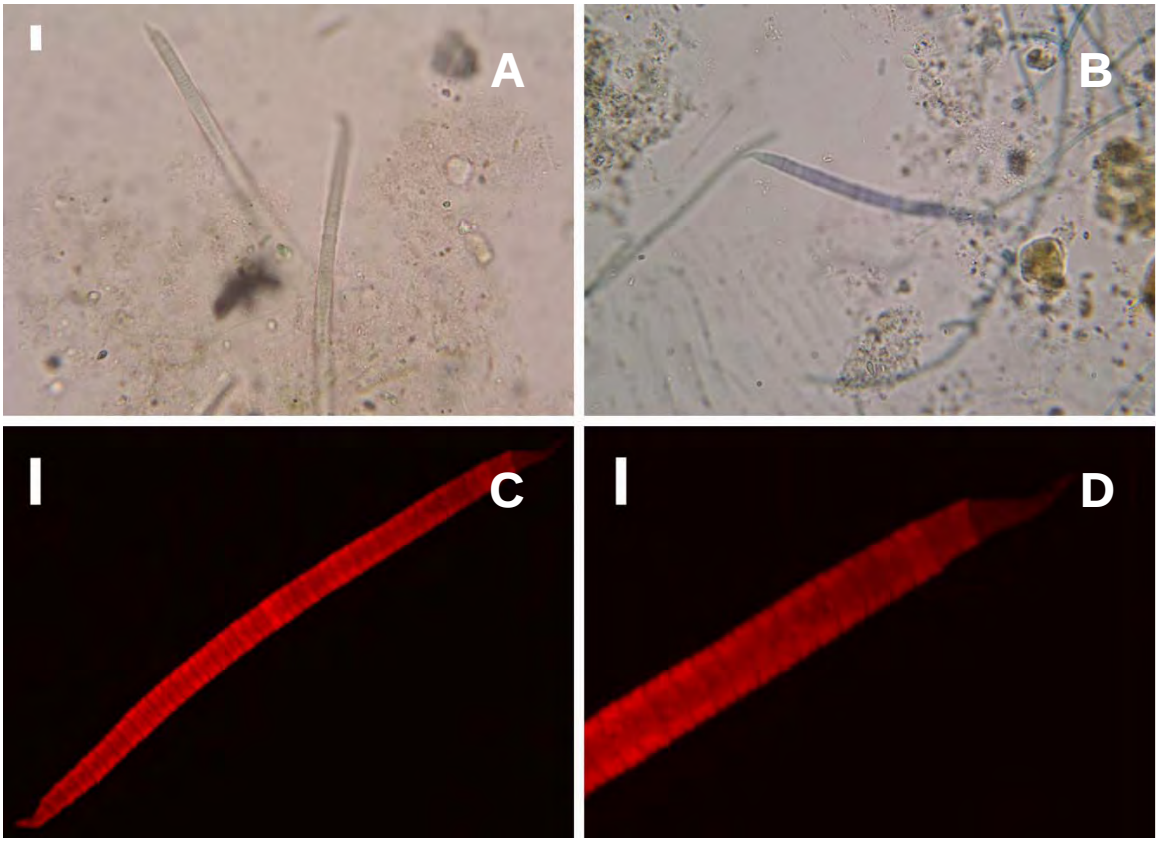

Figure 21. *Oscillatoria lloydiana*. Note the differentiation of the apical cell. (A-B) light microscopy, same scale bar for each picture. (C-D) confocal microscopy. Scale bars = 10 µm.

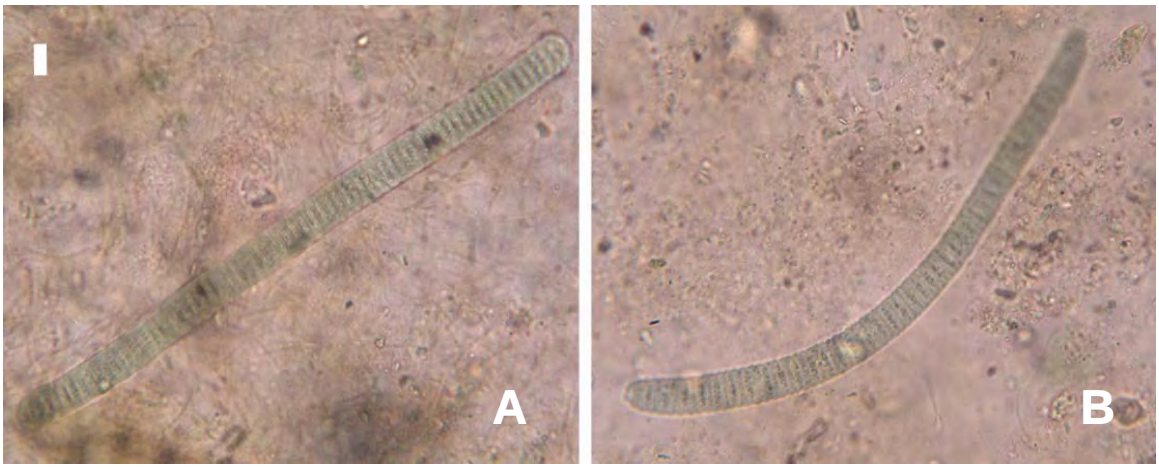

Figure 22. *Oscillatoria nigro-viridis*. (A-B) Light microscopy with the same scale bar (10 µm) for each picture.

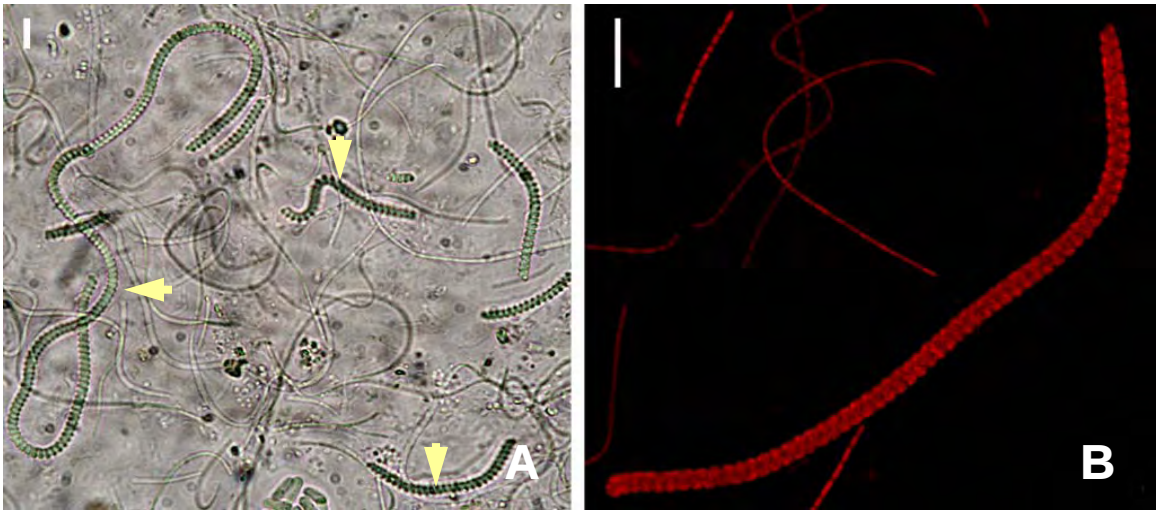

Figure 23. *Spirulina subsalsa*. (A) Mixed bacteria under light microscopy. Yellow arrowheads show *S. subsalsa*. (B) Confocal microscopy. Scale bars = 10 µm.

DISCUSSION

The species composition, density of photosynthetic organisms and levels of secretion of polysaccharides by bacteria change seasonally in response to changes between summer and winter in salinity, water cover, light intensity and temperature (Jorgensen and Revsbech, 1983). Laguna Candelaria, in the Cabo Rojo Salterns, is exposed to constant variations in water depth because of the seawater entrance and the amount of rainfall that it receives during dry or rainy seasons. This can lead to variations in the concentration of salts, varying also the biodiversity of cyanobacterial species in the microbial mats.

During this study, two events of dry season and one in the rainy season were studied. The cyanobacterial species richness and biovolume were analyzed in three stations along a gradient of salinity during each hydroperiod. The statistical tests showed significant differences in the cyanobacterial biovolume among the dry season of January and the other 2 hydroperiods, while there were no significant differences between the dry season of April and the rainy season in November. Even when these last hydroperiods were similar in terms of cyanobacterial biovolume, they had differences in the species richness due to the physicochemical parameters.

As expected, during the rainy season, the water depth was high in the majority of the stations, with salinities ranging from 63-78 ppt. Meanwhile, during the dry season, the water depth decreased, increasing the salinity to values ranging from 81-97 ppt at the majority of the stations. The negative relation of these two factors was similar to the one reported by Casillas-Martínez *et al*.

(2005) (when the rainfall decreased, the salinity increased), but the range of salinity during the dry season was lower in our study. In contrast to the work of Casillas-Martínez *et al*. (2005), the increase in salinity allowed the dry hydroperiod to have higher species richness and biovolume of cyanobacteria in relation with the rainy one, thus, our hypothesis is accepted. There are two reasons that can explain this behavior: 1) there are cyanobacteria that have the capacity of acclimation to extreme conditions (Komárek, 2003), while 2) multicelular organisms are often excluded from hypersaline environments (Stal, 1994). Thus, the absence or limited activity of grazers (or competitors) can promote the development of the cyanobacteria that can tolerate higher salinities in the microbial mats. During both hydroperiods, *Microcoleus* was the dominant species in all stations, followed by *Aphanothece*. *Chlorogloea* and *Oscillatoria* were also present in both hydroperiods, but they were more abundant during the dry one. *Aphanocapsa*, *Cyanosarcina*, and *Spirulina* appeared only during dry conditions, thus, increasing the diversity in this hydroperiod.

In January, the dry season was marked by 0 cm of water depth in Station 2, and by less than 1.5 cm in Stations 1 and 3. The salinity at that moment exceeded 90 ppt (salinity in Station 2 could not be measured). After adding the treatments, the salinities decreased to less than 50 ppt in the majority of the cases, especially in Station 2. This promoted the overgrowth of the coccoid cyanobacteria *Aphanothece*, changing the composition of the mat for that station (formerly dominated by a filamentous one). Thus, a dry season (April) and a rainy season can be similar in abundances, but when conditions are too extreme in the

dry season (i.e. water levels depleted and high salinity, as in January), the algal composition in the mat changes dramatically. A shift in the abundant type of cyanobacteria in the microbial mat may represent a change in the whole trophic chain. Some grazers such as ciliates, copepods, and other metazoans prefer to eat filamentous cyanobacteria than coccoid ones. This is because coccoids often have a thick sheath of mucilage with no autotrophic activity which implies that the grazer will spend energy eating something that is not going to provide as much energy as a whole photosynthetic cell with less or no mucilage. Shifts in the community of grazers may imply shifts in other feeders, like insects or the crustacean *Artemia*, which function as food for the migratory birds as well. Thus, it will be important to avoid extreme events like the dry season in January.

In general, the biodiversity in Laguna Candelaria is similar to those in other salterns. Works from Sabkha, Spain, Sinai, Mexico, and Chile reported the filamentous cyanobacterium *Microcoleus chtonoplastes* as the dominant one (Krumbein *et al*., 1977; Friedman and Krumbein, 1985; Javor and Castenholz, 1988; Esteve *et al.*, 1994; Urmeneta *et al*., 2003; Dermagasso *et al.*, 2003). Moreover, Krumbein *et al.* (1977) and Jorgensen and Revsbech (1983) described *M. chtonoplastes* as the dominant cyanobacteria during the rainy season in winter, but there was a shift to coccoid forms like *Aphanothece* and *Aphanocapsa* during dry seasons in summer. The most similar description of biodiversity to Laguna Candelaria's was the one from the Gavish Sabkha, where they reported the presence of cyanobacterial species such as *Aphanocapsa litoralis*, *Johannesbaptistia pellucida*, *Lyngbya aestuarii*, and *Oscillatoria nigro-*

viridis. Esteve *et al.* (1994) found the coccoid cyanobacteria to be the more abundant during the higher salinities. In this study, even when the coccoid *Chlorogloea* appeared more frequently at higher salinities, that statement is not true because *M. chtonoplastes* was still the most dominant species at the highest salinities.

Microcoleus chtonoplastes is discussed as a key species in many of the microbial mats for being one of the pioneers in the colonization. Its polysaccharide capsule has uronic acid, which functions as glue for sediments (Stal, 1994). *Oscillatoria* and *Lyngbya* are also considered to live in young microbial mats; they provide nutrients to the sediments by nitrogen fixation (Gemerden, 1993; Stal *et al*., 1994). These filamentous cyanobacteria begin to form a network, which trap sediment particles and contribute to the consolidation of the mat. Then, coccoid cyanobacteria, as well as diatoms, bind to the microbial mats by means of their polysaccharides. Nevertheless, *M. chtonoplastes* has more probabilities to prevail in the mat because it remains metabolically active during the nights in the mats (Gemerden, 1993), and also accumulates iron in its polysaccharide sheath. The accumulation of iron may protect the organism against deleterious concentrations of sulfide and also may scavenge oxygen, avoiding toxic conditions inside the cell (Stal, 1994).

In this study, we observed that cyanobacterial communities in Laguna Candelaria are similar to other hypersaline lagoons (Table 2). Its algae succession was the expected one. In addition to *M. chtonoplastes*, patches of *Oscillatoria* were found near Station 2, indicating the formation of young mats.

This was possibly the reason for that station to change in composition when a strong disturbance occurred in January, because the mat was not well consolidated and stabilized. Nevertheless, in stations 1 and 3 nitrogen fixers were not abundant, indicating a more mature status. *M. chtonoplastes* and *A. granulosa* prevailed, even when the disturbances occurred, and the appearance of new species during the dry seasons was probably due to favorable changes in salinity, rather than by an unstable status of species in the microbial mat.

CONCLUSIONS

1. The addition of seawater and distilled water (treatments) did not induced significant changes in cyanobacterial biovolume in the mats.

2. Significant differences in cyanobacterial biovolume between dry and rainy seasons may occur when the water levels are depleted. The created disturbance may change the composition of the mat, from one dominated by filamentous cyanobacteria to one dominated by coccoid forms.

3. Species richness and biovolume of cyanobacteria increased with high salinities possibly due to a reduction in grazing pressure.

RECOMMENDATIONS

A. To have, at least, three replicates of the treatments in the same station (9 aquaria).

B. To compare the biodiversity of cyanobacteria during hydroperiods of little rainfall and high levels of seawater, and high rainfall and low levels of seawater with the hydroperiods discussed in this work.

C. To measure the pigments to know the algal composition in the mat (proportion of cyanobacteria:diatoms:green algae).

D. Fix cyanobacteria with 2% of formaldehyde. Komárek (2003) explained that a higher percent of this preservative can affect the morphology of the cells.

E. Obtain pure cultures to do molecular tests to corroborate the identification of the species, in addition to study their physiology.

F. Different studies can be done, like:
   a. Studying the diet of the grazers in Laguna Candelaria to see if they prefer coccoid or filamentous cyanobacteria.
   b. Comparing the abundance of algae with the abundance of insects and the amount of birds that inhabit at the Refuge.
   c. Comparing the communities from Laguna Candelaria with the ones that live in Fraternidad.
   d. Studing the vertical distribution of the cyanobacteria.
   e. Performing spectrometry on the mats, making classifications according to the pigment absorbance and transmittance, and following up by determing the temporal and spatial changes in the algal composition.

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CHAPTER II: Diatoms

INTRODUCTION

The diatoms are unicellular algae that possess an unusual, and unique, feature of a cell wall composed of silicon dioxide, the principal component of most types of glass (Stoermer and Julius, 2003). This cell wall is resistant to many changes (e.g. pH and temperature) and can maintain its form during long periods [fossil records show that benthic forms existed more than 60 millions of years ago (Round *et al*., 1990)]. For that reason, diatoms have been used in paleolimnology as indicators of environmental changes over time (Mandra, 1972; Mori 1986; Winkler, 1988; Round, 1990; Anderson, 1990; Fritz *et al*., 1991; Kashima, 1994; Vaultonburg and Pederson, 1994; Dixit and Smol, 1995; Reid, 1995; Chavez and Haberyan, 1996; Stoermer, 1998).

The identification and classification of diatoms can be done with live material, with emphasis in the form of the chloroplasts, but it principally relies in the characteristics of the silica cell wall. The size, ornamentation, number of striae and the form of the valves is extremely important for the identification to genera and species. The frustule (whole cell wall) is composed of two valves: the epitheca and the hypotheca (Figure 24). The hypotheca is slightly smaller than the epitheca to fit one valve into the other (like a Petri dish or a shoe box). The valves are joined by the girdle bands, also composed of silica. The first girdle band, the valvocopulae, can form an extension to the inner part of the valve, named septa, which can be used to distinguish some genera (e.g. *Tabellaria*). In the same form, the mantle of the valve can make an extension or pseudo-septum,

named partecta (Figure 25), which is divided in loculi and sometimes have chambers. The arrangement and size of the loculi can be useful to identify some species (e.g. *Mastogloia* spp.). The raphe is also important for the classification of diatoms (Figure 24). Its principal function is to help in the locomotion of the cell. There are frustules with no raphe (araphids), with raphe in both valves (raphids) and with a raphe in just one valve (monoraphids). The position of the raphe can vary with the genera. Usually the raphe is located at the center, but sometimes it is surrounding the whole valve or, as in *Nitzschia* or *Hantzschia* spp., it is located at one side of the valve and has to be supported with a structure named fibulae. Diatoms can have other structures [e.g. processes (rimoportulae and fultoportulae, see Figure 26), linking and separation spines, apical porefield, among others] depending in the type of environment in which they develop.

Diatoms can live as symbionts of cyanobacteria (Venrick, 1974; Villareal, 1991), other algae or plants. Also, they can be found solitary or in colonies in most places that contain water: oceans, freshwater, damp soils, rocks, sand, extreme environments, among others. In freshwater and marine habitats, they are of particular importance because can be used as water quality indicators (Patrick, 1973; Lowe, 1974; Descy, 1979; Schoeman, 1979; Lange-Bertalot, 1979; Zolan, 1981; Kobayasi and Mayama, 1989; Guzkowska and Gasse, 1990; Eloranta, 1994; Reid *et al*., 1995; Kelly *et al*., 1995; Dokulil *et al*., 1997; Kelly, 1998; Gomez, 1998; Spaulding and Elwell, 2007). Besides, detection of certain marine or freshwater species (e.g*. Pseudonitzschia* spp., *Amphora coffeaeiformis*) can indicate the presence of domoic acid, a neurotoxin that causes the amnesic

syndrome (Maranda *et al*., 1990; Fritz *et al.*, 1992; Horner and Postel, 1993; Villac *et al*., 1993; Wekell *et al*., 1994; Vrieling *et al*., 1996; Horner *et al*., 1997; and Cusack *et al*. 2002).

In the ocean, as the diatoms dominate the productive zones, it is estimated that they contribute with up to 45% of the total oceanic primary production (Mann, 1999) and 20-25% of the worldwide net primary production (Werner, 1977). Besides of the large amount of oxygen that they produce, they are great primary producers, as they serve as food for many organisms. Among them, we can find ciliates, dinoflagelates, copepods, rotifers, mussels, shrimps larvae, and other grazers and filter-feeders (Castenholz, 1961; Werner, 1977; Tillmann, 2004).

Diatoms can also be found in hypersaline environments. Some studies describe their distribution and salinity tolerance in this kind of habitat (Gerloff *et al*., 1978; Ajaili *et al*., 1986; Noël, 1986; Clavero *et al*., 1994; Compère, 1994; Gell and Gasse, 1994; John, 1994a; Clavero *et al*., 2000). Generally, they are found forming microbial mats in the benthos. Benthic diatoms not only contribute to oxygen production, but sometimes function as primary colonizers, stabilizers of sediment by the secretion of mucilage, and also form a barrier against desiccation, allowing the existence of greater microbial diversity (Siqueiros-Beltrones, 1988). It is also thought that diatoms contribute to the stromatolite accretion of older microbial mats by adding their siliceous frustules and by trapping other mineral grains such as calcite in their attachment structures and substances (Winsborough and Seeler, 1986).

Studies about diatoms in Puerto Rico

Hagelstein (1938) made a survey of the diatoms in Puerto Rico and the Virgin Islands. He reported diatoms from different habitats, including freshwater, brackish, and marine specimens. Later, Margalef (1957, 1961, and 1962) included the diatoms in reports of the phytoplankton on the coasts of Puerto Rico, and Lyons (1973) reported some diatoms in a study of Yabucoa Bay. A taxonomic study on the marine diatoms within the suborders Coscinodiscineae and Rhizosoleniineae was conducted by Navarro (1981) and later, Romero and Navarro (1999) reported the new species *Cocconeis caribensis* for La Parguera in Lajas. Bryan (2001) also reported on the diatoms of the Mameyes River for the USDA Forest Services. Recently, Hunter (2007) reported some diatoms as environmental indicators in three bioluminescent bays in Vieques.

Diatoms in Cabo Rojo Salterns

There is only one study (Navarro, 1988) that described the diatoms of the upper layer of the mat in the Cabo Rojo Salterns. Navarro identified and characterized the species *Licmophora normaniana* (Grev.) Wahrer, *Mastogloia braunii* Grun. [described previously by Hagelstein (1938) from San Juan harbor] and *Nitzschia lanceolata* W. Sm. He also noticed the presence of *Amphora* spp. in the samples. As we thought that the species richness had increased since that work, this study was designed to update Navarro's report by identifying and characterizing the diatoms in the superficial layer of the microbial mat in Laguna

Candelaria, and also provide a taxonomic key and an illustrated guide to these diatoms. Species of diatoms living in the hypersaline microbial mats from Cabo Rojo salterns must be adapted to high salinities, but grazers may not, thus, reducing the impact of predation by them. Our hypothesis was that if hydroperiods and/or stations have high salinities, then the diatom species richness will be high.

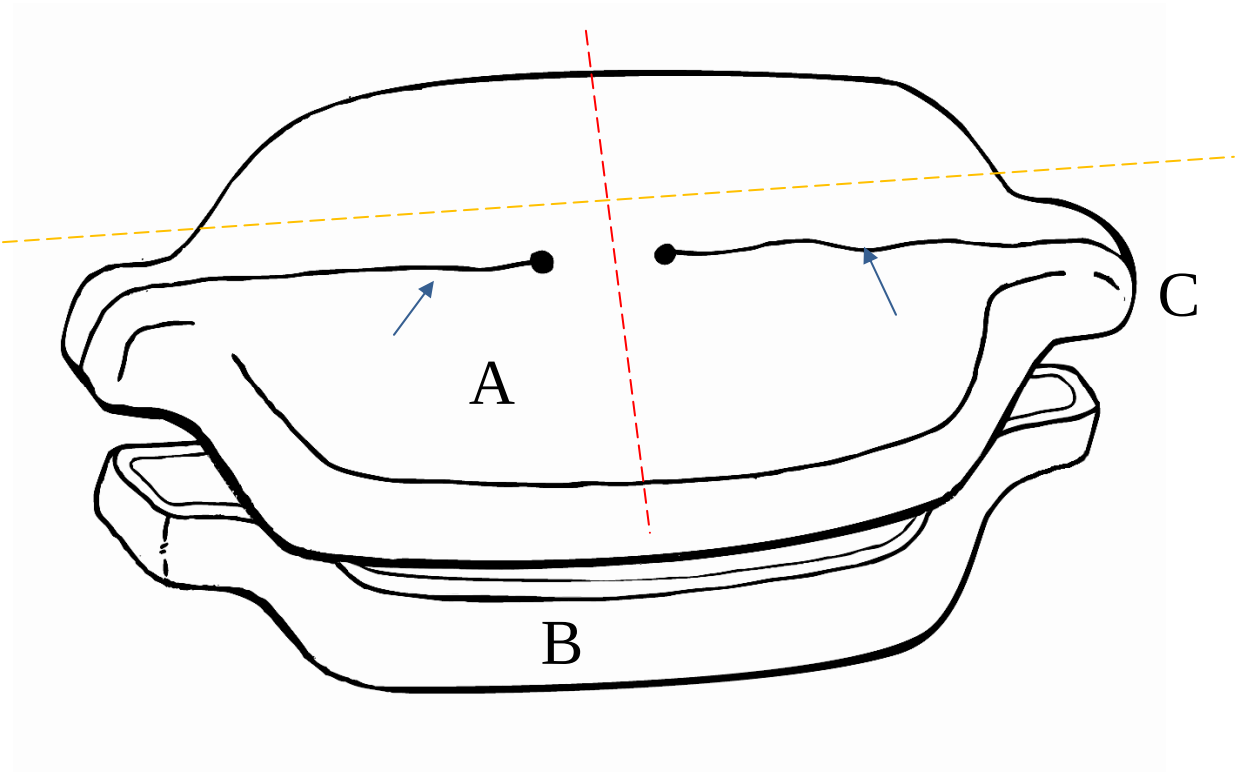

Figure 24. Diagram of a diatom frustule. The slightly bigger valve (A) is the epitheca, while the smaller is the hypotheca (B). The girdle bands (not shown) are located between both valves. The epitheca also shows a canal, named raphe (blue arrows). This frustule has capitate ends (C). Apical axis is shown with an orange dashed line, and the transapical axis is shown with a red dashed line. Drawing by Alex R. Rivera Hernández.

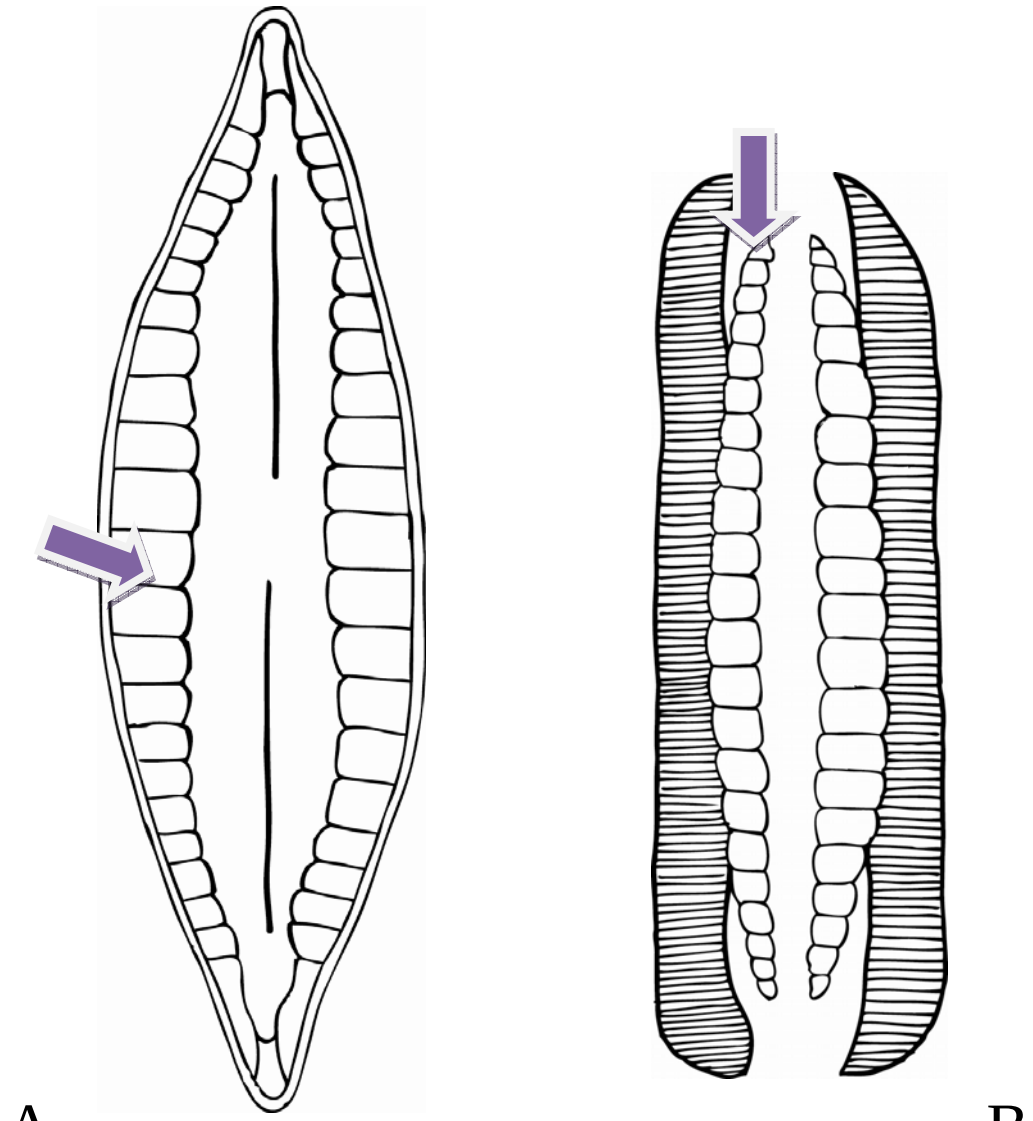

Figure 25. Partecta (arrows) in valve view (A), and girdle view (B). Drawing by Alex R. Rivera Hernández.

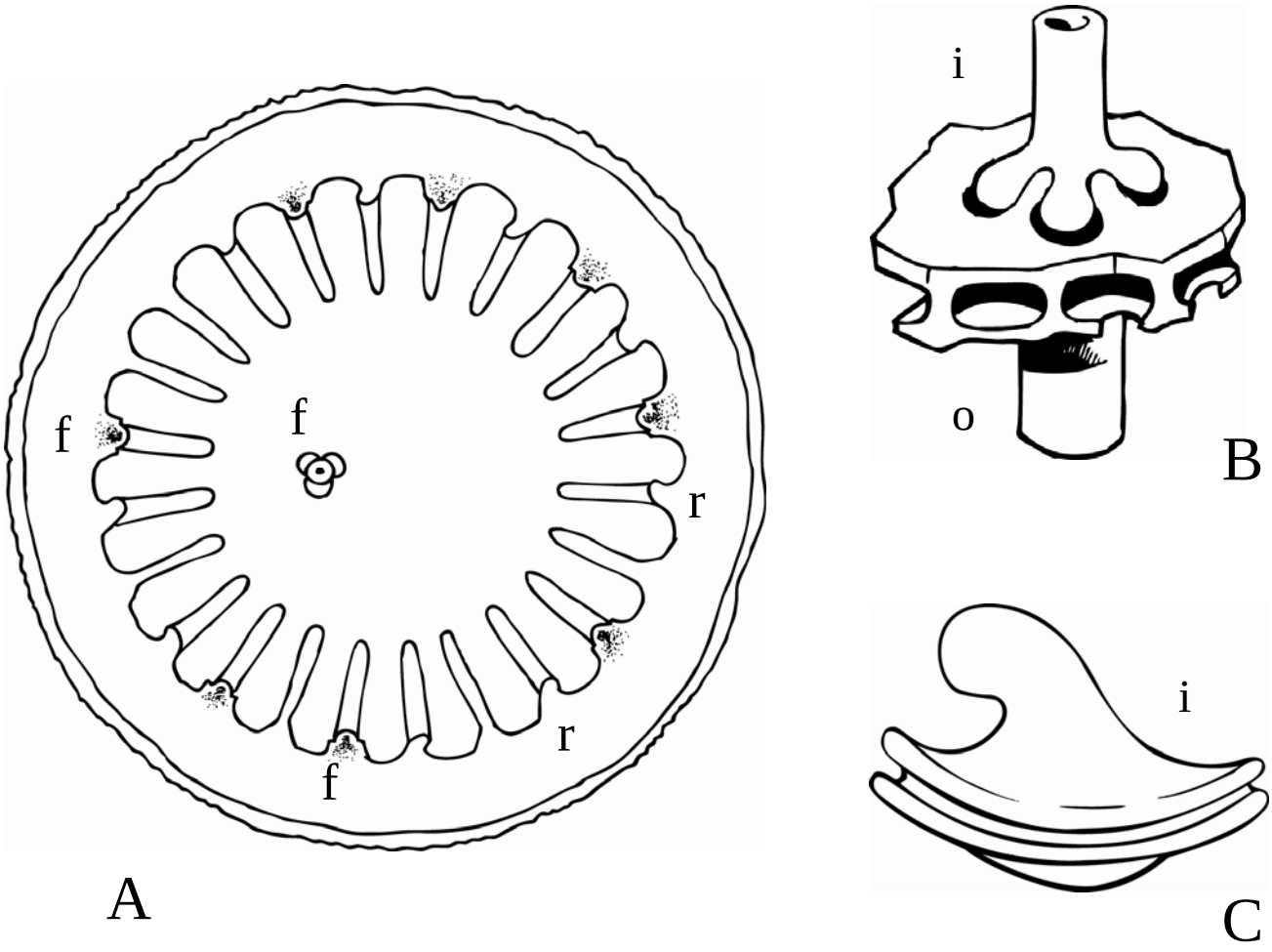

Figure 26. Process in a diatom. Figure A shows the processes (f = fultoportulae, r = rimoportulae) in the inner view of a valve, while B and C show a closer view of the fultoportula or strutted process, and rimoportula or labiate process, respectively. Note that the fultoportula has an extension to the outside of the cell, which can be seen as a tube or hole when observing the outer side of the valve. o = outer side; i = inner side. Drawing by Alex R. Rivera Hernández.

MATERIALS AND METHODS

Sampling

Samples of diatoms were taken from the same aquaria as the samples of cyanobacteria. Three sub-samples of 5 ml from the upper layer of the microbial mat were taken from each aquarium, simultaneously with the samples for cyanobacteria. The samples were fixed in formaldehyde at a final concentration of 4% and transported to the laboratory at room temperature.

Processing

I. Cleaning samples

The samples were cleaned out from the organic matter following a process of oxidation (Navarro, 1981). First, the samples were centrifuged in tubes with a capacity of 15 ml, at 2000 rpm during 5 min. The objective was to remove the formaldehyde, salts, and debris. This step was repeated at least 6 times. The supernatant was discarded within each centrifugation and distilled water was added. After centrifugation, most of the water was removed and 2 volumes of $KMnO_4$ at 10% were added. At this time, the samples turned to a purple color. Immediately, the samples were placed in darkness for a period of 24 hours. Then, 5 drops of concentrated H_2SO_4 were added per milliliter of sample and $KMnO_4$. After this, we added H_2O_2 was added, drop by drop, until the sample became colorless. By this step, all the organic matter was oxidized, and the separation of valves may have occurred, event that helps in the identification

of species. To remove the acids, the samples were centrifuged again, under the same conditions as at the beginning.

Another oxidation protocol was done to see if it works better than the one previously explained. The samples were centrifuged at least 6 times, at 2,500 rpm (712.72 xg), for about 10 minutes, to remove the formaldehyde. Then, they were placed in beakers with 25 ml of 30% H_2O_2. After covering each beaker with a watch glass to prevent evaporation, the samples were placed on a hotplate set on “high” for 30 min. Then, 30 ml of concentrated nitric acid were added. The exothermic reaction occurred immediately, boiling and producing foam. The reaction was stopped by adding distilled water. The samples were allowed to precipitate and settle for about 24 hr. The supernatant was removed and the samples were transfered into tubes to clean the samples by 6 centrifugations, using the same conditions as to remove formaldehyde. This process was performed at the Iowa Lakeside Laboratory, University of Iowa, under the supervision of Dr. Sarah Spaulding and Dr. Mark Edlund (diatomologists).

II. Permanent slides preparation

Permanent slides were prepared using Naphrax® as the mounting medium. First, an aliquot of 0.1 ml was transferred to a coverslip, and put in a slide-warmer at 37°C. When the sample was dry, a slide was warmed on a hot plate and a drop of Naphrax® was placed on the slide. Using forceps, the coverslip was turned upside down, joining the sample with the Naphrax® on the slide. We waited until the medium stopped making bubbles (toluene evaporation) and then,

pressed down to stick the coverslip into the medium (it was a little bit separated because of the bubbles). The slide was removed from the hot plate using forceps, and placed on a tray to cool. This process was done for each sample. Each sample was observed using the compound light microscope.

For the observation of samples using a scanning electron microscope (SEM), a few drops of a sample were added to a coverslip and allowed to dry. This was done for all the samples in the control treatment. Then, each coverslip was placed on a carbon tape, over a metal stub. The samples were coated with gold, then placed in the SEM where the sample was scanned.

Morphological Analyses

For the classification of the diatoms, their morphological features had to be considered. This includes the form (whole valve and ends), symmetry, length (apical and transapical axes for the pennates, or diameter for the central diatoms), if the diatom had raphe, as well as its form and direction, the quantity of striae in 10 µm (ventral and dorsal areas) and the ornamentation of the striae. It was also important to notice the details of the central area in each diatom. Additional characteristics were seen for some genera (*e.g*. the partectum in *Mastogloia* spp., and the fultoportulae and/or rimoportulae in *Cyclotella* and *Licmophora*). These characteristics were compared with the description of diatoms in the literature (Patrick and Reimer, 1966 and 1975; Navarro, 1988; Krammer and Lange-Bertalot, 1999; Siqueiros-Beltrones, 2002; Kociolek and

Spaulding, 2003). All the diatoms were classified to genus and, in some cases, to species level.

Species Richness Analysis

The species richness of the diatoms at the different stations and among hydroperiods was recorded and compared. The graph and descriptive statistics (mean and S.D.) were obtained using InfoStat v.5.

RESULTS

An overall species richness of 4.19 ± 0.92 (mean ± S.D.) diatoms was found. The highest species richness of diatoms was observed during the dry hydroperiod in April (4.78 ± 1.09, see Appendix 5C), while the station with the highest value was Station 2. Nevertheless, the species richness for Station 3 had the highest species richness value, and this occurred during the dry season of January (Figure 27).

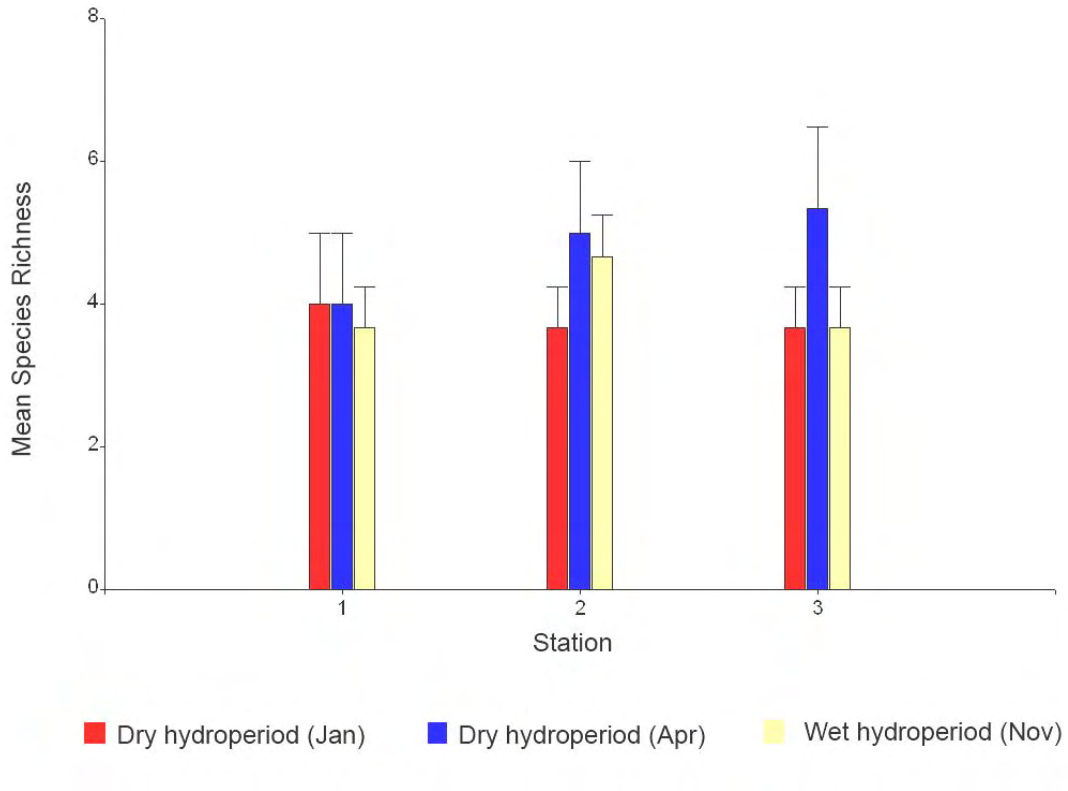

Figure 27. Mean species richness of diatoms for each station for each hydroperiods. Note that the highest values of species richness were observed during the dry hydroperiod of April (blue). Lines above the bars = S.D.

Overall, 19 species of diatoms belonging to 13 genera were found in Laguna Candelaria during the preliminary studies and during the sampling periods (Table 4). Two procedures were performed on separate subsamples to clean the valves for identification. Both cleaning procedures (with sulfuric acid or with nitric acid) functioned well, but the one from Navarro (1981) worked better because it left less organic material. Thus, the diatoms described below were cleaned using that protocol.

Identification and Characterization

I. Taxonomic Key

The following taxonomic key was generated using the morphological features of the cell, such as the valve form, position of the raphe, size, or other distinctive structures in the frustule. A description of each species is also presented.

1a. Pennate form 2
1b. Centric form ..... *Cyclotella* sp.

2a. Frustule with no raphe 3
2b. Frustule with one or two raphes 5

3a. Valve with chambers and spines ..... cf. *Opephora* sp.
3b. Valve with no chambers nor spines 4

4a. Arcuated valve of 8-9 µm wide in its broader part .. ..
..... *Licmophora normaniana*
4b. Valve with a head pole 3 times broader than its foot pole ..... *Licmophora* sp.

5a. Frustule with one raphe ..... *Cocconeis scutellum*
5b. Frustule with two raphes 6

6a. Raphe supported by keels 7
6b. Raphe not supported by keels 9

7a. Marginal raphe 8
7b. Raphe on sigmoid alae ..... *Entomoneis* sp.

8a. Heteropolar ovate valve ..... *Surirella* cf. *fastuosa*
8b. Lanceolate valve ..... *Nitzschia lanceolata*

9a. Valve symmetric to the apical and transapical axis 10
9b. Valve only symmetric to the transapical axis or not symmetric at all 14

10a. Valve with partecta 11
10b. Valve without partecta 13

11a. Valve with capitate ends ..... *Mastogloia* cf. *corsicana*
11b. Valve with round ends 12

12a. Partecta with wider loculi at the center ..... *Mastogloia braunii*
12b. One or two partecta at each side of the valve (thickened at the center) ..... *Mastogloia crucicula*

13a. Central striae shorter in one side of the valve, the other striae are radially arranged ..... *Navicula* sp. 1
13b. Central stria shorter in one side and interrupted in the other; the rest of the striae not radially arranged ..... *Navicula* sp. 2

14a. Wedge shape valve 15
14b. Sigmoid valve 18

15a. Valve with costae ..... *Rhopalodia* cf. *gibberula*
15b. Valve without costae 16

16a. Valve with capitate ends ..... *Amphora acutiuscula*
16b. Valve without capitate ends 17

17a. Ondulated raphe ..... *Amphora* cf. *arcus*
17b. Straight raphe ..... *Amphora* sp. 3

18a. Striae parallel to the apical and transapical axis ..... *Gyrosigma* sp.
18b. Striae diagonal to the apical and transapical axis ..... *Pleurosigma* sp.

II. Description of diatom species

A. Fragillariophyceae (pennate, araphids)

1. *Licmophora normaniana* (Greville) Wahrer in Wahrer, Fryxell & Cox 1985 Fig. 28

This species has an arcuated valve, asymmetrical to the apical and transapical axis. No raphe is present, although a hyaline line (axial area) is visible in its place. In the head pole, it has a rimoportula (labiate process). There are 14 striae in 10µm. The valve has an approximate length of 235 µm and a width of 8.5 µm.

Navarro (1988) described this species previously for the Cabo Rojo Salterns. In the present study, it was a rare species. It appeared at least once at each station, before the treatments, and after 72 hours, but it was not present after 24 hours. In previous works, this species may be classified under the genus *Campylostylus* (Gerloff *et al*., 1978; Campere, 1994).

2. *Licmophora* sp. Fig. 29

This species has the same shape than *L. normaniana*, but it is shorter and has a broader head pole. It was only seen in the preliminary studies, forming aggregates of cells. No measurements could be taken.

3. cf. *Opephora* Fig. 42

The valve is symmetrical to the apical axis. The striae are composed of small chambers. There are 10 striae in 10µm. The development of spines was noticed, which is rarely seen for this genus. The valve length is of 8.08µm and the width is 2.46µm. This was a usually rare species and it appeared only in the preliminary studies.

B. Bacillariophyceae (pennate, raphids)

1. *Amphora acutiuscula* Kuetzing, 1844 Fig. 30

The valves are wedge-shaped, symmetrical to the transapical axis and asymmetrical to the apical axis. It has an arched dorsal margin and a straight ventral one. The raphe is eccentric, straight, with the proximal ends slightly deflected to the dorsal margin and the distal ends forming a hook towards the same margin, at the level of the last stria. Also, a silicified structure can be seen in the raphe area. Dorsal striae are punctuate-lineate, slightly radiated, from 21 to 24 in 10µm. The ventral striae are parallel to the ventral side, from 37 to 38 in 10µm, separated from the raphe by a thin linear axial area. The central area is defined by a separation of the ventral striae. The ends of the valve are capitate, separated from the rest of the valve. The valve has a length of 20.3 to 20.8 µm and 3.9 µm wide. This species was present in all

stations before and after 72 hours of treatment, but after 24 hours of treatments it was only present in station 2.

2. *Amphora* cf. *arcus* Gregory 1855 Fig. 31

The symmetry of this species is the same of *A. acutiuscula*. It is more arched at the dorsal margin than the previous species and the ventral margin is straight to slightly undulate. The ends of the valve are rostrate. The raphe is undulated, with its proximal ends toward the ventral margin and the distal ends toward the dorsal one. Dorsal striae range from 18 to 19 in 10µm while ventral striae range from 20-21 in 10µm. This species is bigger than *A. acutiuscula*, with a length of 46-47µm and a width of 10µm. This species was only found in station 2.

3. *Amphora* sp. 3 Fig. 32

The symmetry of this species is similar to the previously described species. It differs from *A.* cf. *arcus* in the shape of the ventral margin and the raphe, both being straight. However, the raphe forms a slight hook in the distal ends. This species have approximately 16 dorsal striae in 10µm and 18 ventral ones in 10µm. The valve ends are rostrate. The length of the valve varies from 49.5 to 61.5µm and the width varies from 10.5 to 11µm. As *A.* cf. *arcus*, this species was only found in station 2.

4. *Cocconeis scutellum* Ehrenberg 1838 Fig. 33

This species is a monoraphid species. The valve is symmetrical to the apical and transapical axis. It has an elliptical shape, with no obvious ends. The striae are slightly radiate, with a count of 17 in 10µm. Each stria has approximately 17 single punctae, but at the margins the striae become double or triple-punctated. The valve has a length of 18µm and a width of 10.5µm. *C. scutellum* was rare, found only in station 1.

5. *Entomoneis* sp. Fig. 34

This species has a panduriform shape in girdle view (valve view is difficult to observe because it has an elevation). The wings of the valve can not be seen in a single focus. The raphe is sigmoidal (can only be seen in valve view), and the striae appear as small dashes. This species was observed only during the preliminary studies. No measurements could be taken.

6. *Gyrosigma* sp. Fig. 35

This species has a sigmoid valve (asymmetrical to the apical and transapical axis). The ends are slightly beaked. Striae are punctuated, forming apical (although almost indistinguishable) and transapical rows, with a count of 21 transapical striae in 10µm. The central area is elliptical, with the proximal ends of the raphe straight. The raphe has a sigmoid form, always at

the center of the valve. The valve has an approximate length of 80μm and is 15μm wide. This species was rare, found once in station 1.

7. *Mastogloia braunii* Grunow 1863 Fig. 36

The valve is lanceolate, with rounded ends. It is symmetrical to the apical and transapical axis. The raphe is undulated, with two twists. Both distal ends of the raphe form a hook in the same direction. The valve has two depressions that, joined with the central area, form an “H” shape. The striae are punctated in the margins, divided from the central linear striae by a hyaline longitudinal line (the border of the “H” shape). The axial area is narrow, straight, bordered by a longitudinal line of single punctae. There are 16-19 striae in 10μm, and 14 transverse punctae in 10μm. The loculi of the partecta are wider in the center than in the ends. The length of the valve range from 35-81μm and the width can vary from 11-17μm. *M. braunii* was the third most abundant species in all stations and treatments through time. Navarro (1988) also described this species for the Cabo Rojo Salterns.

8. *Mastogloia crucicula* (Grunow) Cleve 1895 Fig. 37

The valve is elliptical. The symmetry is the same as the previous species described for this genus. This species was found with four partecta, but it can also be find with one to three of them (See Figure 33). The valve has a length of 12.61μm and a width of 7.15μm. There are approximately 19 punctated

striae in 10µm, with 20 punctae in 10µm.This species was rare in the samples, found only in station 1.

9. *Mastogloia* cf. *corsicana* (Grunow) H. Peragallo & M. Peragallo 1897

Fig. 38

This species has an elliptical shape with capitate ends. As the previous species of this genus, it is symmetrical to the apical and transapical axis. The raphe has a twist near the central area. There are 10-11 striae in 10µm. It has a length of approximately 29µm and a width of 11µm. This was a rare species found only in the preliminary studies.

10. *Navicula* sp.1 Fig. 39

The valve is lanceolate, with rostrated ends. It is symmetric in the apical and transapical axis. The central striae are shorter in one side of the valve than in the other. Striae are radially arranged and a change in its direction can also be seen at two points in one side of the valve (“Y” shape). There are 10-18 striae in 10µm. The raphe is central, straight, with a bifurcation in its proximal end and forming a hook to the same direction in both distal ends. The valve have a length of 25-28 µm and a width of 5-6µm. This species was the second most abundant species in all the stations and treatments through the time.

11. *Navicula* sp. 2 Fig. 40

The valve of this species is also lanceolate and symmetric. The striae are not radially arranged, and an interruption in the central stria of one side of the valve can be seen. There is a shorter stria in the opposite side of the interrupted one. There are 15 striae in 10µm. The raphe is straight and forming a hook in the distal ends. The valve length is of 26µm and it is 5.6µm wide. This species was in the same proportion as *Navicula* sp.1. Thus, it is also the most abundant species in the samples in all stations and treatments through the time.

12. *Nitzschia lanceolata* W. Smith 1853 Fig.41

This species is symmetrical to the apical and transapical axis. As its name suggests, it has a lanceolate form. The striae are punctated, although the puncta is sometimes indistinguishable. There are 29-31 striae in 10µm. The raphe is located in the margin of the valve, supported by a fibula. The marginal raphe in both valves is located at opposite sides in the frustule. The valve length varies from 40 to 50µm and the width from 5 to 7µm. *N. lanceolata* was the most abundant species in all stations and treatments through the time. This species was previously described by Navarro (1988) for this location.

13. *Pleurosigma* sp. Fig. 43

As *Gyrosigma*, this species has a sigmoid valve, but the striae are diagonal to the apical and transapical axis. There are approximately 11 striae in 10µm. The raphe is also sigmoidal. This species was the largest one found in the samples. The valve has a length of 135µm and a width of 21µm. This was a rare species found only in station 1.

14. *Rhopalodia* cf. *gibberula* (Ehrenberg) Otto Müller 1895 Fig. 44

The valve is wedge-shape, with the ventral margin almost straight and the dorsal margin strongly arched. The raphe is located along the dorsal margin. *R.* cf. *gibberula* has approximately 17 costae and about 32 rows of alveoli in 10µm (more costae and alveoli rows than the amount described for the species). This was a rare species, found only in the preliminary studies.

15. *Surirella* cf. *fastuosa* Ehrenberg 1840 Fig. 45

This species has a heteropolar ovate valve, with a broad rounded headpole and a narrower slightly cuneate pole. It is symmetrical to the apical axis and asymmetrical to the transapical axis. The raphe is marginal (one raphe at each margin of the valve), supported by rib-like fibulae. The valve is slightly folded, so the marginal and ventral areas can be distinguished. There are 13-14 striae in 10µm. The length of the valve is of approximately 3.9µm and has a width of 2.3 µm. This species appeared only once in station 1.

C. Coscinodiscophyceae (centric)

1. *Cyclotella* sp. Fig. 46

The valve is circular, with a clear and undulated central area. The marginal area is composed of ribs that differentiate the margin and central area. The inner part of the valve presents 12-13 peripheral fultoportulae and one fultoportula in one side of the central area. All fultoportulae can be visible in the outer side of the valve as little holes. In light microscopy, they can just be seen as tiny dots. The diameter of the valve is of 8.5 - 9.0µm, and there are about 10 areoli in 10µm. This species was found in once at station 2.

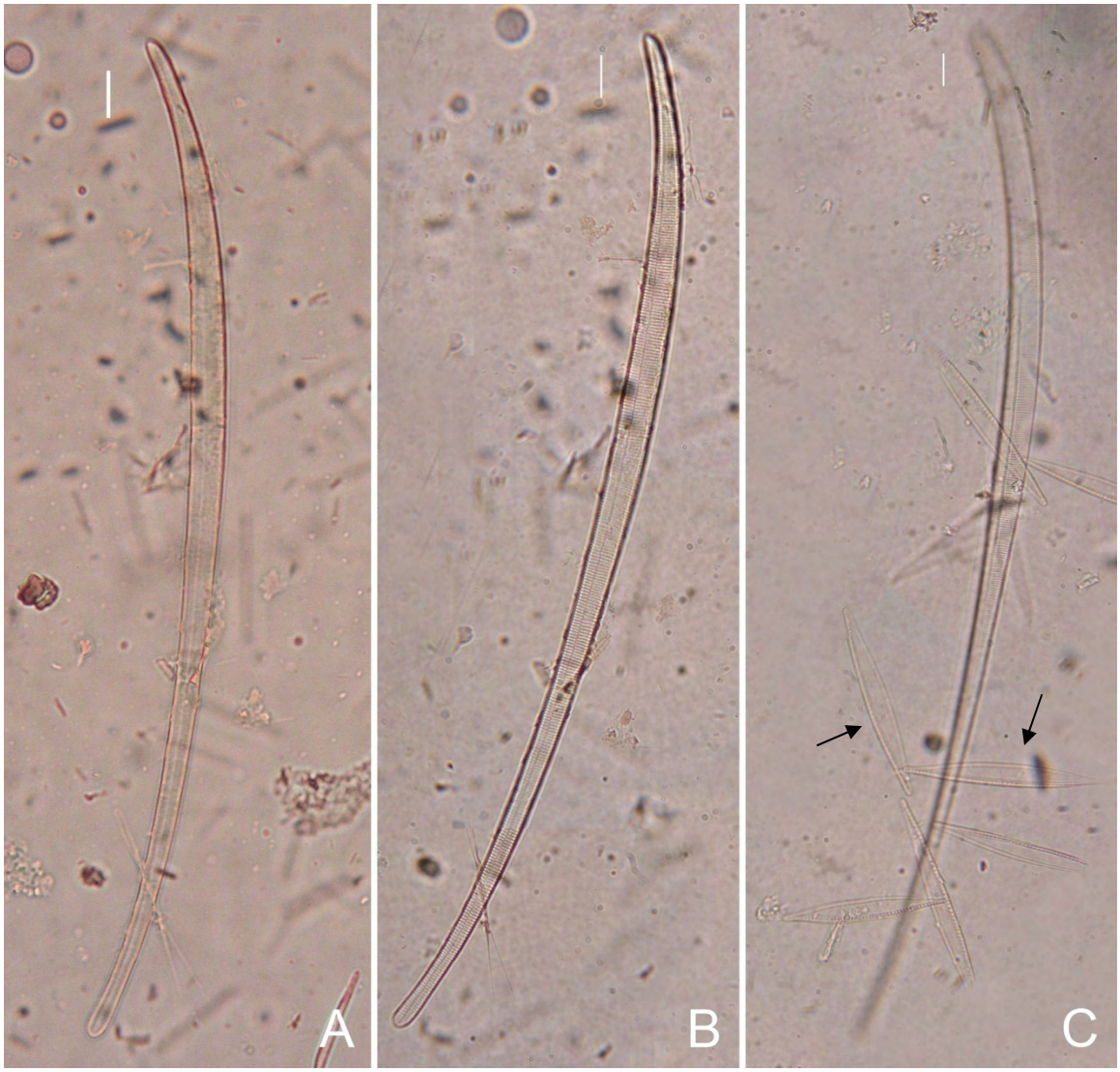

Figure 28. *Licmophora normaniana*, light microscopy. Observe the pseudoraphe along the apical axis. Some *Nitzschia* can also be seen in figure C (arrows). Scale bars = 10 µm.

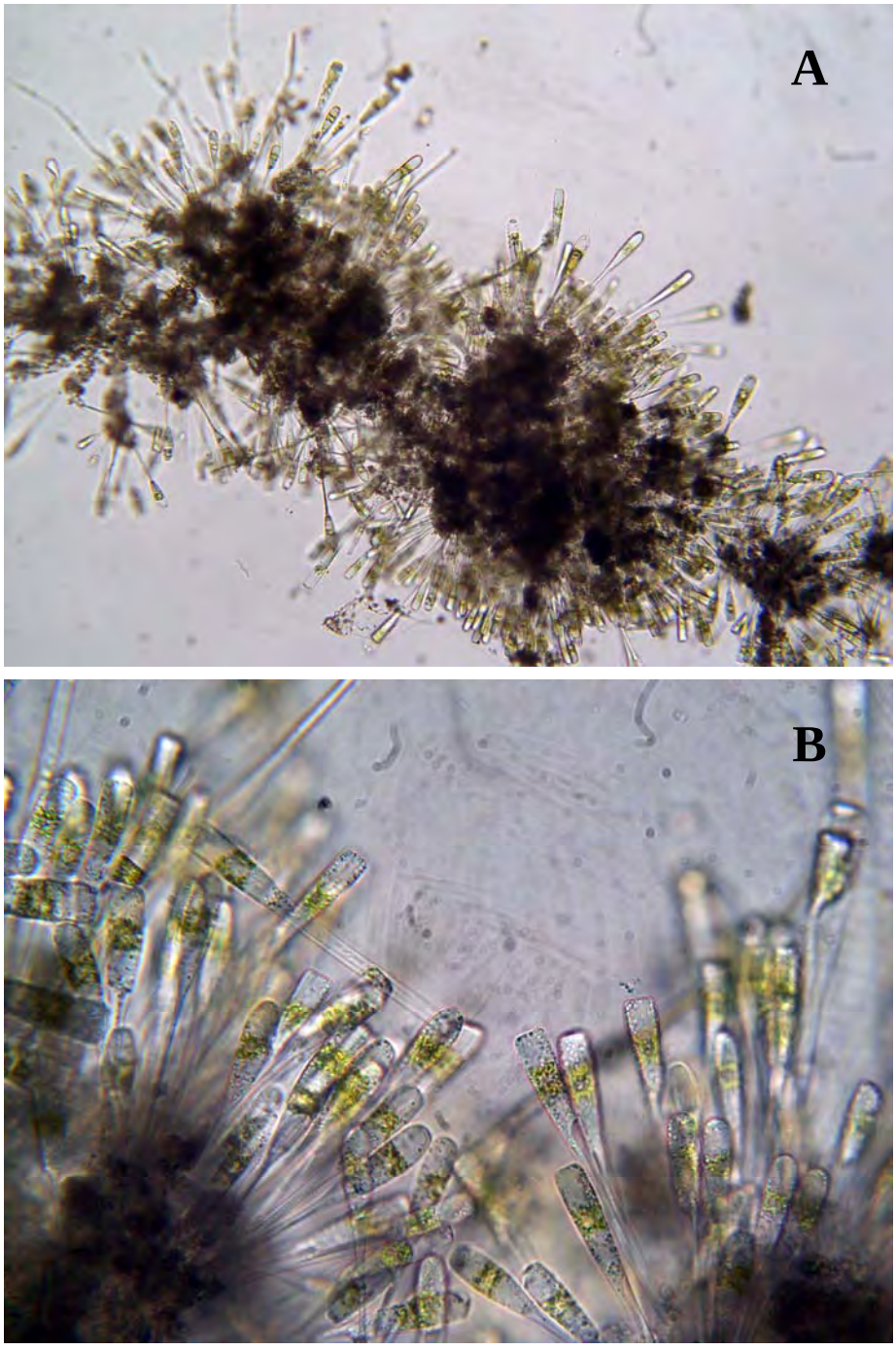

Figure 29. *Licmophora* sp. Live specimens forming aggregates. Figure B is a closer view of A.

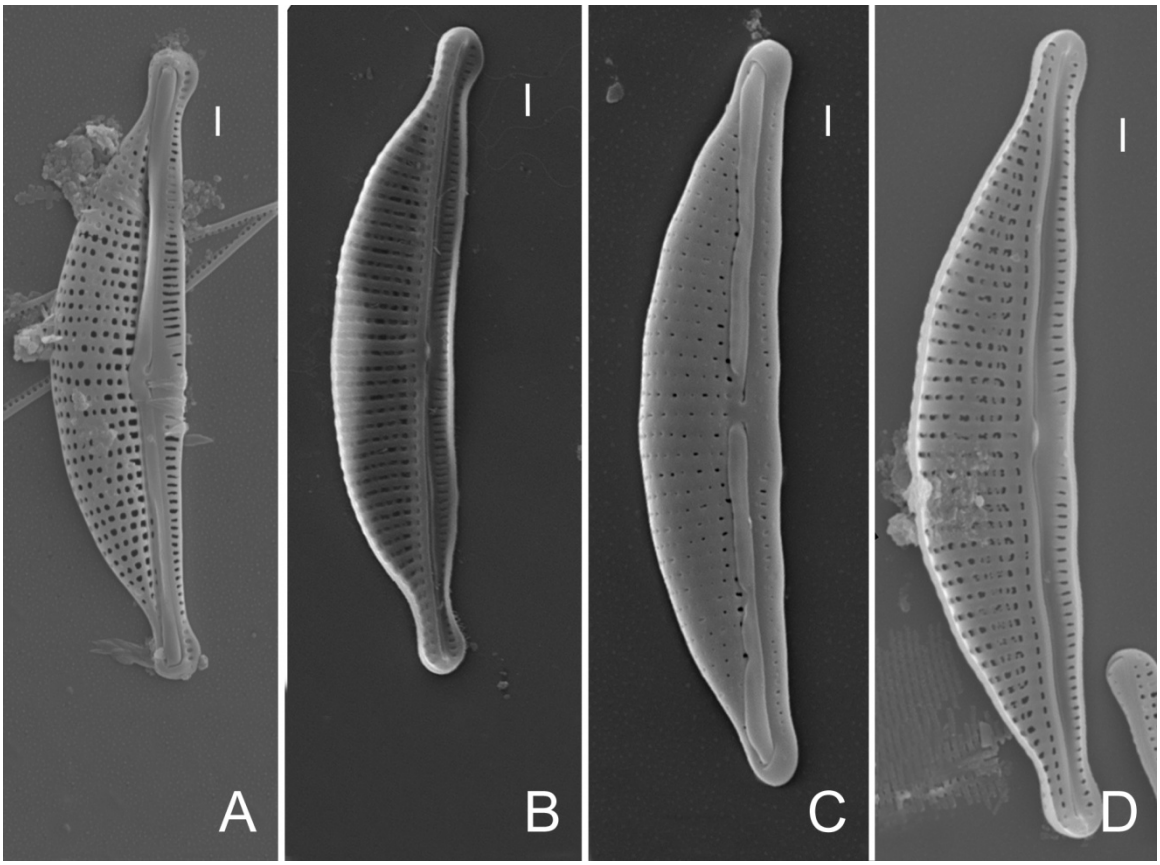

Figure 30. *Amphora acutiuscula*. A-D are SEM images. A and C show the face of the valve. Observe the silica structure around the raphe. B an d D are pictures from the interior of the valve. Notice a difference in the form and number of ventral and dorsal striae in each picture. All scale bars = 1 µm.

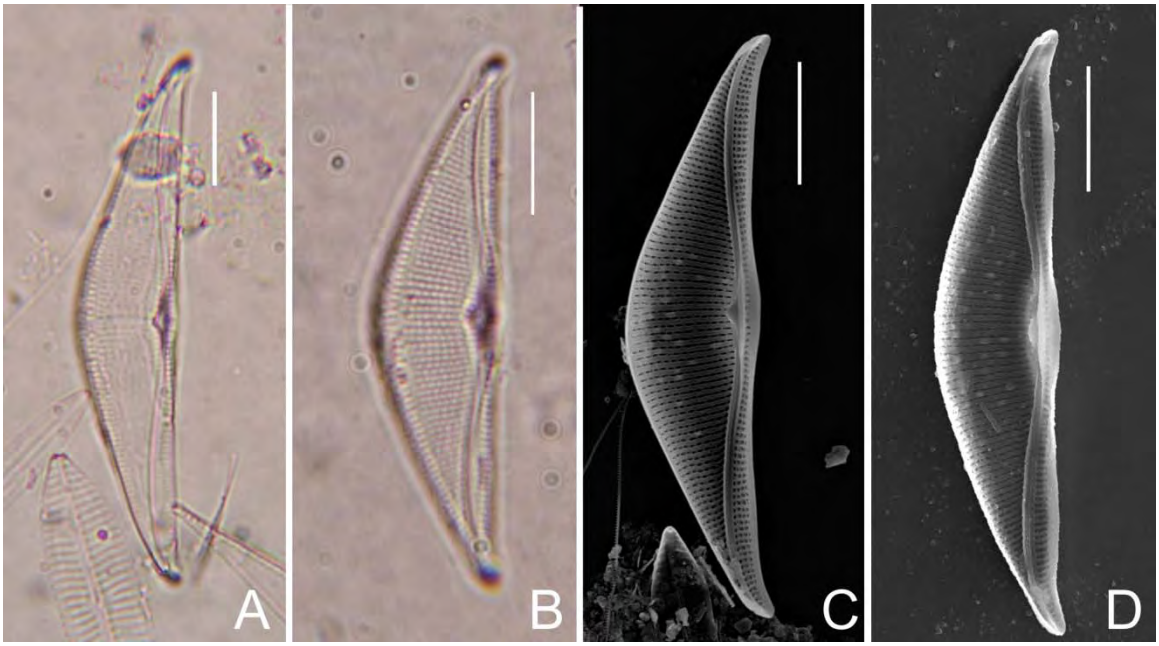

Figure 31. *Amphora* cf. *arcus*. A-B are pictures taken with light microscopy. C-D are SEM pictures. All pictures show the interior of the valve. All scale bars = 10 µm.

Figure 32. *Amphora* sp. 3. All pictures were taken using light microscopy. Observe the straight raphe (dashed lines). All scale bars = 10 µm.

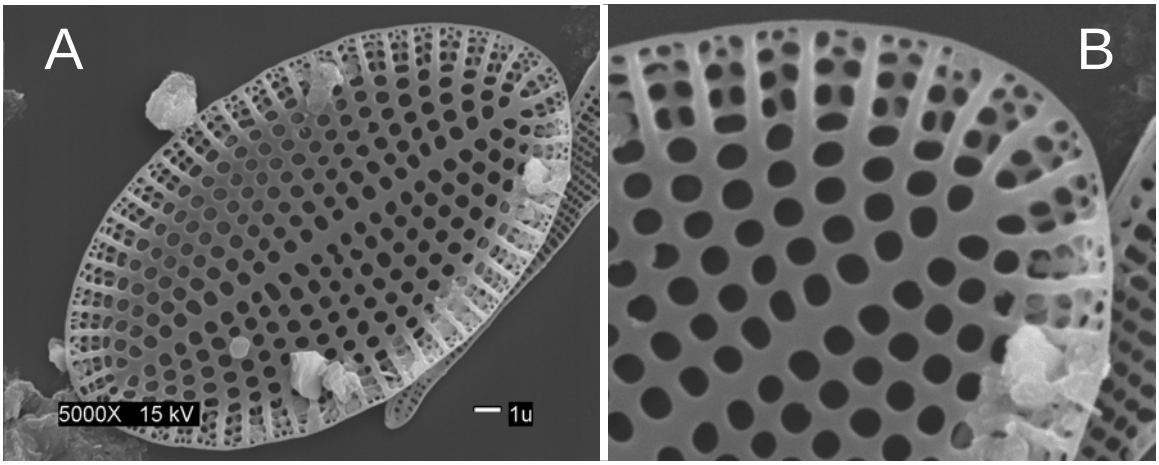

Figure 33. *Cocconeis scutellum*. This SEM picture is an inner view of the valve. Figure B is a closer view from A. Observe the differences of the striae in the center and marginal area.

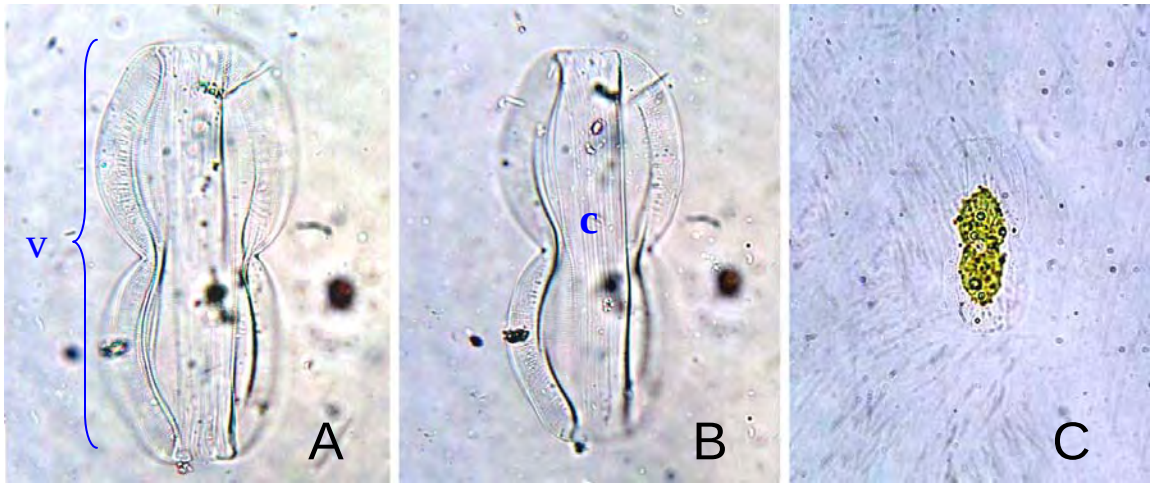

Figure 34. *Entomoneis* sp. Figures A and B are the same picture in different focus. Observe the short striae in the valve (v), and the bands in the cingulum (c). Figure C is a live specimen with chloroplast. All pictures were taken with light microscopy.

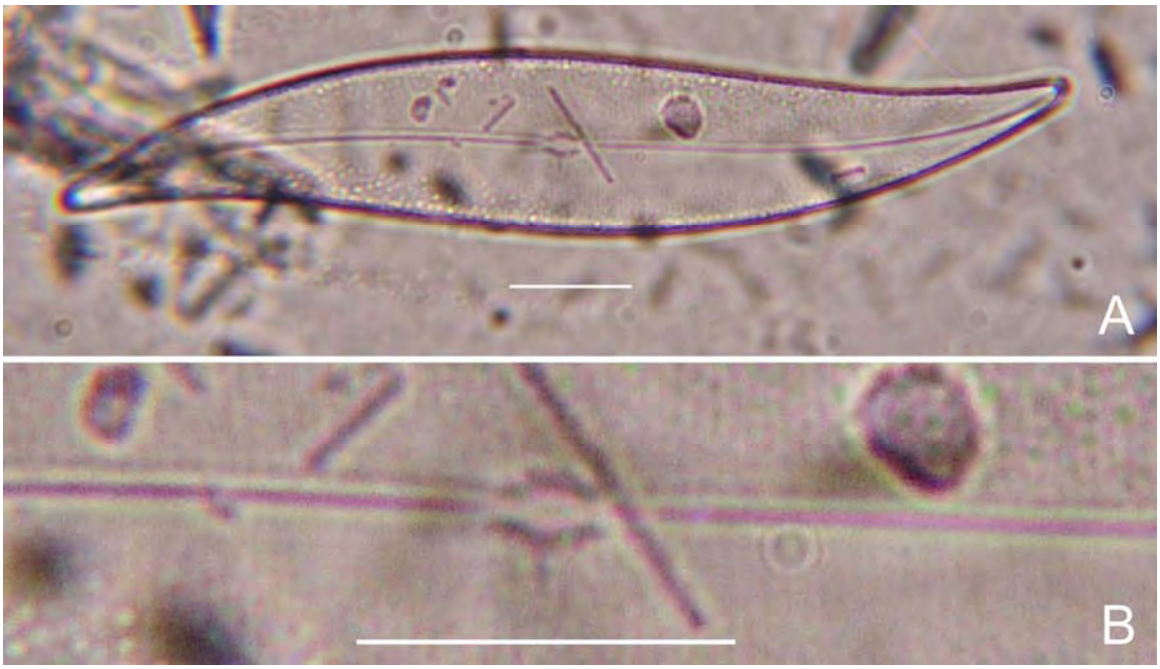

Figure 35. *Gyrosigma* sp. Both pictures were taken under light microscopy. Figure A shows the whole valve. Figure B is a closer view of the striae pattern, parallel to the transapical axis. Scale bars = 10 µm.

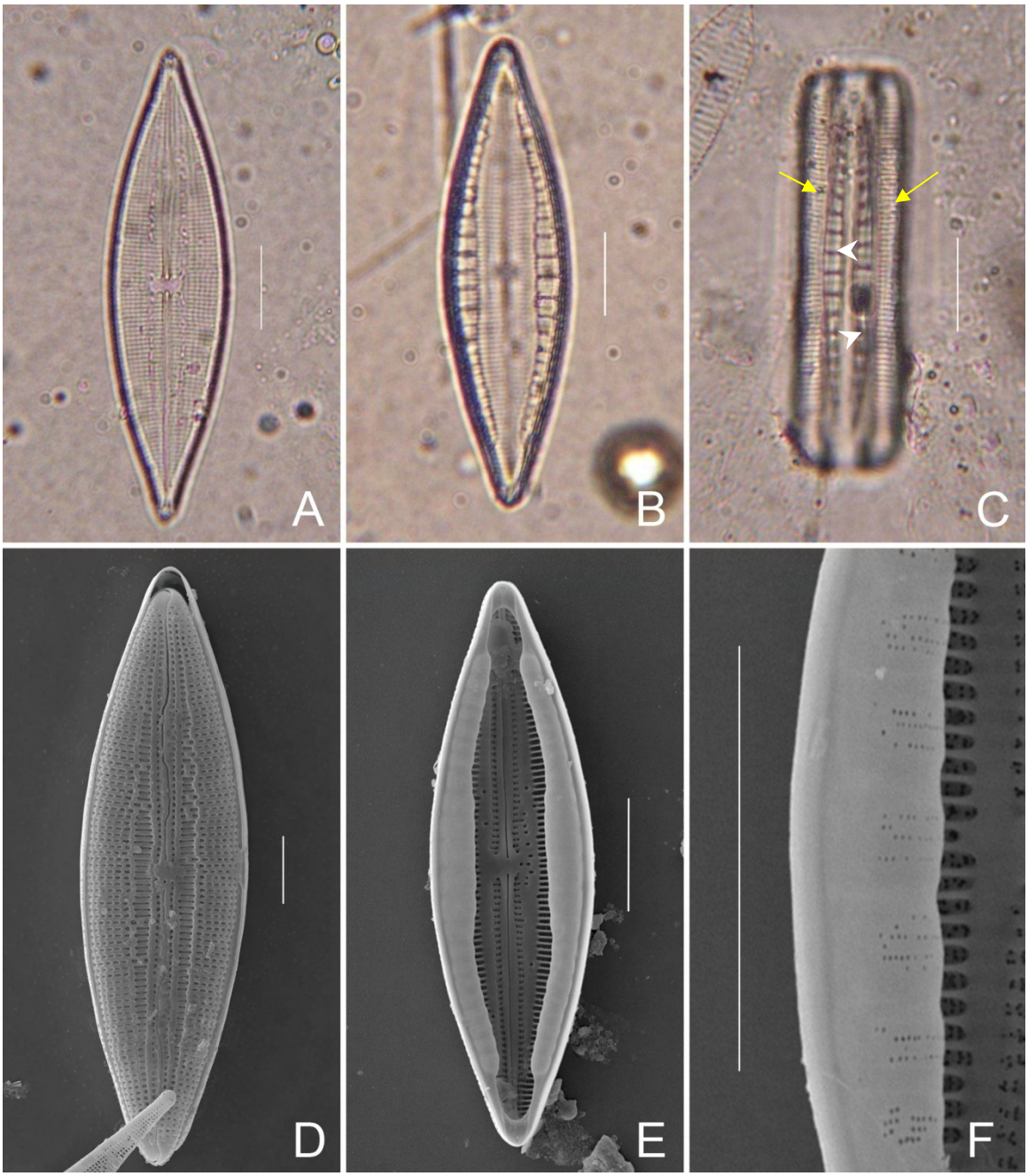

Figure 36. *Mastogloia braunii*, under light microscopy (A-C), and under SEM (D-F). A and D show the face of the valve. Note the “H” shape, formed by a depression in the valve and the twists in the raphe. B and E show the partecta, where the wider ones are at the center. Figure C is a girdle view, where the mantle of the valve (arrows) and the partecta (arrowheads) can be seen. Details of the partecta can be seen in Figure F. All scale bars = 10 µm.

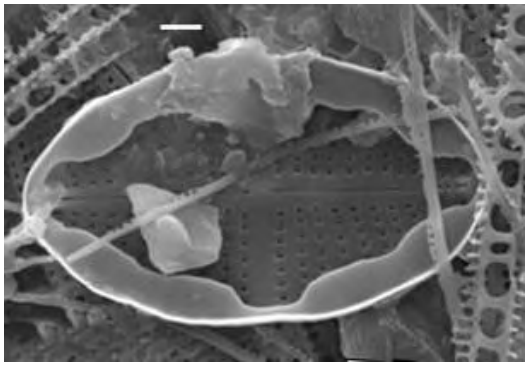

Figure 37. *Mastogloia crucicula* under SEM. Four partecta can be seen, as well as the punctuated striae. Observe that the valve is almost elliptical, with no evident ends. Scale bar = 1 µm.

Figure 38. *Mastogloia cf. corsicana*. The depression at both sides of the valve can be seen (dashed lines), as well as the capitate ends of the valve and the twists in the raphe (arrowheads).

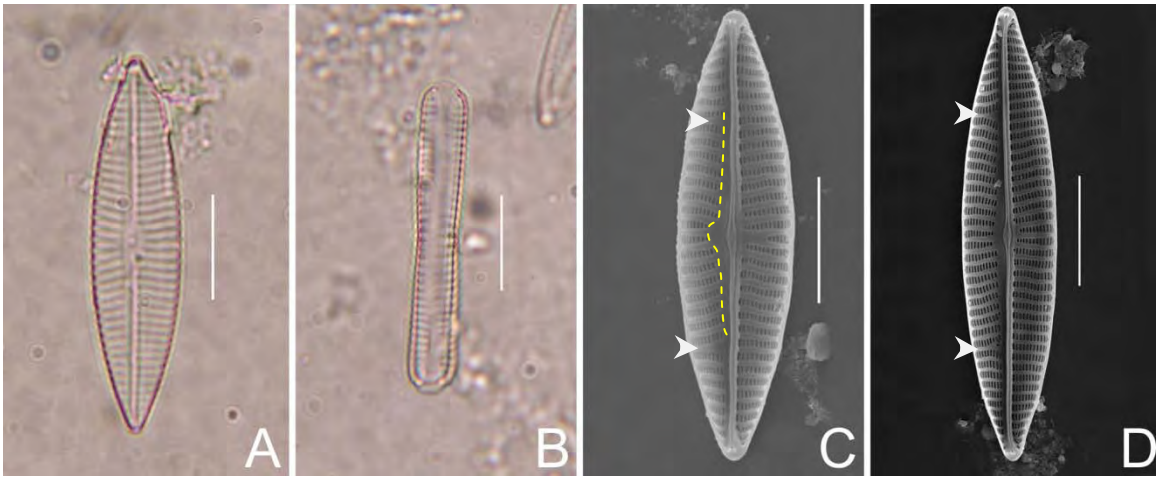

Figure 39. *Navicula* sp. 1. A-B are pictures taken under light microscopy. C-D are SEM images. Figure A shows the valve face. Observe the radiated central striae. We can also observe a girdle view of this species in figure B. Figures C and D show the interior of the valve. Observe the deviation ("Y-shape") of the striae (arrowheads) and the shorter striae in one side of the valve (dashed lines). All scale bars = 10 µm.

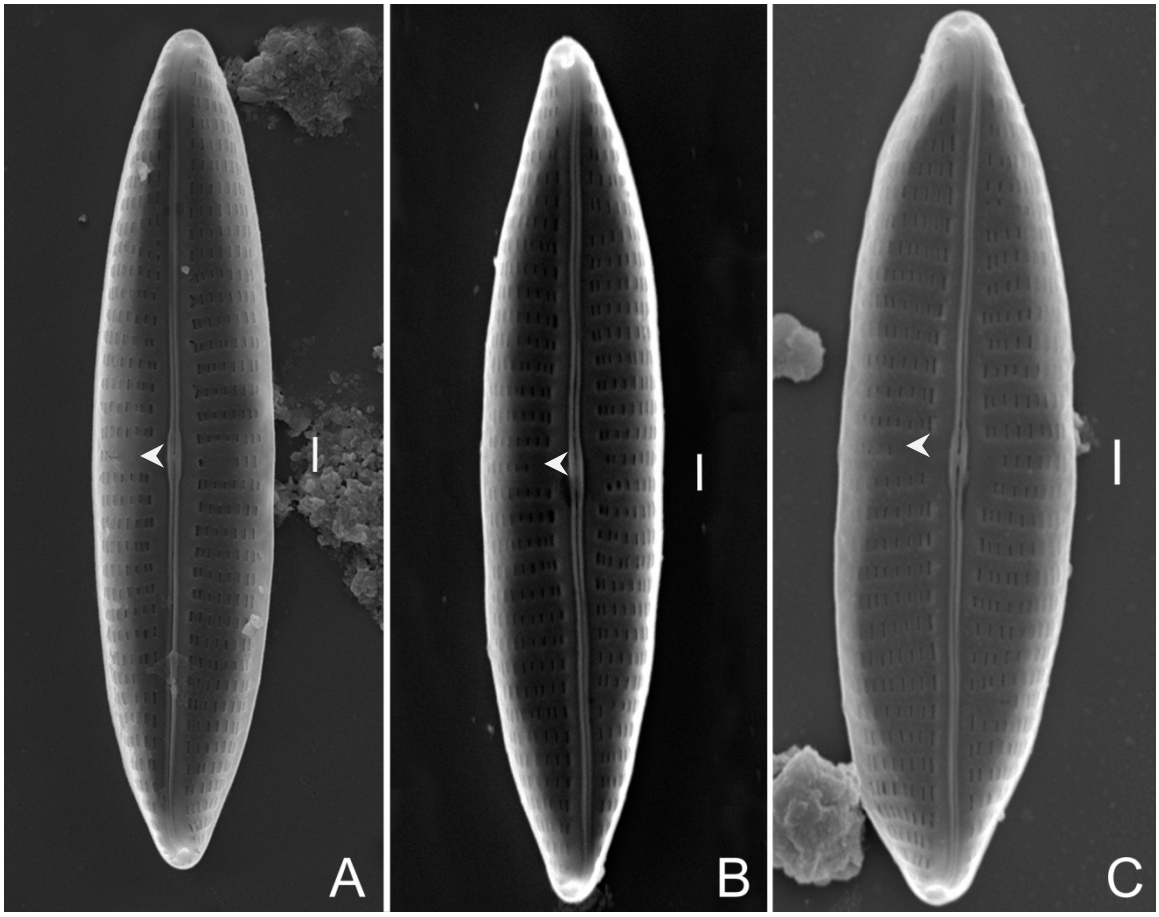

Figure 40. *Navicula* sp. 2. Figures A-C are SEM images. All of them show the interior of the valve. Observe that the central stria is shorter in one side (arrowheads) than in the other. The axial area is almost equal in both sides and the striae are parallel to the transapical axis. All scale bars = 1 µm.

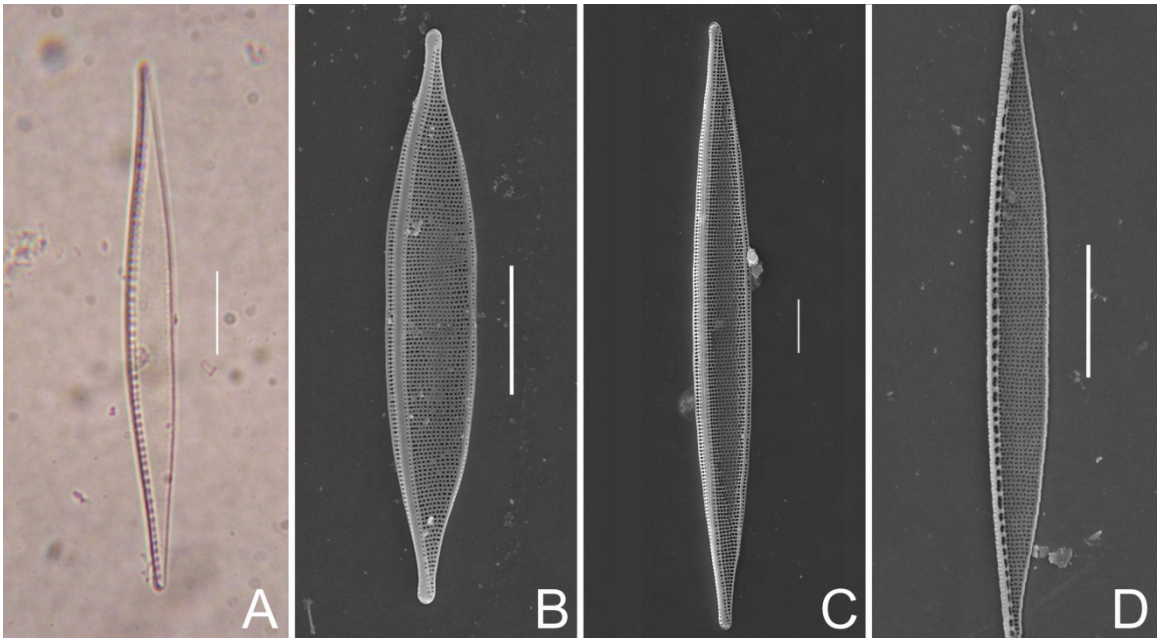

Figure 41. *Nitzschia lanceolata*. Figure A shows a valve under light microscopy. The fibula can be seen at the margin of the valve. Figures B-C are SEM images. B and C show the face of the valve and the puncta in the striae. Figure D shows the interior part of the valve, as well as the puncta in the striae. The fibula can also be seen clearly. All scale bars = 10 µm.

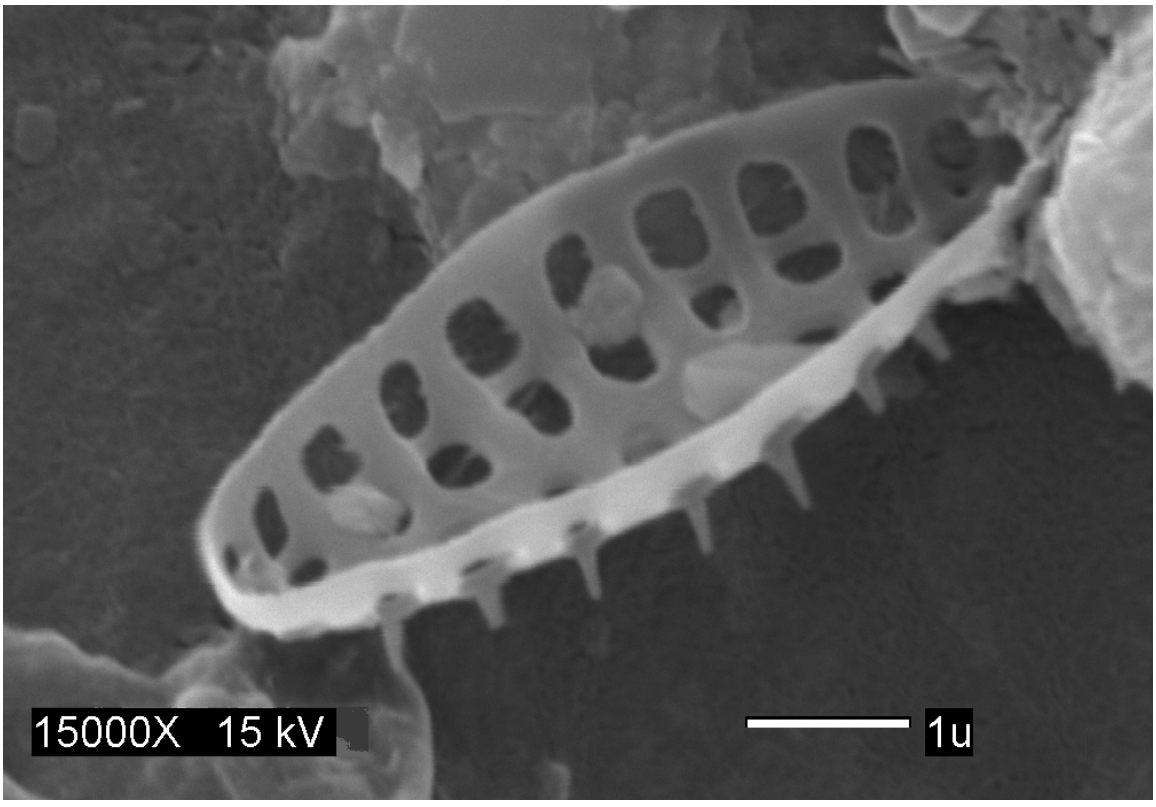

Figure 42. cf. *Opephora*. Observe the chambers that compose the striae. Also, some spines can be seen.

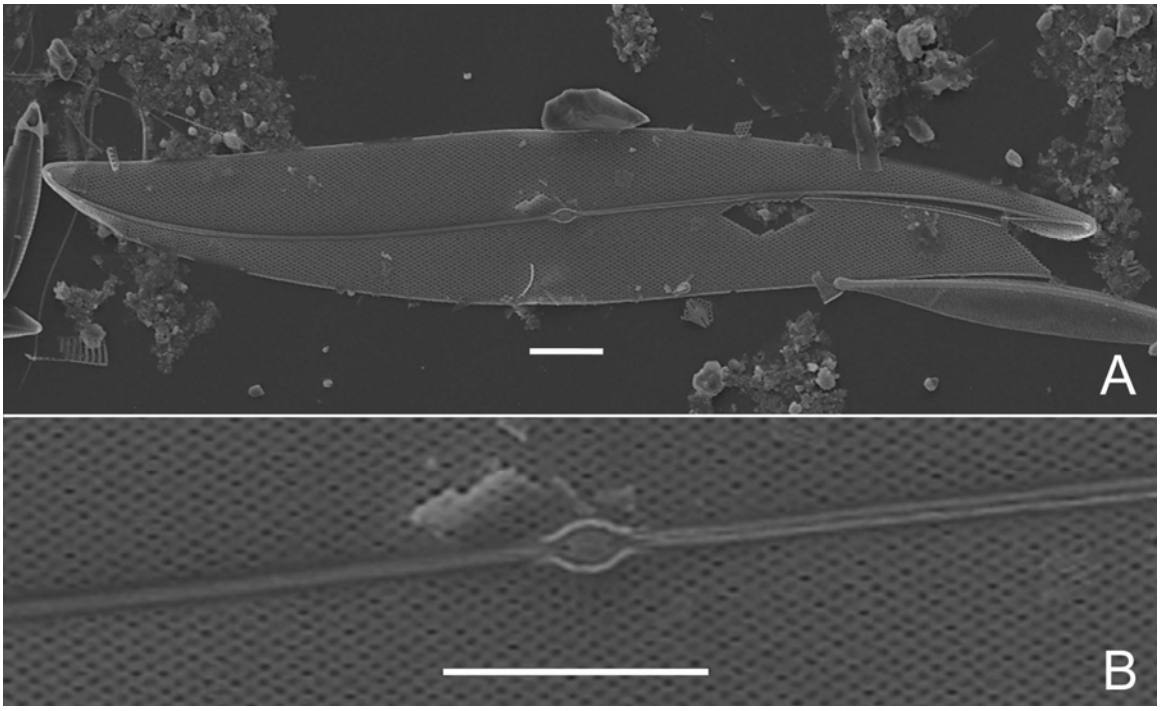

Figure 43. *Pleurosigma* sp. A and B are SEM images. The sigmoidal shape of the valve can be seen in figure A, while the transversal pattern of the striae is shown in figure B. Also, figure B shows the central nodule in more detail, with the proximal ends of the raphe straight.

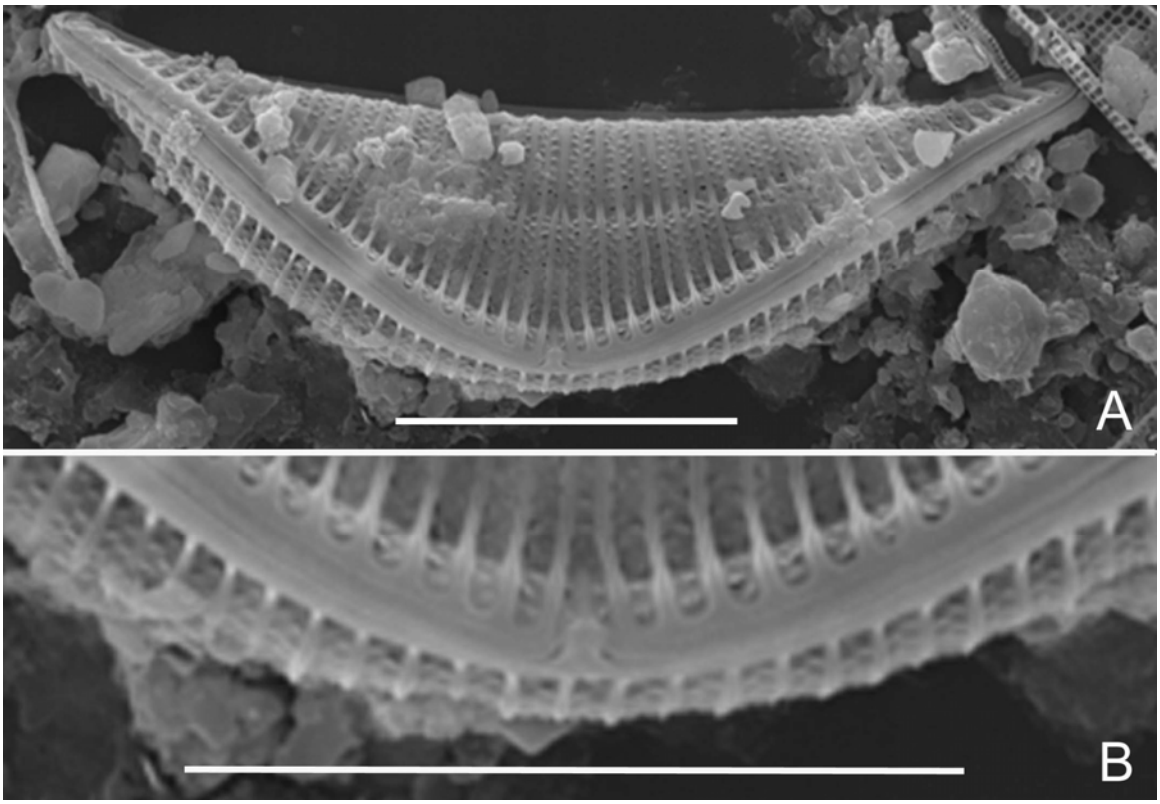

Figure 44. *Rhopalodia* cf. *gibberula*. This image was taken with SEM. Observe the wedge-shape form, as well as the marginal dorsal ribs (Figure A). The raphe can be seen along the dorsal margin (Figure B).

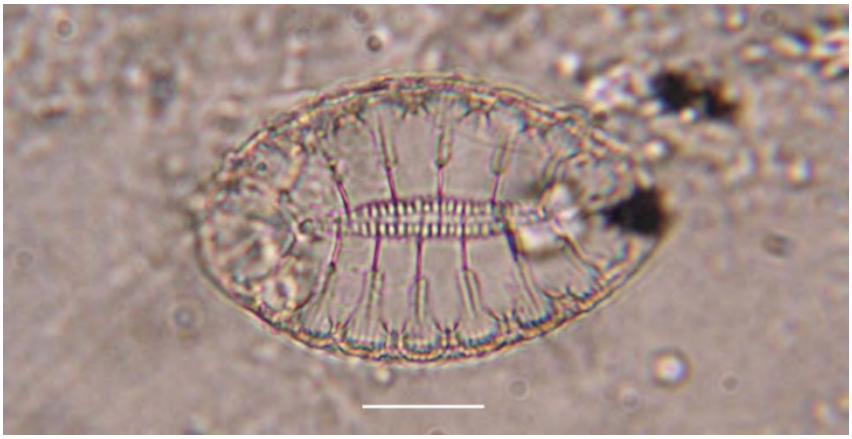

Figure 45. *Surirella* cf. *fastuosa*. Observe the ovate shape as well as the ribs in the margins. The right end is more or less cuneate. Also, a distinctive ornamentation in the central area can be observed.

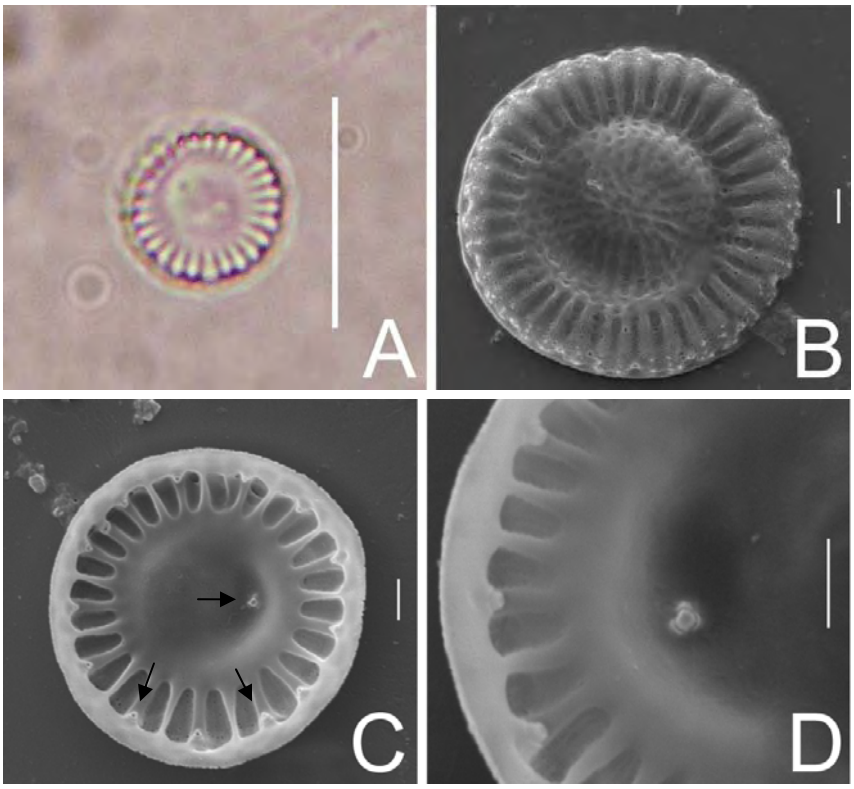

Figure 46. *Cyclotella* sp. A. Picture taken under light microcopy. B-D are SEM pictures. B shows the face of the valve, while C shows the interior. Note the marginal chambers, the fold in the central area and the fultoportulae (arrows). Picture D shows a closer image of the peripheral fultoportulae and the central one. Scale bar in picture A = 10 µm. Scale bars from B-D = 1 µm.

Table 4. Diatom species identified in Laguna Candelaria, Puerto Rico, and reports of their presence worldwide.

Diatom	Observed only in preliminary studies	Observed during the sampling periods	Previously recorded for the Cabo Rojo Salterns	Previously recorded elsewhere in Puerto Rico	Previously recorded for brackish water in the Caribbean	Previously recorded in other salterns	References
Amphora							
acutiuscula		+		+	+	+	Hagelstein (1938), Noël (1986), Siqueiros-Beltrones (1988)
cf. *arcus*		+				+	Clavero *et al.* (2000), Noël (1986), Winsborough and Seeler (1986)
sp. 3		+					
Cocconeis scutellum		+		+	+	+	Foged (1984), Hagelstein (1938), Noël (1986), Siqueiros-Beltrones (1988)
Entomoneis sp.	+					+	Clavero *et al.* (2000)
Gyrosigma sp.		+					
Licmophora							
normaniana		+	+			+	Campere (1994)*, Gerloff *et al*. (1978)*, Navarro (1988)
sp. 2	+						
Mastogloia							
braunii		+	+	+	+	+	Campere (1994), Foged (1984), Gerloff *et al*. (1978), Hagelstein (1938), Navarro (1988), John (1994b)
cf. *corcicana*	+				+	+	Foged (1984), John (1994b)
crucicula		+		+	+	+	Foged (1984), Hagelstein (1938), John (1994b)
Navicula							
sp.1		+					
sp. 2		+					

Diatom	Observed only in preliminary studies	Observed during the sampling periods	Previously recorded for the Cabo Rojo Salterns	Previously recorded elsewhere in Puerto Rico	Previously recorded for brackish water in the Caribbean	Previously recorded in other salterns	References
Nitzschia lanceolata		+	+	+	+	+	Elrich and Dor (1985), Foged (1984), Hagelstein (1938), Navarro (1988)
cf. *Opephora*	+						
Pleurosigma sp.		+				+	Siqueiros-Beltrones (1988)
Rhopalodia cf. *gibberula*	+			+	+	+	Blinn (1991), Elrich and Dor (1985), Foged (1984), Gerloff *et al*. (1978), Hagelstein (1938), Winsborough and Seeler (1986)
Surirella cf. *fastuosa*		+		+	+	+	Gerloff *et al*. (1978), Hagelstein (1938), Noël (1986), Siqueiros-Beltrones (1988)
Cyclotella sp.		+					

* Described as *Campylostylus normanianus*.

DISCUSSION

Among the 19 taxa described for Laguna Candelaria, four of them predominated in most stations: *Nitzschia lanceolata*, *Mastogloia braunii*, *Navicula* sp. 1, and *Navicula* sp. 2. The former two were also described by Navarro (1988) as abundant for the same area. *Licmophora normaniana* was present but not abundant in this study; Navarro (1988) described them as abundant.

The diatom flora described in this study is similar to those described for other salterns in the world. From 19 species reported for Laguna Candelaria, 10 are also described from different salterns from México, France, Spain, Australia, Sabkha, Jordan, or Egypt (Gerloff *et al*., 1978; Elrich and Dor, 1985; Noël, 1986; Winsborough and Seeler, 1986; Siqueiros-Beltrones, 1988; Blinn, 1991; Campere, 1994; John, 1994b; Clavero *et al*., 2000). These species include *Amphora acutiuscula*, *A. arcus*, *Cocconeis scutellum*, *Licmophora normaniana*, *Mastogloia braunii*, *M. corcicana*, *M. crucicula*, *Nitzschia lanceolata*, *Rhopalodia gibberula* and *Surirella fastuosa*. Gerloff *et al.* (1978) described species from the genus *Nitzschia* as the dominant diatom genus in the microbial mats from Jordan. This agrees with our observations, even when other species are not the same. John (1994b) described the *Mastogloia* species associated with stromatolites in Shark Bay, Australia. The three species described in this study (*M. braunii*, *M. corcicana*, and *M. crucicula*) are all included in his reports from Shark Bay. The

other taxa observed in this study were not identified to the species level, but the genera are also mentioned in the literature from salterns.

Diatom species richness was similar to that of the cyanobacteria. If we compare Figures 5 and 27, we can observe that the species richness of both groups for Stations 1 and 2 were directly proportional among hydroperiods. Nevertheless, there was a difference in Station 3, where the highest value of species richness occurred during the dry season of April. This was not directly proportional to the data from cyanobacteria, but it follows the same explanation given in the previous chapter: dry hydroperiods promote a higher diversity of microalgae, which in this case were the diatoms, thus, our hypothesis is accepted.

A taxonomic key and an illustrated guide were provided in this study to facilitate future investigators the identification of diatoms from Laguna Candelaria by the observation under light microscopy or SEM. To complete this information it is important to note some features that can not be observed in the obtained pictures. The first one is the rimoportula in *L. normaniana*, located at one of the poles of the valve. The other is the variation of the partecta in *M. crucicula*. In this study it was observed with 4 partecta, but it can also be seen with only 2 or 3 of them. For the better illustration, images provided by Navarro are presented in figures 47 and 48.

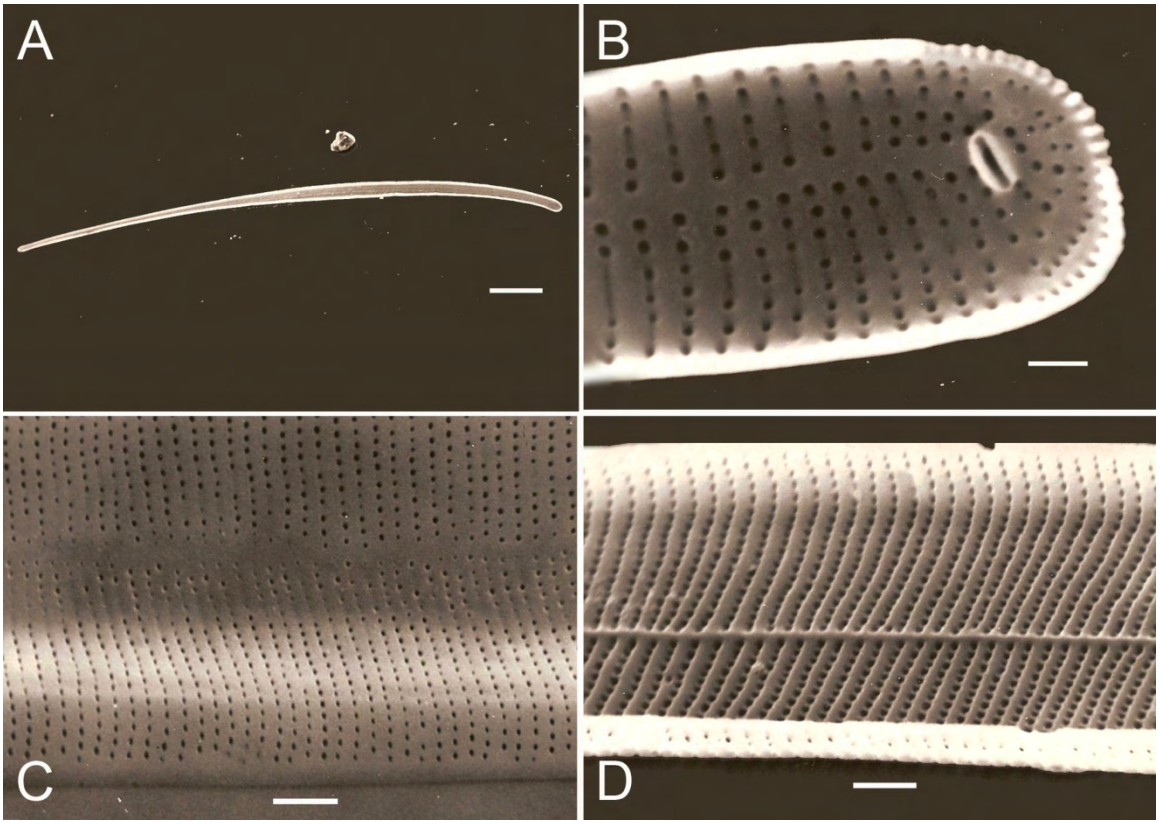

Figure 47. *Licmophora normaniana* after SEM. A) Whole frustule, scale bar = 40 µm; B) Rimoportula in one of the poles of the valve, scale bar = 1 µm. C and D show the pattern of the striae for face and the inner part of the valve, respectively. All images provided by Dr. Navarro.

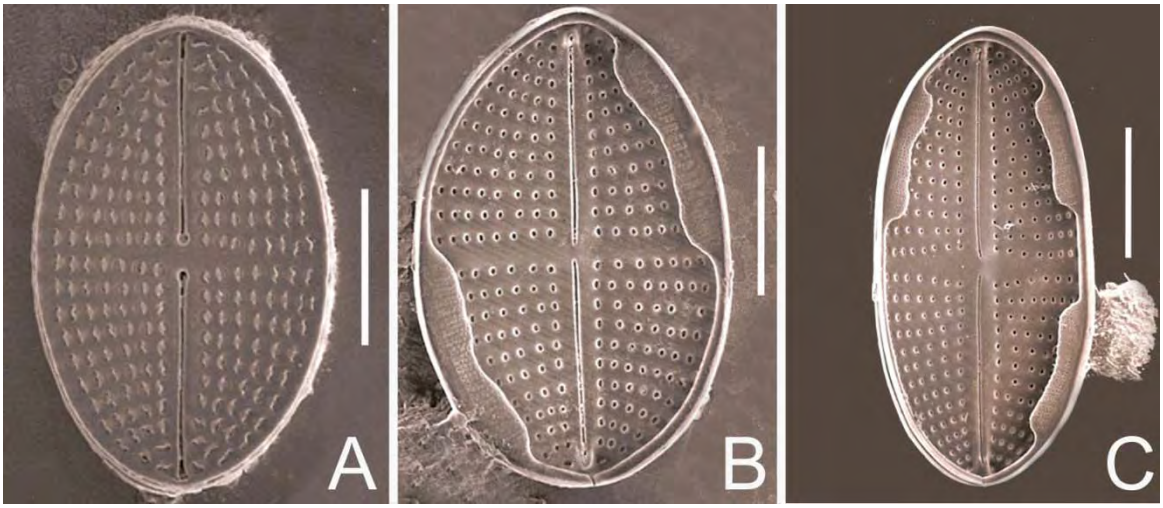

Figure 48. *Mastogloia crucicula* after SEM. The valve face is shown in figure A. This species can vary, having two (B), three (C), or four partecta (as seen in the samples from this study). Scale bars: A and B = 3 µm; C = 4 µm. All images provided by Dr. Navarro.

CONCLUSIONS

1. The pennate diatoms *Nitzchia lanceolata*, *Navicula* spp. and *Mastogloia braunii* dominated the diatom community in the microbial mats.

2. The diatom species richness increased with high salinities probably due to a reduction in grazing pressure.

RECOMMENDATIONS

1. Live specimens should be observed to determine if they can tolerate disturbances in environmental dilutions.

2. Spectrometry of the pigments in the samples should be done to compare the abundance of diatoms in relation to the cyanobacteria and other algae.

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CHAPTER III: Ciliates

INTRODUCTION

Ciliates are simple eukaryotic organisms, classified into the Kingdom Protoctista. As their name suggests, the majority of these organisms have cilia at some time during their life cycle (Small and Lynn, 1985). Nevertheless, as Small and Lynn (1985) suggested, the presence of nuclear dimorphism is what distinguishes them from other protists. A ciliate may have at least one macronucleus, controlling the physiological functions of the cell, and at least one micronucleus, which contains the genetic information.

The classification of ciliates is primarily based in their infraciliature. The cortex of the cell can be divided into the somatic and oral regions. The somatic region has different functions such as locomotion, sensing the environment and attachment, while the oral region is specialized in acquiring the food and ingesting it (Lynn and Small, 1990). The infraciliature in both regions can vary, but it is basically composed of a kinetosome (or basal body) and infracilliary fibrils (kinetodesmal fibril and the transverse and postciliary microtubular ribbons). Other organelles such as the lorica, extrusomes, the contractile vacuole, food vacuoles, cytoproct, or cytopyge are also important in the identification of ciliates. Such morphological and physiological variations can be noticed by staining the cells (e.g. silver protein stain), and by observing the samples with a phase contrast microscope. Sometimes observation through the scanning electron microscope is necessary. A general illustration of a ciliate is shown in Figure 49.

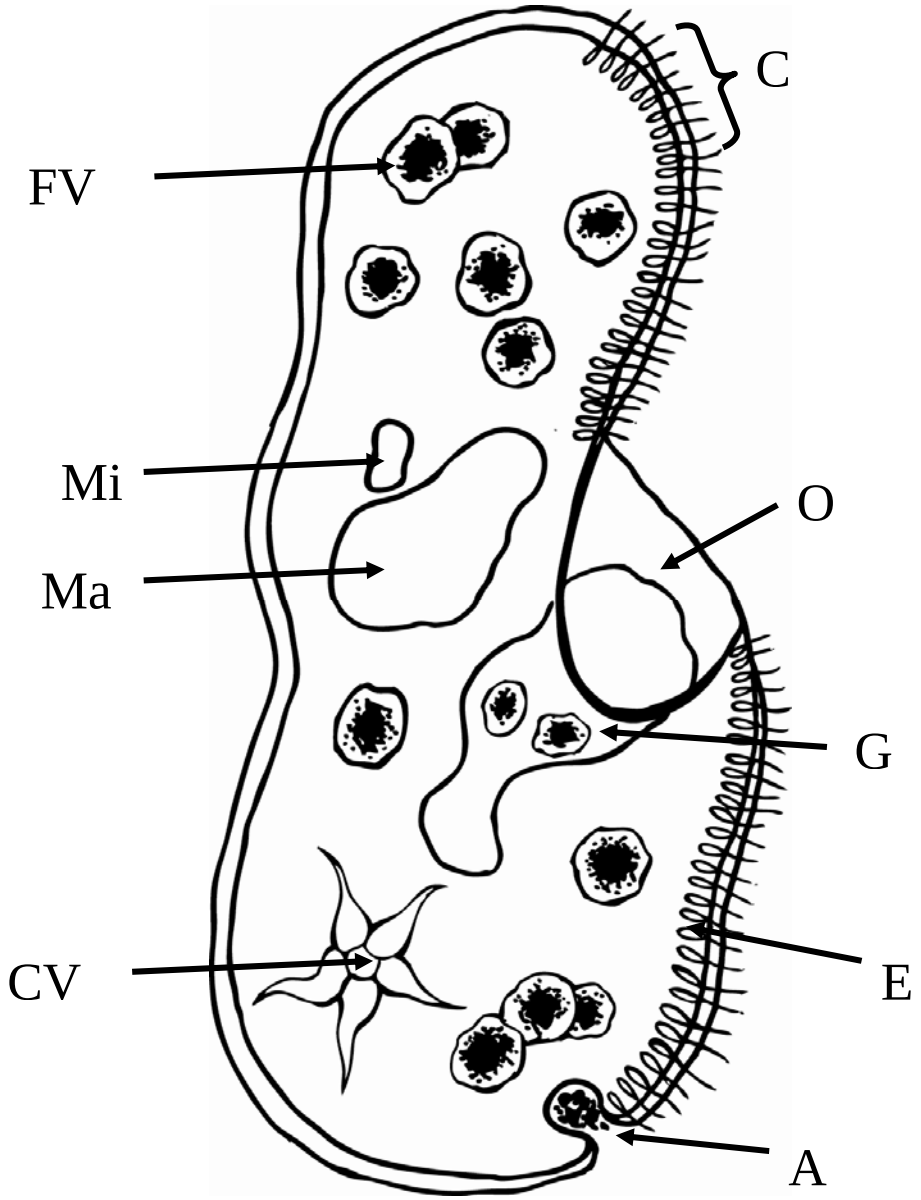

Figure 49. General drawing of a ciliate. C = ciliates, O = oral groove, G = gullet, E = extrusomes, A = anal pore or cytopyge, CV = contractile vacuole, Ma = macronucleous, Mi = micronucleous, and FV = food vacuoles. Drawing by Alex R. Rivera Hernández.

Ciliates can be found in any type of environment, from tundra to deserts (Foissner, 1987). Lynn and Small (1990) described at least four of these environments: the benthos, terrestrial soils, the plankton, and symbiotic associations. In these habitats, they function as grazers, feeding on bacteria, picoplankton, other protist, phytoplankton, and some metazoans, contributing to the mineral cycling and being important mediators of energy transfer in trophic webs (Lynn and Small, 1990; Elloumi *et al*., 2006).

There are some studies that describe their distribution in extreme environments, especially hypersaline ones. Based on their observations from a hypersaline lagoon in Spain, Esteban and Finlay (2003) proved that “each and every ecosystem supports a ‘seedbank’ of microbial species that are imported by random dispersal”. After a series of dilutions, they recorded 24 species of ciliates, from which 14 were from freshwater environments. Post *et al*. (1983) studied the organisms accidentally introduced on the growth medium of the green alga *Dunaliella salina*. The salt crust that is used to prepare the medium contains some organisms that can affect negatively the production of this alga. They found 14 ciliates, from which at least 5 species (i.e. *Blepharisma salina*, *Cladotricha sigmoidea*, *Euplotes* sp., *Fabrea salina*, and *Nassula* sp.) were feeding on algae. Salvadó *et al*. (2001) studied the effects of wastewater with high salt content in organisms that live in sludge, and found *Vorticella* spp. to be the most tolerant organisms in the treatment.

One of the most studied halophilic ciliate is *F. salina*. Elloumi *et al*. (2006) described the ciliate community in ponds of different salinities in Tunisia and

found that heterotrichs (especially *F. salina*) were among the most halophilic organisms. Pandley and Yeragi (2004) described the optimum growth conditions of *F. salina*, ranging from food to temperature and salinity. In their study, they found that egg-custard is the food that best promotes the growth of this ciliate, followed by cultures of *D. salina*. Other biological studies of *F. salina* have been directed to describe its phototaxis with changes in temperature, UV-B radiation and certain ions (Marangoni et al., 1995; Martini et al., 1997; Puntoni et al. 1998). Another hypersaline organism, *Blepharisma intermedium*, was described by Al-Rasheid *et al*. (2001) in a hypersaline oasis in Saudi Arabia.

Studies about ciliates in Puerto Rico

Acosta-Mercado and Lynn (2002, 2004) conducted studies on ciliates in soils in Cambalache and Maricao Commonwealth State Forests, and in the rizosphere of different tropical plants, respectively. Bamforth (2007) also studied ciliates from ground soils in Luquillo Experimental Forest. Studies of ciliates from seawater are limited with is one related to symbiotic ciliates in sea urchins of the Caribbean (Jones and Rogers, 1968) and another about ciliates affecting Caribbean corals (Cróquer *et al*., 2005). Currently, there are no records of ciliates from extreme environments in Puerto Rico. Thus, this study pretended to describe the biodiversity of the ciliates in the microbial mat of Laguna Candelaria, and also to identify and characterize the most common ones in the stations and among hydroperiods. Our hypothesis was that if dilutions treatments (distilled water or seawater) are applied, then the species richness of ciliates should

increase. This was based in the idea that high salinities will reduce the grazing pressure (*i.e.* ciliates).

MATERIALS AND METHODS

Sampling

To identify and describe the most common ciliates in Laguna Candelaria, 3.5 ml from the upper layer of the microbial mat in the control treatments were obtained. The samples were fixed *in situ* with 1 ml of Bouin's fixative (Lot No. 563-01; Fisher's Cat. No. LC11790-4). This is a solution that contains pycric acid, formaldehyde, and acetic acid, and it is specialized for preserving the ciliates and flagella. Then, the samples were transported to the laboratory at room temperature. Live observations were done during the preliminary studies.

Staining Procedures

Two staining strategies were employed: the quantitative protargol stain (QPS), which is a quantitative method (Montagnes and Lynn, 1993), and a protargol impregnation, which is a qualitative one (Wilbert, 1976). When a trial was done following the QPS protocol, we had difficulties filtering the samples (possibly due to the high salt content or the amount of mucilage in them). Thus, we decided to follow the Protargol impregnation. In general, the Bouin's fluid was removed by centrifuging at least 6 times, at 2000 rpm (456.14 xg) for 3 minutes. Then, we turned on the incubator at 50º C. The samples were placed, separately, in watch-glasses. Once in the watch-glasses, the samples were observed through an inverted light microscope to view the cells and remove debris. A few drops of 1% of Clorox® were added slowly to bleach the samples. When the cells were almost transparent, they were washed five times with distilled water (adding

water to the watch-glass, allowing the samples to precipitate, and then, removing the excess of liquid). Protargol Silver Protein was subsequently sprinkled on the water surface and the samples were placed on the preheated incubator for about 45-60 min. The samples were washed three times with distilled water (as previously described). A few drops of 1% hydroquinone were added until the nuclei and infraciliature became distinct; this process took approximately one minute. The samples were washed with distilled water at least once and 1 ml of 5% $Na_2S_2O_3$ was added to stop the developing process of the hydroquinone. The samples were washed five times again, then centrifuged, and mounted on a phenol-Karo® syrup solution, as described for the cyanobacteria protocol.

Morphological Analyses

The identification of the ciliates was based on the illustrated guide by Lynn and Small (2000) and on literature that described hypersaline species (Post *et al*., 1983; Al-Rasheid *et al.*, 2001; Esteban and Finlay, 2003, among others).

Species Richness Analysis

The species richness of ciliates for each station and among hydroperiods were recorded and the data were analyzed with the program SigmaScan v.2.

RESULTS

The overall species richness of ciliates was of 4.44 ± 1.53 (see Appendix 5D). The hydroperiod which promoted highest species richness was the dry one from April (5.22 ± 1.20). Meanwhile, the site which generally had highest species richness was Station 2 (5.22 ± 1.64), while Station 3 had the lowest value (3.22 ± 1.30) (Figure 50).

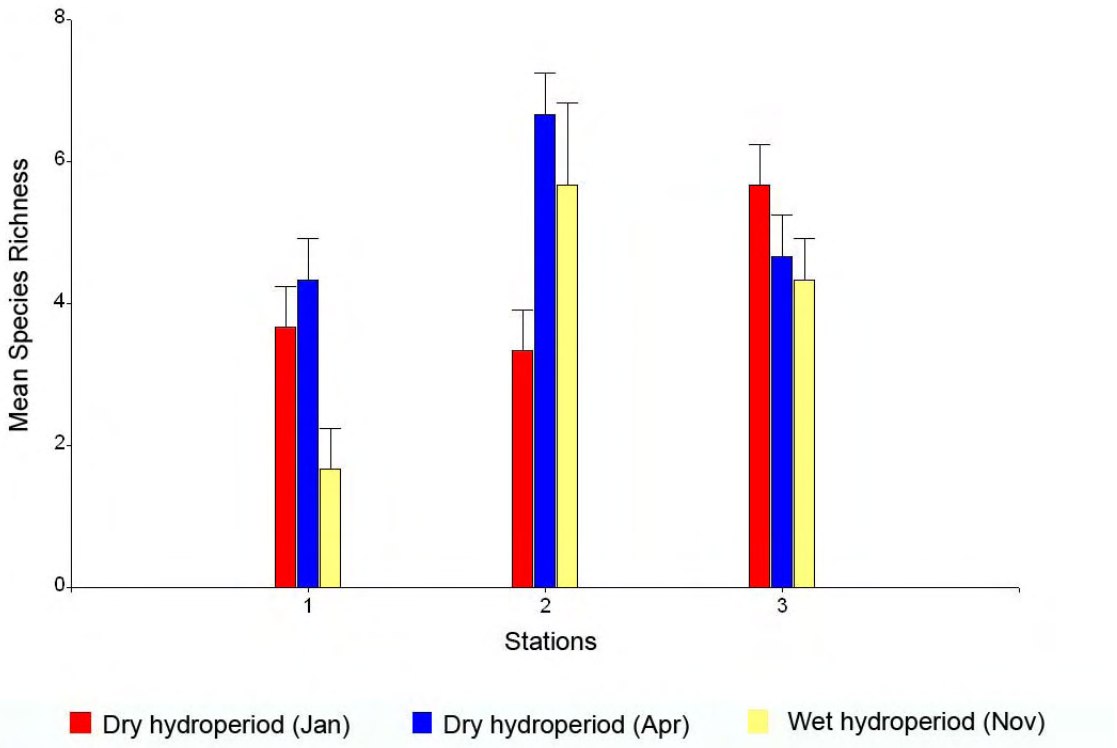

Figure 50. Mean species richness of ciliates for each sampling station and among hydroperiods. Observe that Station 1 and 2 had highest species richness during the dry season of April (blue), while Station 3 had the highest value during the dry season of January (red). Lines above the bars = S.D.

In total, 14 species of ciliates were found in Laguna Candelaria. Out of these, seven species were identified and characterized. The remaining seven species were unidentified because of the lack of literature about ciliates in hypersaline environments and the difficulty in finding an appropriate sample size to characterize oral and somatic features. Nevertheless, an illustrated guide is provided in figures 51 to 58.

Identification and Characterization

I. Subphylum Postciliodesmatophora

A. Class Spirotrichea

1. *Fabrea salina* Henneguy 1890 Figure 51

This species has an ovoid shape, with a pointed anterior end. The oral ciliature can be distinguished, forming a clockwise spiral. A cytopyge is also visible. The cell length vary from 63-104 µm and the width from 50-72 µm.

This organism is described as a usually hypersaline ciliate (Esteban and Finlay, 2003; Pandley and Yeragi, 2004; Elloumi et al., 2006). Our results agree with the literature, because in this study, it was the most dominant species of ciliates in station 3, which was the most saline site.

2. *Blepharisma* sp. Figure 56

This organism has a pyriform and spindle shape. The anterior end is narrower than the rest of the cell. It measures about 175-180 µm long and 45-50 µm wide. It is usually similar to the *B. halophila* described from Hutt Lagoon, Australia (Post et al., 1983). In that work, it was described as a *Dunaliella* feeder.

II. Subphylum Cyrtophora

A. Class Nassophorea

1. *Nassula* sp. Figure 52

The cell is more or less rounded, with ciliates around it. It has a large cyrtos, and sometimes, an extrusome can be seen. The length of the cell is of 93-95 µm and it has a width of 51-60 µm.

Nassula was described for the hypersaline Hutt Lagoon in Australia as a coccoid blue-green algae and bacteria eater (Post *et al*., 1983). This genus was abundant in Station 2, only where the coccoid cyanobacteria dominated in the microbial mat during Hydroperiod I.

2. *Euplotes* sp. Figure 53

This organism has an ellipsoid cell. The presence of cirri of about 10 µm long around the cell is evident, as well as the two

caudal cirri. Also, it has a “C-shaped” macronucleous. The cell is approximately 40 µm long and 23 µm wide.

This genus was described for Hutt Lagoon, Australia, as a halotolerant species that feeds on bacteria. In our study, *Euplotes* sp. appeared in Stations 1 and 2, showing that it can live in a wide range of salinity.

B. Class Oligohymenophorea

1. *Plagiopyla* sp. Figure 54

The cell is densely ciliated. It has a subapical deep cytostome that extends from right to left. The cell length is of approximately 95 µm and has a width of 64 µm.

Small and Lynn (1985) described it for anaerobic habitats. Thus, it is possible that we captured this species after a vertical migration from the deep layers of the mat. It was only found in station 2.

2. *Vorticella* cf. *globosa* Ghosh, 1925 Figure 55 a, b

The cell is rounded, with a contractile myoneme. A “C-shape” nucleus can be observed. Its length vary from 26.2 to 32.5 µm and the width from 20 to 25.8 µm.

This species was only found in Station 3.

3. *Vorticella* sp.2 Figure 55c

The cell is cylindrical, with a hyaline stalk. It also presents a “C-shaped” nucleous. The cell high is about 30 µm and it has a width of 25.7 µm.

This species appeared once, also in Station 3.

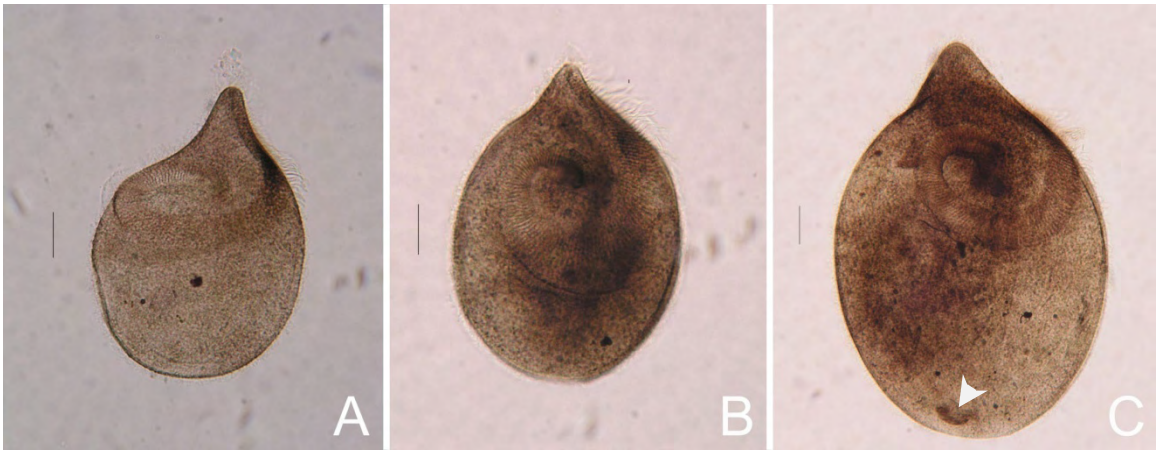

Figure 51. *Fabrea salina*. Observe the oral ciliature forming a clockwise spiral and the cytopyge (arrowhead in Figure C). Protargol impregnation after Wilbert. All scale bars = 10 µm.

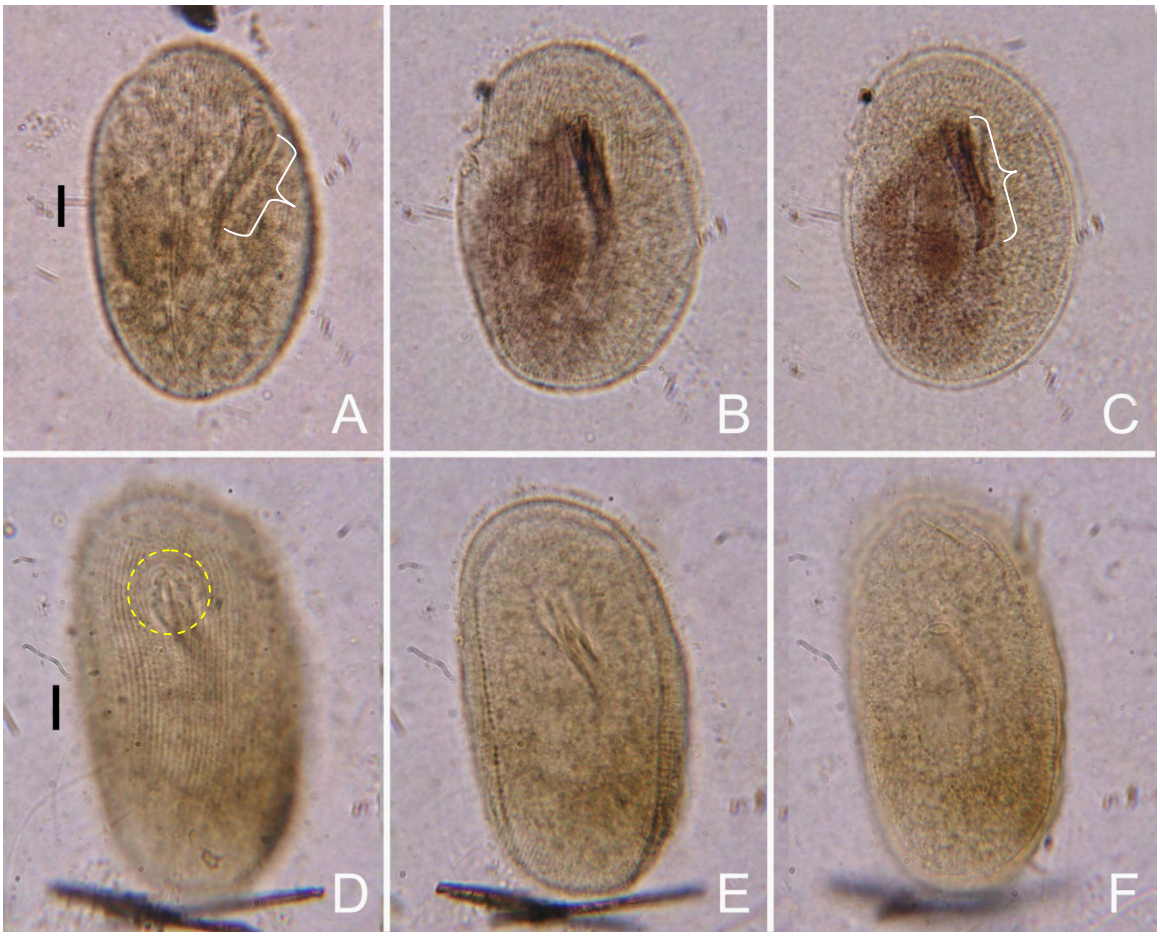

Figure 52. *Nassula* sp. in different planes. Observe the cyrtos (}) in Figures A and C. The cytopharyngeal basket (dashed circle) can be seen in Figure D. Protargol impregnation after Wilbert. Scale from A is the same for B and C. Scale from D is the same for E and F. All scalebars = 10 µm.

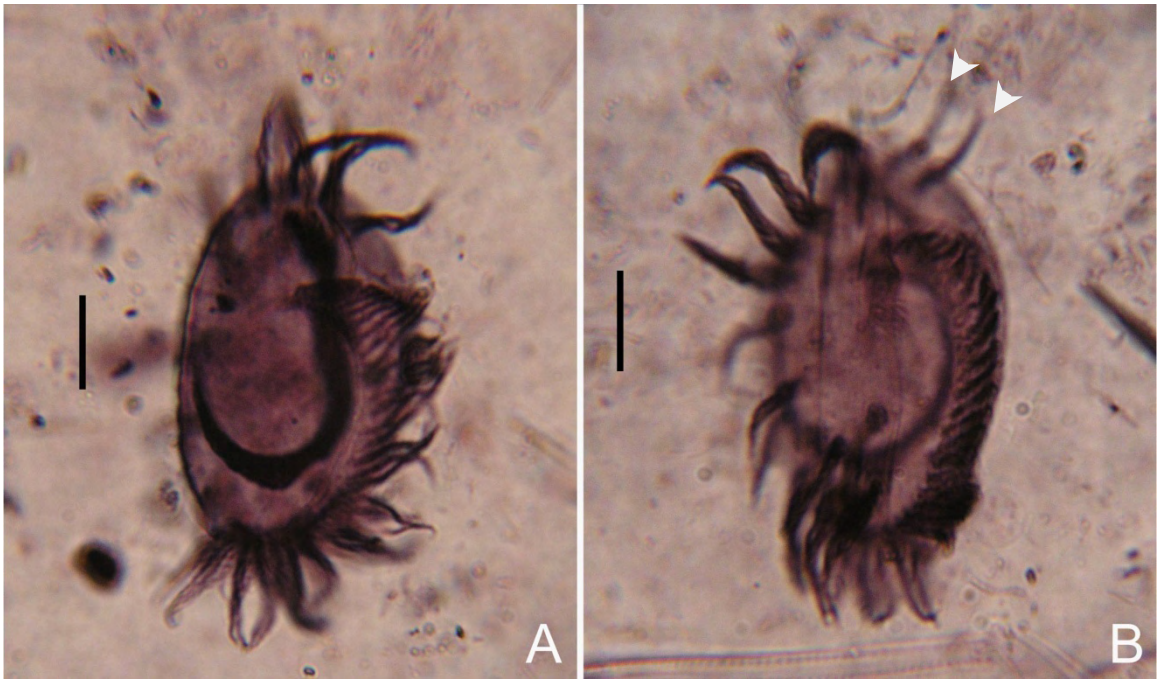

Figure 53. *Euplotes* sp. Observe the "C-shaped" macronucleous in Figure A and the numerous cirri in both pictures. The caudal cirri are indicated by arrowheads in Figure B. Quantitative protargol stain protocol. All scale bars = 10 µm.

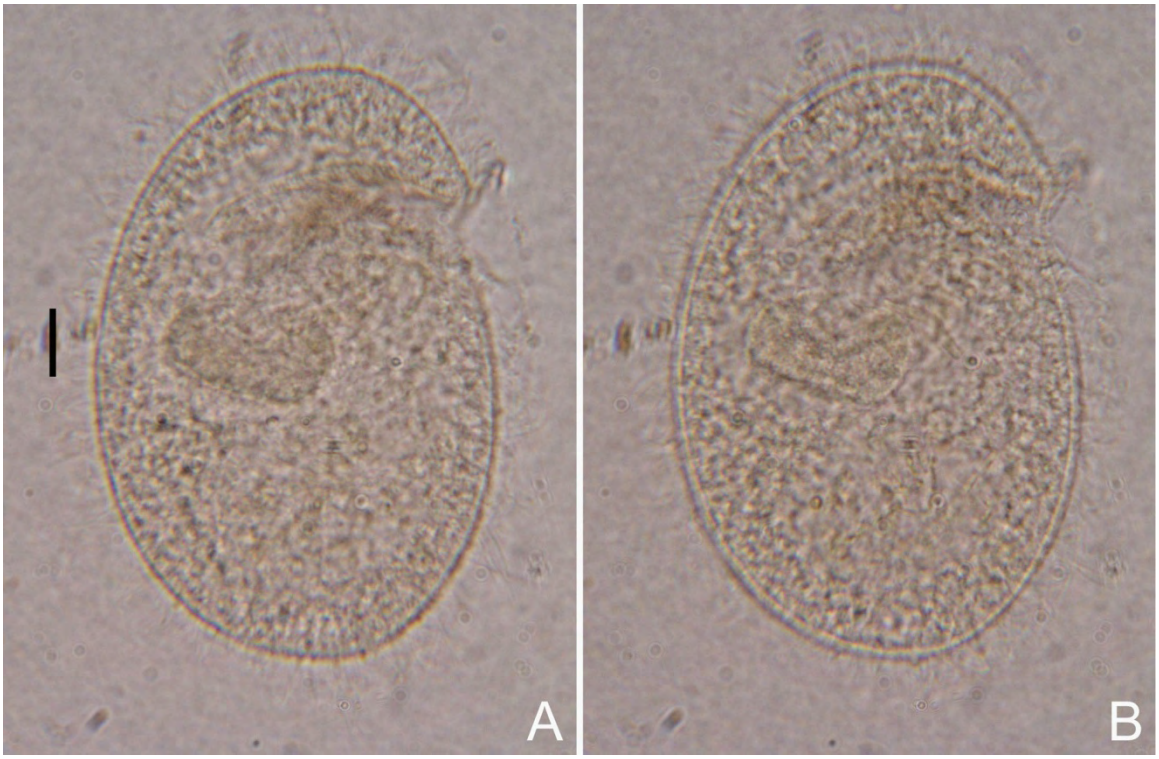

Figure 54. *Plagiopyla* sp. A and B are the same pictures in different focus. Observe the deep cytostome (A) and the macronucleous (B). Protargol impregnation after Wilbert. Scale bar = 10 µm.

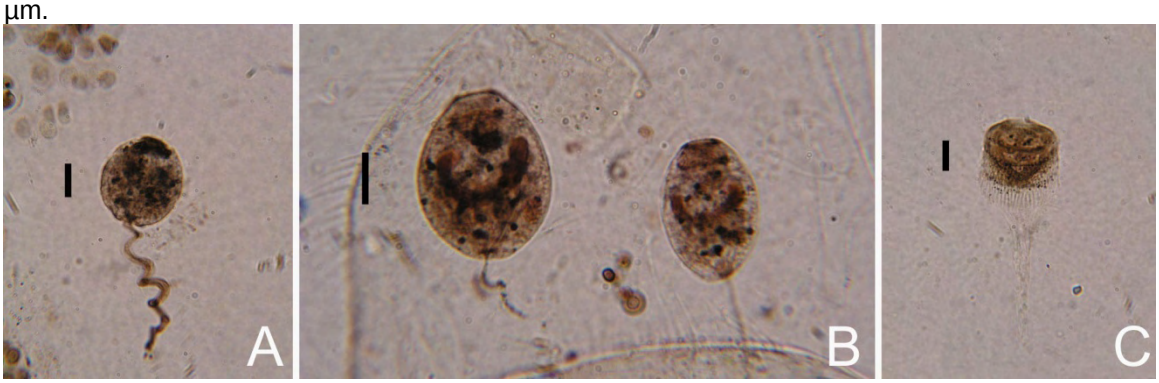

Figure 55. *Vorticella* cf. *globosa* and its contractile myoneme (A and B); Figure C shows *Vorticella* sp. 2. All pictures show the "C-shaped" macronucleous. All scale bars = 10 µm.

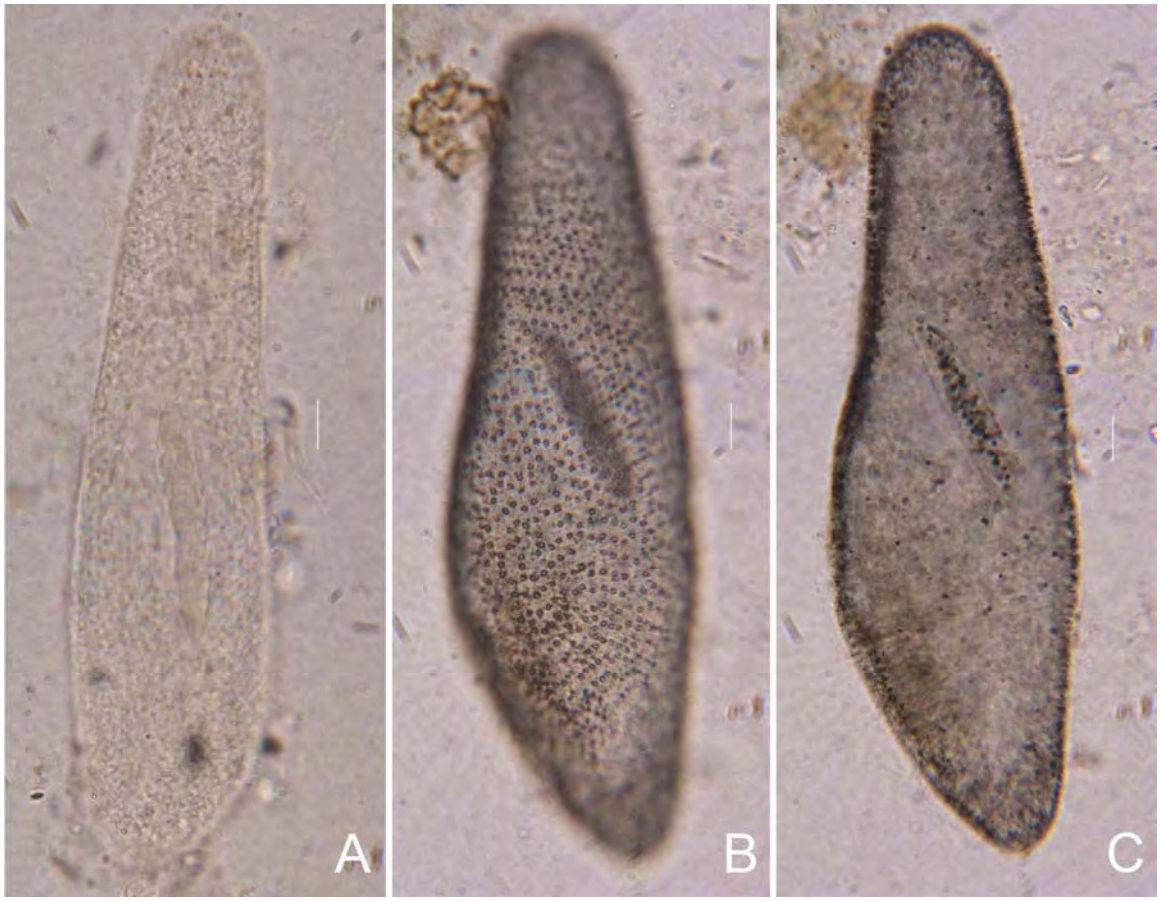

Figure 56. *Blepharisma* sp. (A) Stained by the qualitative protocol. (B and C) Same picture in different planes. Stained by the quantitative protargol stain protocol. All pictures were taken under light microscopy. All scale bars = 10 µm.

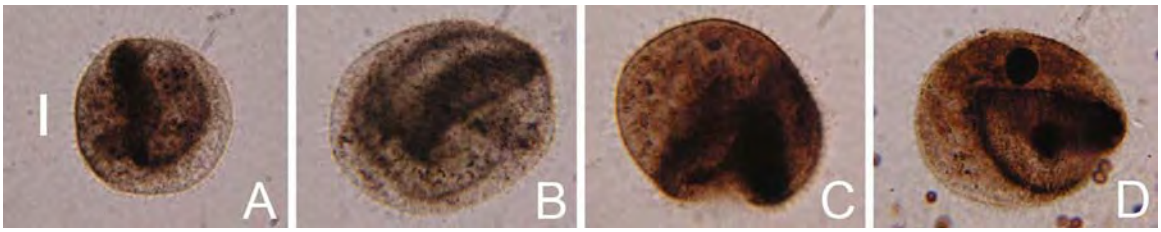

Figure 57. Unknown sp. 1. All pictures were taken under light microscopy. All scale bars = 10 µm.

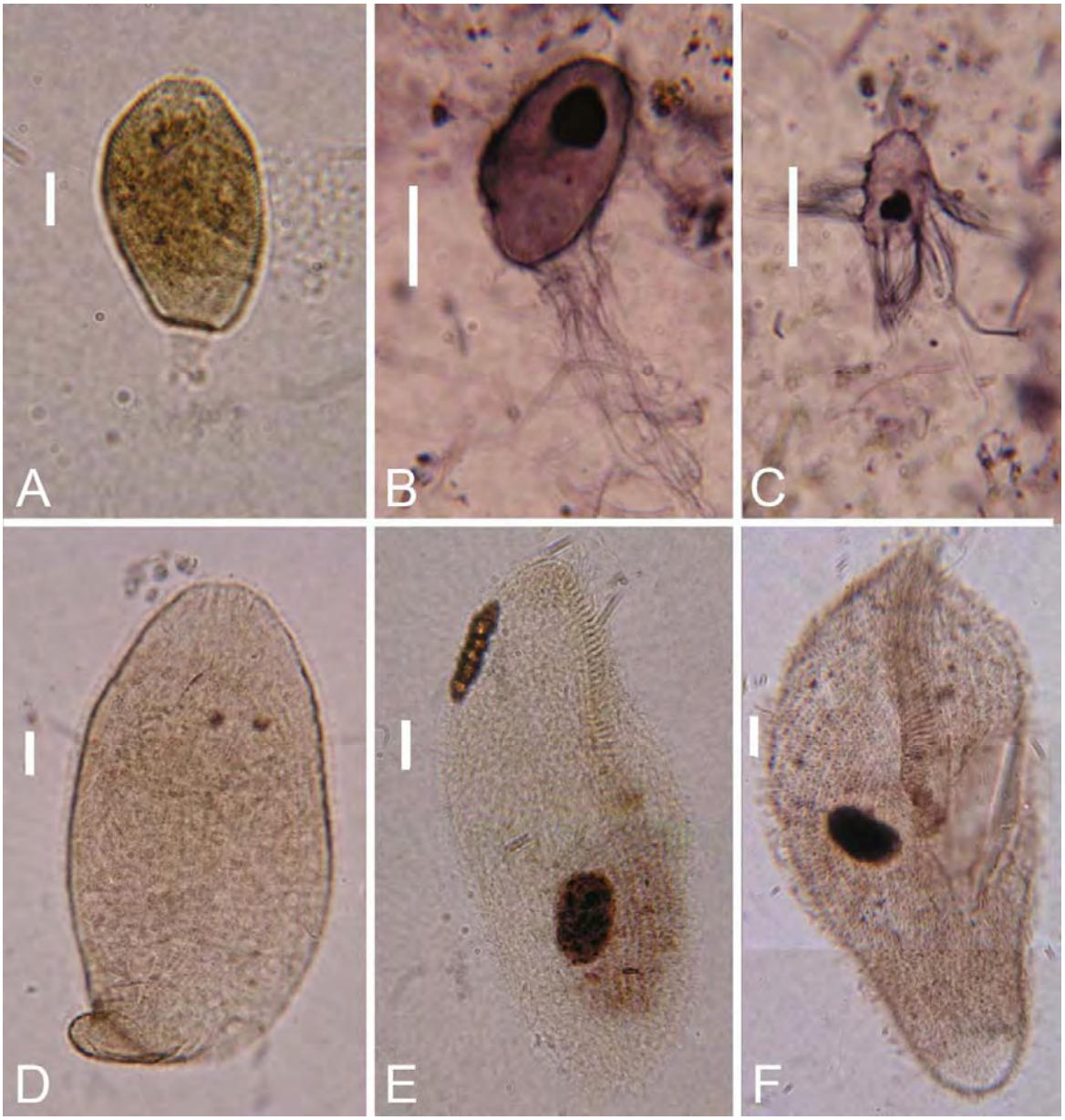

Figure 58. Unknown species. All pictures were taken under light microscopy. A, D, E, and F were stained by the qualitative protocol, while B and C were stained with by the quantitative protargol stain. All scale bars = 10 µm.

DISCUSSION

This study is a preliminary description of the ciliates that inhabit Laguna Candelaria. Each of the identified species is a new record for this area. Nevertheless, *Blepharisma* and *Euplotes* are considered cosmopolitan organisms and they were also reported previously from edaphic environments in the Luquillo Experimental Forest (Bamforth, 2007).

The species richness of ciliates is directly proportional to the cyanobacterial populations in the area and in most cases, is similar to the diatom species richness (compare Figure 49 with Figure 5 and 27). This data implies that cyanobacteria and diatoms may serve as food for the ciliates. Thus, a range of grazers can be present when a variability of food is available. Thus, dry hydroperiods, which generally had greater salinities, are not affecting the ciliate communities as we first thought in our hypothesis, i.e. that the rainy season can provide a suitable environment for these species. The majority of the ciliates that inhabit the sampling stations must be adapted to high concentrations in salinity, thus the hypothesis is rejected.

Most of the identified ciliates are also described from other salterns in the world. The saltern with the most similar diversity to the one described for Laguna Candelaria is the Hutt Lagoon in Australia (Post et al., 1983). In that work, they found *Fabrea*, *Blepharisma*, *Euplotes* and *Nassula* in their samples. From these species, the most common in our samples were *F. salina* and *Nassula* sp. The feeding information (on coccoid algae) from Post *et al*. (1983) agreed with our

observations. *Nassula* sp. was most common ciliate in Station 2, where a bloom of coccoid algae occurred during the dry season of January. Post *et al.* (1983), Esteban and Finlay (2003), and Elloumi *et al*. (2006) described *F. salina* as adapting to hypersaline environments and *Euplotes* as a halotolerant ciliate, which corroborates the observations in this study.

The *Blepharisma* that we observed in this study is similar to the *B. halophila* described from Hutt Lagoon (Post *et al*. 1983). Nevertheless, it was not abundant in our samples, thus, more information is needed to confirm this identification. Al Rasheid *et al*. (2001) described *B. intermedium* from hypersaline lagoons as having a slender filiform macronucleous (131 µm ± 12.9), but this species is bigger and more slender than the one found in this study. *Vorticella* had been described from salterns in Sfax, Tunesia by Elloumi *et al*. (2006). It was also described by Salvadó *et al*. (2001) as one of the genera that resisted high concentrations of salts in experiments conducted in their laboratory.

The unidentified organisms in this work were seen occasionally, mostly in Station 2 (which is the one with the highest species richness in general). More studies had to be conducted to identify them at a genus or species level.

CONCLUSIONS

1. *Fabrea salina* and *Nassula* sp. were the dominant forms of the ciliate community in the microbial mats.

2. Ciliate speciess richness increased with high salinities, contrary to what was postulated.

RECOMMENDATIONS

1. To compare species within dilution treatments.
2. To stain specimens with the quantitative protargol stain to improve observation of the infraciliature.
3. To quantify biomass changes.
4. To incorporate a model of possible trophic interactions.

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GLOBAL CONCLUSIONS

1. The cyanobacterial species composition in the microbial mats changes from filamentous forms to coccoid ones when the water levels are depleted. Considering that coccoid forms are nutritionally poor, management measures should be taken to avoid total desiccation of the salterns. This will ensure having edible algae and transfer of energy for the next trophic levels in the food web.

2. The species richness of ciliates seems to be directly proportional to the species richness of the cyanobacteria. This may suggest that ciliates are feeding upon cyanobacteria or that environmental conditions favor both communities in a similar way.

APPENDIX

Appendix 1. Rainfall data for the Cabo Rojo National Wildlife Refuge from 1980 to 2006. Data provided by James Padilla, from USFWS.

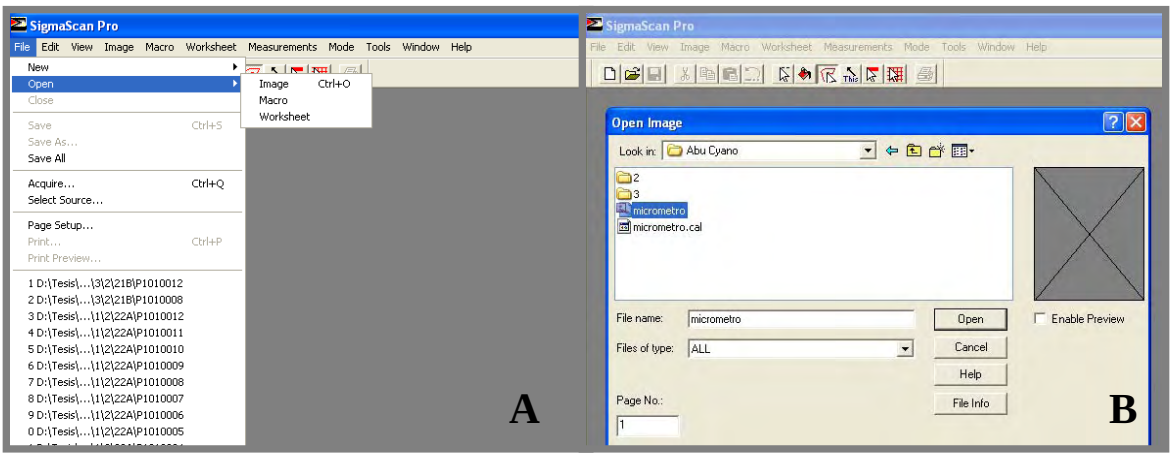
	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total	Average
1980	0	0	0	0	0	0	0	17.0456	51.4646	26.5518	0.6556	23.4377	119.1553	9.929608
1981	13.9315	62.4459	42.614	61.7903	106.6989	4.917	65.7239	40.4833	85.7197	69.8214	65.7239	106.535	726.4048	60.53373
1982	6.3921	4.917	6.556	53.2675	98.34	20.3236	77.6886	31.4688	114.73	42.614	63.7571	15.8983	535.953	44.66275
1983	3.6058	7.7033	84.4085	82.9334	212.4144	2.1307	36.8775	22.946	37.5331	106.8628	82.7695	30.8132	710.9982	59.24985
1984	51.6285	52.1202	19.5041	8.5228	37.8609	16.8817	48.0227	21.4709	131.2839	98.6678	177.5037	21.307	684.7742	57.06452
1985	3.278	14.0954	41.7945	0.8195	105.0599	4.917	14.0954	59.004	51.7924	232.2463	77.3608	1.3112	605.7744	50.4812
1986	3.9336	9.1784	3.4419	14.5871	232.9019	14.4232	13.4398	47.8588	24.585	31.141	51.4646	13.6037	460.559	38.37992
1987	64.2488	12.2925	14.4232	26.7157	44.253	142.9208	13.4398	25.5684	81.95	52.9397	200.9414	23.7655	703.4588	58.62157
1988	1.4751	11.1452	14.751	32.2883	4.917	26.7157	35.2385	217.3314	78.672	39.1721	13.4398	17.2095	492.3556	41.02963
1989	24.7489	35.4024	120.1387	25.5684	25.8962	4.917	56.7094	69.8214	118.8275	83.7529	94.8981	13.9315	674.6124	56.2177
1990	19.0124	17.2095	27.2074	4.4253	29.9937	128.3337	58.6762	28.6825	80.1471	227.3293	46.3837	7.7033	675.1041	56.25868
1991	5.5726	49.0061	44.253	35.4024	30.4854	10.9813	17.2095	25.4045	29.502	10.4896	108.0101	4.917	371.2335	30.93613
1992	166.0307	7.7033	6.3921	56.2177	164.7195	8.5228	11.473	36.7136	38.1887	96.8649	55.0704	18.029	665.9257	55.49381
1993	61.2986	23.1099	6.0643	41.3028	115.2217	25.4045	34.0912	89.4894	39.9916	22.4543	70.1492	32.78	561.3575	46.77979
1994	31.4688	10.1618	24.0933	12.2925	0.8195	6.556	3.6058	19.3402	100.6346	156.3606	64.2488	31.6327	461.2146	38.43455
1995	18.8485	46.7115	5.9004	3.1141	59.004	15.2427	18.8485	82.9334	57.6928	24.4211	82.2778	16.2261	431.2209	35.93508
1996	24.4211	13.9315	11.473	42.9418	67.6907	55.3982	58.0206	47.0393	78.9998	19.8319	97.6844	48.3505	565.7828	47.14857
1997	25.7323	0.8195	16.0622	0	12.7842	9.0145	42.4501	9.834	21.4709	62.7737	87.6865	7.5394	296.1673	24.68061
1998	13.2759	59.4957	2.6224	27.0435	26.8796	40.4833	18.3568	68.5102	253.5533	182.0929	193.2381	59.004	944.5557	78.71298
1999	44.4169	19.1763	5.4087	29.3381	11.8008	16.0622	42.9418	44.5808	80.1471	100.9624	64.5766	18.029	477.4407	39.78673
2000	22.2904	17.3734	13.6037	134.725	60.4791	21.307	32.78	19.8319	123.5806	112.7632	62.282	11.473	632.4901	52.70751
2001	13.112	29.502	1.3112	20.6514	183.4041	18.3568	28.6825	47.8588	46.2198	27.2074	36.2219	30.1576	482.6855	40.22379
2002	0.4917	20.4875	21.9626	53.7592	37.0414	0.9834	11.473	53.4314	43.7613	21.9626	22.1265	9.5062	296.9868	24.7489
2003	4.0975	15.8983	42.9418	182.256	0	0	0	41.3028	13.9315	82.9334	308.9515	0	692.3136	57.6928
2004	0	0	22.2904	37.8609	107.1906	10.4896	34.419	0	0	0	0	0	212.2505	17.68754
2005	0	0	0	19.0124	119.647	27.3713	45.892	26.5518	60.4791	182.0929	39.336	4.7531	525.1356	43.7613
2006	18.5207	15.7344	56.5455	79.8193	54.7426	21.1431	45.7281	85.0641	33.1078	84.0807	25.5684	31.4688	551.5235	45.96029

	Jan.	Feb.	Mar.	Apr.	May.	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total	Average
Total	641.8324	555.621	655.7639	1086.65	1950.246	653.7971	865.883	1279.567	1877.966	2198.391	2192.326	599.382	14557.43	1213.12
Average	23.7715	20.5785	24.28755	40.2465	72.23134	24.21471	32.0697	47.39138	69.5543	81.42188	81.19727	22.1993	539.1642	44.93035

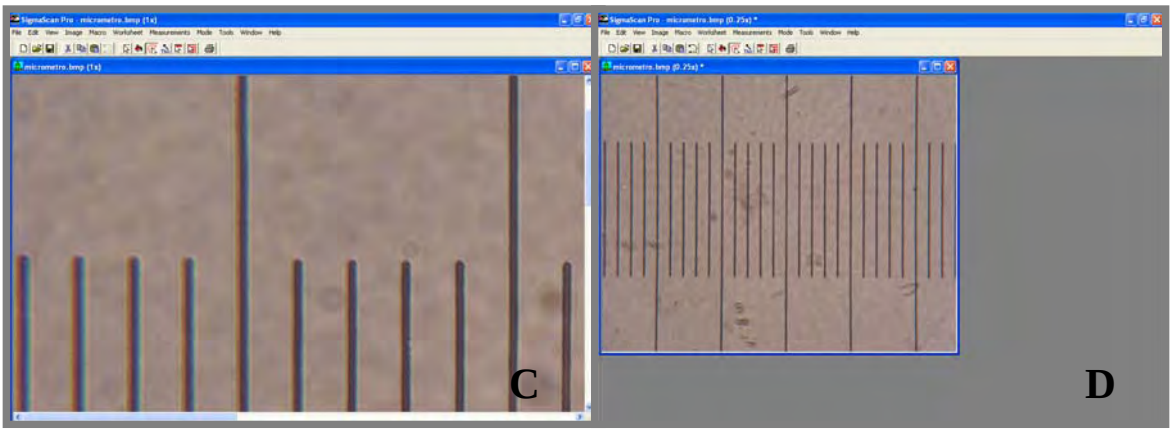
All measurements are in cm^3.

Appendix 2. Protocol for the use of SigmaScan Pro v. 5

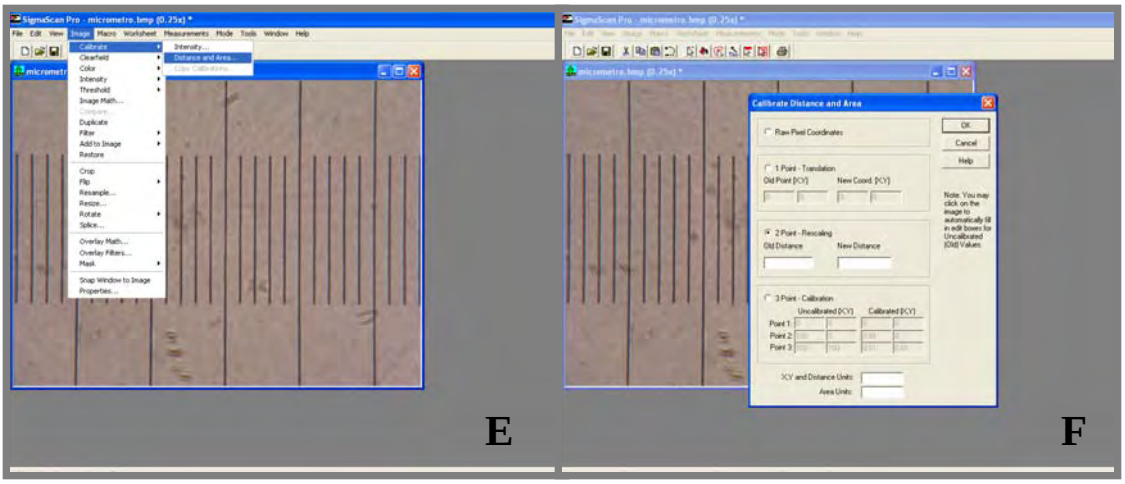
First, it is necessary to calibrate the program. For that, a digital photo of a stage micrometer was taken in a magnification of 40X. The photo was opened, selecting "**Open Image"** from the "**File"** Menu (Figure A) and then, the name of the image, followed by the "**Open"** button (Figure B).



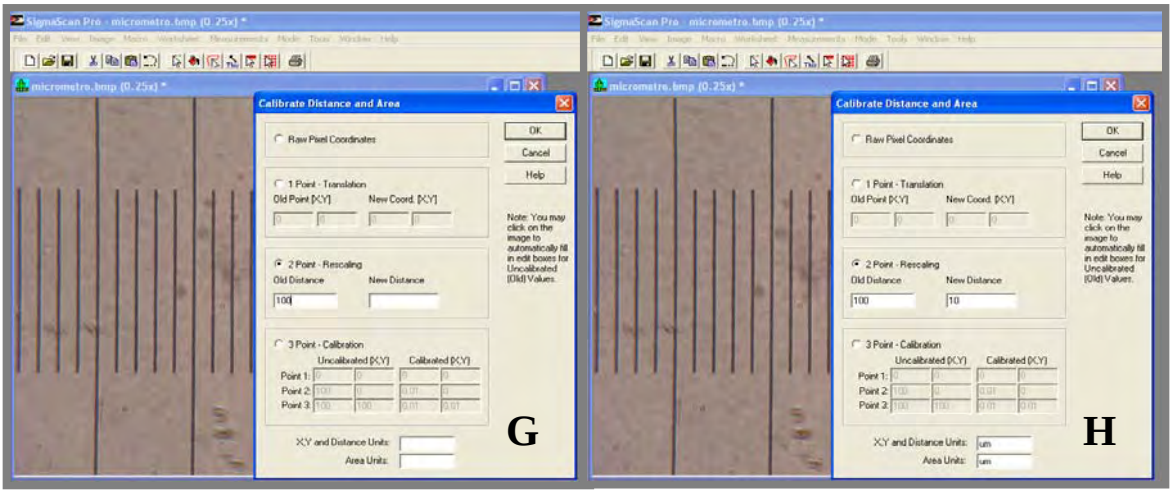
By pressing the buttons Ctrl + 2, the picture was reduced from its original size (Figure C) to a size where the whole picture can be seen (Figure D). (Ctrl + 1 increased the size again).



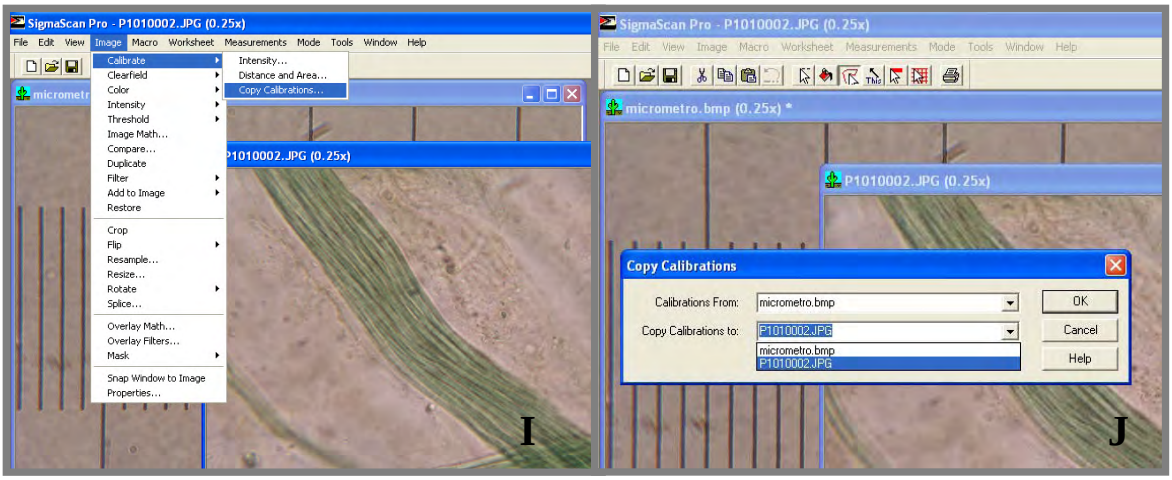
The calibration started by selecting "**Calibrating" – "Distance and Area",** from the "**Image"** menu (Figure E). A window name "Calibrate Distance and Area" appeared, where the option "2 Point – Rescaling" was selected (Figure F).



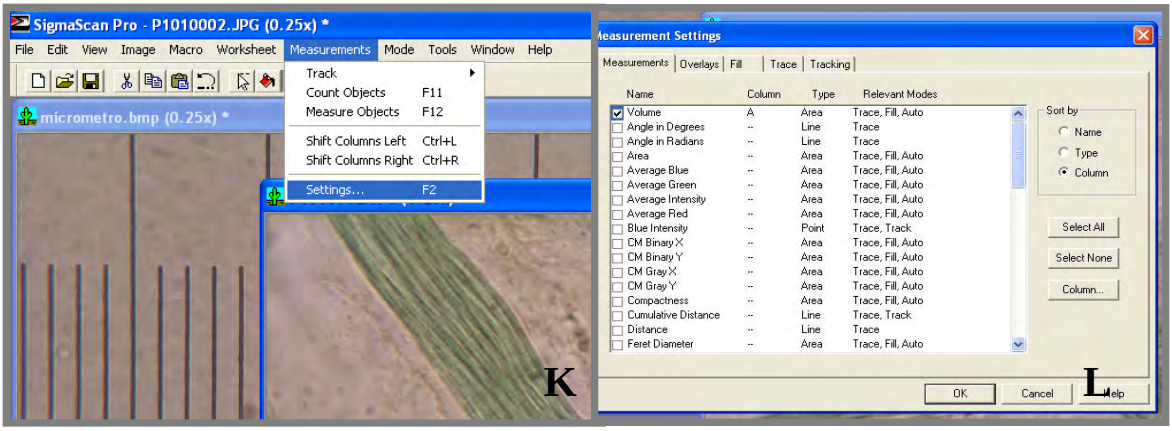
Immediately, the cursor took a "C" shape. By clicking on one of the lines of the photo of the stage micrometer and then to the next line, the number of pixels appeared within the two lines was recorded in the "**Old Distance**" space in the window before mentioned (Figure G). In the "**New Distance**" space it was necessary to write the known distance of the stage micrometer. Then, the measurement units were specified and the "**OK"** button was pressed (Figure H).



In order to measure distance, area or volume, each of the images needed to be calibrated. For that, the images were opened. For each image, the "**Calibrate"** – "**Copy Calibration"** option from the "**Image"** menu had to be selected (Figure I). A window appeared, allowing to copy the calibration from the photo of the stage micrometer to the new photo (Figure J).

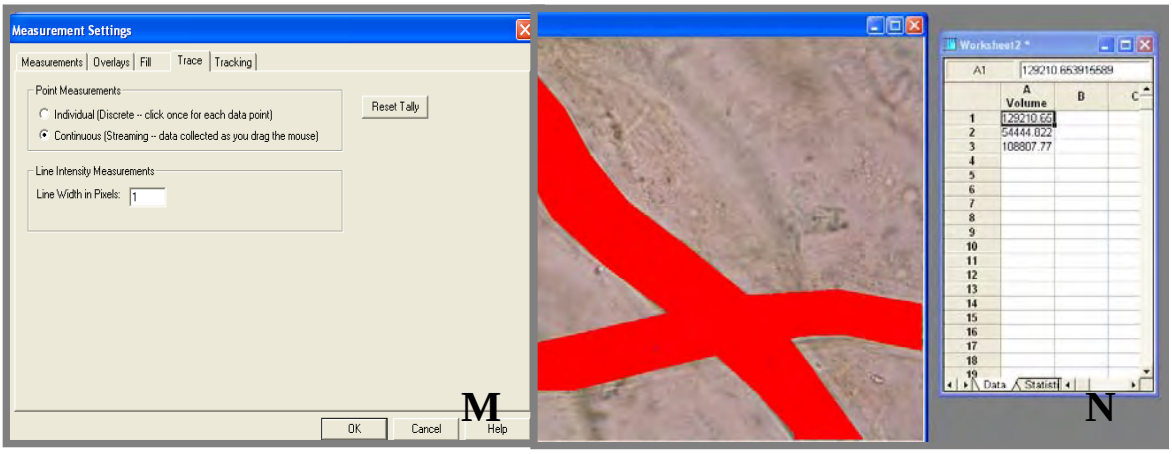


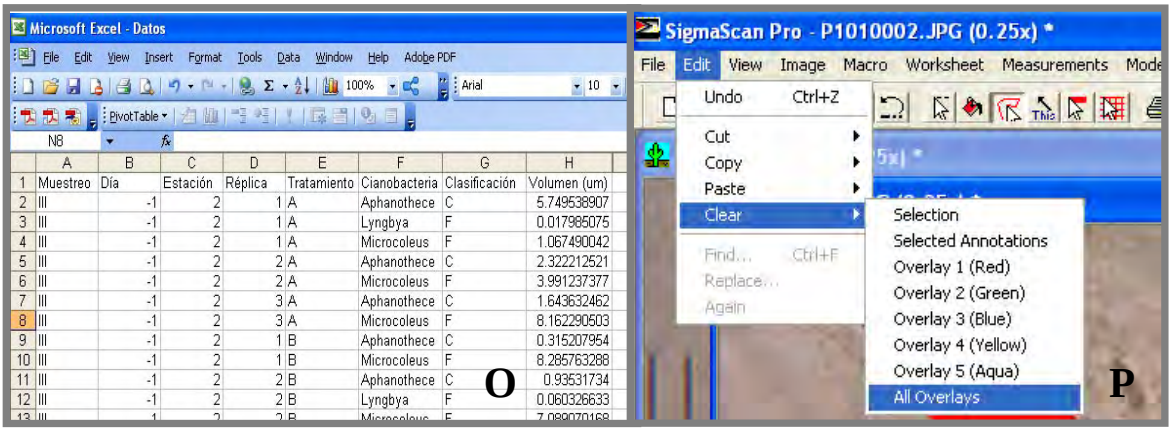
When all the photos were calibrated, the measurement options had to be set. For that, the "**Settings"** option under the "**Measurement"** menu was selected (Figure K). A window appeared with all the options that we can measure (Figure L). The "**Volume**" option was selected. Also in this window, other options like the column in which we want the data to appear can be selected.



Then, in the Trace file, the "**Continuous"** option was selected, and the "**OK"** button pressed (M). (If it is the distance what you want to measure, you must select "**Individual**", instead of "**Continuous**").

The pictures were ready. Each of the species of cyanobacteria had to be measured separately. This is, there can be 15 opened photos of the same sample, with the same calibration. For each one, just one species (e.g. *Microcoleus chthonoplastes*) can be measured. All the data began to appear in a table (Figure N). When all the specimens of that species were measured for the 15 photos, the data were copied and pasted in excel, where we did a summation and the total was recorded in a table (Figure O).





Back to the SigmaScan Program, once every specimen of the first species were measured, the overlays were deleted by going to the "**Edit**" menu and selecting "**Clear**" - "**All Overlays**" (Figure P). Then, the table with the measurements had to be closed (Pressing the "X" the right-superior part of the table) and the process was repeated for every other species in the samples.

Appendix 3. Physical and chemical parameters in each aquarium during all hydroperiods

Hydroperiods	Day	Station	Treatments	S (ppt)	T (˚C)	O.D. (ppm)	Water depth (cm)
I	-1	1	A	96	32.00	*	1.5
I	-1	1	B	98	32.50	*	1.5
I	-1	1	C	97	33.00	*	1.5
I	-1	2	A	*	31.00	*	0.0
I	-1	2	B	*	31.00	*	0.0
I	-1	2	C	*	31.00	*	0.0
I	-1	3	A	89	26.00	*	1.0
I	-1	3	B	89	26.50	*	1.0
I	-1	3	C	93	93.00	*	1.0
I	1	1	A	2	23.50	*	15.0
I	1	1	B	20	24.50	*	10.7
I	1	1	C	16	25.00	*	16.0
I	1	2	A	2	22.50	*	10.9
I	1	2	B	18	22.50	*	10.0
I	1	2	C	8	23.00	*	5.0
I	1	3	A	11	22.50	*	8.0
I	1	3	B	24	22.50	*	13.1
I	1	3	C	18	23.00	*	7.5
I	3	1	A	8	22.00	*	13.4
I	3	1	B	31	23.00	*	8.5
I	3	1	C	93	22.00	*	15.0
I	3	2	A	8	21.00	*	7.0
I	3	2	B	26	21.00	*	6.0
I	3	2	C	38	22.00	*	3.0
I	3	3	A	26	21.00	*	7.0
I	3	3	B	30	21.00	*	11.0
I	3	3	C	56	21.00	*	6.5
II	-1	1	A	85	26.63	3.6	6.7
II	-1	1	B	85	26.47	3.8	6.5
II	-1	1	C	85	26.31	3.8	6.5
II	-1	2	A	87	25.47	1.0	1.5
II	-1	2	B	87	25.93	0.8	1.5
II	-1	2	C	87	25.81	0.8	1.5
II	-1	3	A	81	21.40	1.6	4.0
II	-1	3	B	81	21.00	0.6	3.5
II	-1	3	C	81	21.10	1.0	4.4
II	1	1	A	6	24.70	2.4	12.4
II	1	1	B	44	23.41	4.8	11.0
II	1	1	C	91	23.48	4.2	12.5
II	1	2	A	6	22.84	4.6	8.7
II	1	2	B	43	22.43	4.8	6.0
II	1	2	C	92	22.43	2.0	4.5
II	1	3	A	7	22.26	8.0	4.5

Hydroperiods	Day	Station	Treatments	S (ppt)	T (˚C)	O.D. (ppm)	Water depth (cm)
II	1	3	B	48	21.56	3.8	3.5
II	1	3	C	86	21.68	2.0	4.4
II	3	1	A	11	27.68	3.6	10.5
II	3	1	B	52	27.31	4.8	9.2
II	3	1	C	97	26.84	4.8	11.0
II	3	2	A	12	28.11	3.2	3.5
II	3	2	B	60	28.60	5.4	2.0
II	3	2	C	4.8	29.44	0.0	2.0
II	3	3	A	34	25.93	0.2	4.5
II	3	3	B	66	25.09	0.4	4.5
II	3	3	C	46	25.30	0.8	3.5
III	-1	1	A	64	30.00	5.0	10.0
III	-1	1	B	61	30.00	5.8	10.0
III	-1	1	C	66	30.00	5.0	10.0
III	-1	2	A	65	25.00	0.0	8.0
III	-1	2	B	65	25.00	0.0	8.0
III	-1	2	C	63	25.00	0.0	8.0
III	-1	3	A	66	23.00	0.0	7.4
III	-1	3	B	65	23.00	0.0	7.4
III	-1	3	C	65	23.00	0.0	7.4
III	1	1	A	4	27.00	4.8	12.5
III	1	1	B	37	26.50	3.2	12.4
III	1	1	C	66	26.50	2.0	12.4
III	1	2	A	2	25.00	4.4	11.0
III	1	2	B	34	25.00	1.6	12.0
III	1	2	C	65	25.00	0.0	10.9
III	1	3	A	10	25.00	1.8	5.2
III	1	3	B	41	25.00	2.2	6.5
III	1	3	C	67	25.00	0.2	7.5
III	3	1	A	10	24.00	1.4	11.3
III	3	1	B	44	24.00	1.8	11.0
III	3	1	C	73	24.50	3.0	11.3
III	3	2	A	9	23.00	1.8	9.5
III	3	2	B	41	23.00	0.0	10.3
III	3	2	C	73	23.00	0.2	8.5
III	3	3	A	25	22.00	0.8	4.6
III	3	3	B	61	22.00	0.2	5.8
III	3	3	C	78	22.00	1.0	5.9

Day -1: before treatments; Day 1: 24 hrs after treatment; Day 3: 72 hrs. after treatments; Treatment A: distilled water; Treatment B: sterilized seawater; Treatment C: ambient water from the station; The asterisk (*) indicate that the water depth was not sufficient to permit the data collection. Hydroperiod I = dry season of January; Hydroperiod II = dry season of April; and Hydroperiod III = rainy season of November.

Appendix 4. Cyanobacterial biovolume (μm^3) after measuring each genus with SigmaScan Pro v.5

Hydroperiod	Day	Station	Treatment	*Aphanocapsa*	*Aphanothece*	*Chlorogloea*	*Chroococcus*	*Cyanosarcina*	*Lyngbya*	*Microcoleus*	*Oscillatoria*	*Spirulina*
I	-1	1	A1	0.216839866	1.180104604	0.134189036	0.021488733	0.081733976	0.052149076	6.464097779		
I	-1	1	A2	0.103067365	1.824892412		0.021975861			1.939095193		
I	-1	1	A3	0.364117489	0.566536683			0.008142823		3.576383444		
I	-1	1	B1		2.306230743	0.134141566				1.810047366		
I	-1	1	B2		1.635050794	2.125488512		0.08442976	0.022514296	2.329828416		
I	-1	1	B3		0.618041966	0.189300466		0.018763451	0.174940315	2.526302547		
I	-1	1	C1		0.75603569	0.142227564			0.03551381	6.094173159		0.001533661
I	-1	1	C2	0.230558385	0.716855032	1.823439455	0.019884076			3.64866981		
I	-1	1	C3	0.055564131	1.798639639				0.027112768	7.639040664		
I	-1	2	A1		12.26745574				0.022640887	0.068898646	0.056467556	
I	-1	2	A2		5.520765328				0.032694181		0.006262235	
I	-1	2	A3		20.85682954						0.001672248	
I	-1	2	B1		6.780171817						0.053811546	
I	-1	2	B2		12.11053744				0.000846109			
I	-1	2	B3		2.144240481						0.05362237	
I	-1	2	C1		8.023940606	0.322659636					0.034350355	
I	-1	2	C2		6.782995082					0.018120475	0.013586735	
I	-1	2	C3		5.321557602					0.378711088	0.018190519	
I	-1	3	A1		0.246602012				0.027000159	13.30328505		
I	-1	3	A2		0.246602012				0.027000159	13.30328505		
I	-1	3	A3		0.246602012				0.027000159	13.30328505		
I	-1	3	B1		0.350337465	1.153182904			0.005503716	6.025233786		
I	-1	3	B2		1.152046062					5.908350196	0.001451443	
I	-1	3	B3		0.560776714	1.729519644			0.049792978	8.46440521		
I	-1	3	C1		0.359747799	2.16538332				6.933851374		
I	-1	3	C2		0.903635557				0.019152872	6.361613428		
I	-1	3	C3		0.523018762	0.751448489			0.053962007	4.446511569		
I	1	1	A1		2.132268732					9.696098911		
I	1	1	A2		1.477151891	0.003131939	0.020486545			5.184706399		

Hydroperiod	Day	Station	Treatment	*Aphanocapsa*	*Aphanothece*	*Chlorogloea*	*Chroococcus*	*Cyanosarcina*	*Lyngbya*	*Microcoleus*	*Oscillatoria*	*Spirulina*
I	1	1	A3	0.369509084	3.025905525					10.32313512		
I	1	1	B1	0.851751833	1.438763843					1.646154788		
I	1	1	B2	0.147757694	0.637323372	1.350990108		0.402306378	0.011990166	2.447593583		
I	1	1	B3	2.168432666	1.695018165	0.265556583			0.022627795	4.257996538		
I	1	1	C1	1.03892587	2.007267856					1.626076512		
I	1	1	C2		0.993061924			0.03049783		2.227983302		
I	1	1	C3		0.608853456	0.549617991				5.29335875		
I	1	2	A1		4.758987925					0.739826593	0.013440364	
I	1	2	A2		2.04432082					0.261122455	0.10802736	
I	1	2	A3		5.081357245					0.166327447	0.10530682	
I	1	2	B1		3.951647709					0.450023098	0.018234323	
I	1	2	B2		3.989498358			0.048245393		0.347192884	0.023295252	
I	1	2	B3		3.695061222					0.054337926	0.028976323	
I	1	2	C1		10.24067644			0.032366049		0.429546638	0.0042007	
I	1	2	C2		17.40353841							
I	1	2	C3		4.349069853					0.038208384	0.02358264	
I	1	3	A1		0.211959704					33.07589739		
I	1	3	A2		3.016900535					23.10139257		
I	1	3	A3		0.810302594					22.15622095		4.97409E-05
I	1	3	B1		3.236094688		0.039981943			7.793744624		
I	1	3	B2		1.544931806	0.011623871			0.033068885	5.106493169		
I	1	3	B3		1.172171845		0.020972566			12.18523106		
I	1	3	C1		1.163876685				0.014137147	12.55407949		
I	1	3	C2	0.027935603	1.830291945					15.99455064		
I	1	3	C3		0.295778926				0.032680041	9.830513812		
I	3	1	A1		4.400644019				0.025493524	3.311120576	0.057632986	
I	3	1	A2	0.031719432	0.607983029		0.009488478		0.021064133	1.460140509		
I	3	1	A3	1.236634496	0.962459115	3.831899763				1.719008748		
I	3	1	B1		1.867783381	0.500008857	0.027654139	1.500960425		4.06776111		
I	3	1	B2	0.121619963	0.451991677	1.261418578	0.01331445		0.050109339	9.737754707		0.000579386
I	3	1	B3		1.915681156	0.050864466		0.062358826		3.472128978		
I	3	1	C1	0.132331751	1.333098036	1.950088437		0.057821531		3.26653984		
I	3	1	C2	0.029698492	1.75342362	0.116323304				5.227879034		

Hydroperiod	Day	Station	Treatment	*Aphanocapsa*	*Aphanothece*	*Chlorogloea*	*Chroococcus*	*Cyanosarcina*	*Lyngbya*	*Microcoleus*	*Oscillatoria*	*Spirulina*
I	3	1	C3		0.945243073					3.578524932	0.00029267	
I	3	2	A1		5.506974033					0.034067234	0.011420167	
I	3	2	A2		3.904879974				0.010741728	0.191546006	0.018272888	
I	3	2	A3	0.080721712	3.421535564					0.324907953	0.00936643	
I	3	2	B1		4.663948714					1.277700248	0.021033495	
I	3	2	B2		1.301376021						0.021556588	
I	3	2	B3		2.474587504					2.12387416	0.011695802	
I	3	2	C1		5.499315477					0.32993077	0.022729214	
I	3	2	C2		1.031320765	0.0368162				1.295229441	0.240893772	
I	3	2	C3	0.041301355	9.192070652					0.180613465		
I	3	3	A1		0.787595621	1.159748908				3.67593419		
I	3	3	A2		0.592697075		0.0105848		0.020969126	20.25598189		
I	3	3	A3		0.636999112		0.015523071			4.555755897		
I	3	3	B1		0.472889868					3.98593449		
I	3	3	B2	0.066784551	0.544412331				0.039544675	2.415497465		0.000130887
I	3	3	B3		0.454202235					6.363992205		
I	3	3	C1		0.278602823	0.057798564		1.456850718	0.017006183	13.38103262		
I	3	3	C2		2.432103642					5.126761662		
I	3	3	C3		0.002556371				0.249551638	5.950344469		
II	-1	1	A1		0.235	0.139	0.0652	0.125		3.47		0.00221
II	-1	1	A2	0.435	3.25	0.497	0.017			2.38		
II	-1	1	A3	1.85	1.81	0.43	0.0611	0.503		3.49		0.0211
II	-1	1	B1		0.51	0.141	0.0102	0.0317		4.81		0.49
II	-1	1	B2	1.03	1.12		0.0133	0.00309		1.93		
II	-1	1	B3		1.34	0.333		0.0369		1.9		
II	-1	1	C1		0.665			0.00342		2.39		
II	-1	1	C2		0.441	0.0797		0.0276		4.23		
II	-1	1	C3		0.624			0.0109		1.17		
II	-1	2	A1		0.302					4.67	0.0164	
II	-1	2	A2		0.4							
II	-1	2	A3	0.0717	0.836					8.76	0.0309	
II	-1	2	B1	0.0198	0.688		0.0101			5.98	0.163	0.18

Hydroperiod	Day	Station	Treatment	*Aphanocapsa*	*Aphanothece*	*Chlorogloea*	*Chroococcus*	*Cyanosarcina*	*Lyngbya*	*Microcoleus*	*Oscillatoria*	*Spirulina*
II	-1	2	B2	0.133	0.564		0.0419			9.84	0.00553	0.00106
II	-1	2	B3		0.155		0.00687			9.22	0.0208	0.00323
II	-1	2	C1	0.138	0.605		0.0226			9.44	0.0729	0.274
II	-1	2	C2	0.139	0.64					5.37	0.0583	0.0101
II	-1	2	C3		0.214		0.0044			14.9		
II	-1	3	A1	1.06	0.647	0.752	0.00547			5.45		
II	-1	3	A2	1.14	0.542	2.815064		1.63		2.46		
II	-1	3	A3		0.275		0.00749			15.7		
II	-1	3	B1		0.42	0.435	0.00911	0.0289		11.9		
II	-1	3	B2		1.21	1.74		0.231		7.86		
II	-1	3	B3		0.377					10.4		
II	-1	3	C1		0.246					13.2		
II	-1	3	C2		0.151			0.0801		9.05		
II	-1	3	C3		0.142					13.1		
II	1	1	A1		2.52			0.322		12.1		
II	1	1	A2	0.155	0.859	4.27245	0.0725			2.6		
II	1	1	A3		1.55	2.75	0.0241			10.3		
II	1	1	B1		1.05		0.0112	0.0107		3.15		
II	1	1	B2		0.737							
II	1	1	B3	0.0871	0.799	1.37	0.0153	1.13		7.73		
II	1	1	C1	0.567469	1.36		0.0289	0.118		3.28		
II	1	1	C2	0.0887	2.32	0.48		0.537		1.6		
II	1	1	C3		1.3					6.09		
II	1	2	A1		2.01		1.1			2.67	0.1206	
II	1	2	A2		0.133	0.298023	0.0117			28.4		
II	1	2	A3		0.388	0.389	0.0275			4.99	0.8205	
II	1	2	B1	0.0209	0.298					8.49	0.8809	0.0147
II	1	2	B2		0.344					23.5	0.0719	
II	1	2	B3		2.84		0.0303			4.23	3.418	0.105
II	1	2	C1		0.467			0.164		5.12	0.0983	
II	1	2	C2		1.26	10				5.71	0.377	
II	1	2	C3		0.442		0.0303			15	6.23	

Hydroperiod	Day	Station	Treatment	*Aphanocapsa*	*Aphanothece*	*Chlorogloea*	*Chroococcus*	*Cyanosarcina*	*Lyngbya*	*Microcoleus*	*Oscillatoria*	*Spirulina*
II	1	3	A1		2.01	0.0563				15.1		
II	1	3	A2		1.88					11.1		
II	1	3	A3		0.0867	10.7				5.13		
II	1	3	B1		0.145					4.49		
II	1	3	B2	0.0887	0.131					9.47		
II	1	3	B3		0.56			0.22021		11.095292		
II	1	3	C1		0.033					19.3		
II	1	3	C2		0.0519		0.0113	0.167849		23.41673		
II	1	3	C3		0.0805					14.864221		
II	3	1	A1	0.0868	1.19	0.0943	0.115	4.9		7.1		
II	3	1	A2		0.698		0.0832	2.02		14.2		0.00391
II	3	1	A3		0.759		0.124			4.35		
II	3	1	B1	0.0758	1.21		0.0184	0.0704		6.55		
II	3	1	B2		0.546	2.78	0.0356	0.0437		13.3		0.923
II	3	1	B3	0.329	2.39	1.66		0.165		5.56		
II	3	1	C1		0.298	0.0171	0.0655	1.4		9.41		
II	3	1	C2		1.07	8.19	0.0338			5.71		
II	3	1	C3		1.3	1.5	0.0385	0.293		4.46		
II	3	2	A1		3.29					3.9	1.2459	0.0176
II	3	2	A2	1.39	0.524		0.0369			19.6	0.31	
II	3	2	A3	0.303	0.94			0.235	0.0629	12.2	0.486	0.00837
II	3	2	B1		1.23		0.0197			9.01	0.0481	
II	3	2	B2		1.52	0.0916				18.4	0.00862115	0.000701
II	3	2	B3		2.66		0.114			10.4	0.2834	0.0364
II	3	2	C1	0.0702	1.12					4.01	3.1454	0.00247
II	3	2	C2		1.34		0.0111	0.19155		5.47	0.072	0.00183
II	3	2	C3		0.728	0.939	0.0122			13.2	0.072	0.119
II	3	3	A1		1.22		0.00425			3.41	0.0358	0.000282
II	3	3	A2	0.0105	0.355	0.0878				19.1		
II	3	3	A3		0.897	0.449	2.56			19.4		
II	3	3	B1		0.128			0.235		16.5		
II	3	3	B2		2.31					15.5		

Hydroperiod	Day	Station	Treatment	*Aphanocapsa*	*Aphanothece*	*Chlorogloea*	*Chroococcus*	*Cyanosarcina*	*Lyngbya*	*Microcoleus*	*Oscillatoria*	*Spirulina*
II	3	3	B3		0.263					16.1		
II	3	3	C1		0.0062		0.00876			18.6		
II	3	3	C2	0.103	0.124					37.2		
II	3	3	C3		0.0773	1.9				14.8		
III	-1	1	A1		5.749538907				0.017985075	1.067490042		
III	-1	1	A2		2.322212521					3.991237377		
III	-1	1	A3		1.643632462					8.162290503		
III	-1	1	B1		0.315207954					8.285763288		
III	-1	1	B2		0.93531734				0.060326633	7.089070168		
III	-1	1	B3		1.525080647					6.905864637		
III	-1	1	C1		1.139670647				0.025914815	11.1471354		
III	-1	1	C2		0.983610658					9.628902303		
III	-1	1	C3		1.770033		0.015498154		0.01718884	15.04476819		0.005819364
III	-1	2	A1	0.048402703	0.003332798	0.291097521		0.149087853		0.232094348	0.07175179	
III	-1	2	A2			0.108710883					0.364015691	
III	-1	2	A3	0.077018745				0.943006571		0.378266069	0.017353661	
III	-1	2	B2		0.441147415				1.038541483	0.110047448	0.016684477	
III	-1	2	B3							1.212669377	0.061854991	
III	-1	2	C1		0.850477128					15.41019804	0.051912798	
III	-1	2	C2		3.676800652					5.585881751	0.106119757	
III	-1	2	C3							0.149600261	0.07740088	
III	-1	3	A1		0.433949623		0.014900917			40.99905737		
III	-1	3	A2		0.719544465					11.85890744		
III	-1	3	A3		0.374901691				0.00021706	18.69093418		
III	-1	3	B1		0.289717942					14.24036866		
III	-1	3	B2		0.809098461				0.018292961	18.73260105		
III	-1	3	B3		0.69264126					10.97745759	0.0037274	
III	-1	3	C1		0.276015331					14.61646814	0.001373735	
III	-1	3	C2		0.189859551					16.59538662		
III	-1	3	C3		0.369067827				0.019099678	10.08874949		
III	1	1	A1		0.77610828					5.398325242	0.006179195	
III	1	1	A2	1.443563975	7.650974767	0.952510469			0.017506924	2.104536381		

Hydroperiod	Day	Station	Treatment	*Aphanocapsa*	*Aphanothece*	*Chlorogloea*	*Chroococcus*	*Cyanosarcina*	*Lyngbya*	*Microcoleus*	*Oscillatoria*	*Spirulina*
III	1	1	A3	0.119612269	1.01713725				0.037412801	3.018968111		
III	1	1	B1		1.185934295					7.089876792		
III	1	1	B2	0.424733924	1.414132022				0.014920207	8.50482592		
III	1	1	B3	0.026231294	0.818013818					9.439408257		
III	1	1	C1		1.625408845					11.2155497		
III	1	1	C2	0.102363043	3.13678734				0.04802232	14.64210255		
III	1	1	C3		3.315936439	0.674084059				9.628612131		
III	1	2	A1								0.043789348	
III	1	2	B2			0.316091884					0.147166871	
III	1	2	B3		0.036646296					19.71875472	0.011755873	
III	1	2	C1		1.633148296					6.849840809	0.330964414	
III	1	2	C2		0.415203213	0.410670813	0.004922095			3.473637367	0.065630779	
III	1	2	C3							1.848502222		
III	1	3	A1		0.504195831				0.055396147	13.52571625		
III	1	3	A2		0.016043117					11.09494097		
III	1	3	A3		0.036090211					13.47920851		
III	1	3	B1		0.924332267					11.52107545		
III	1	3	B2		0.35470818					15.29400808		
III	1	3	B3		0.250139537			0.232128808		16.48189276		
III	1	3	C1		0.385513525					13.68208933		
III	1	3	C2		0.420061992					13.5185476		
III	1	3	C3		0.193767539					17.36478201		
III	3	1	A1		1.918630809					4.36542186		
III	3	1	A2		3.851259439					9.537383594		
III	3	1	A3		5.692553334					10.8659297		
III	3	1	B1		2.694169371	0.090760165		0.559178273	0.018434373	4.026842099		
III	3	1	B2		0.447466343				4.53584297	16.25264865		
III	3	1	B3	0.678336544	3.709909678					6.537561382		
III	3	1	C1		1.286329318			0.160422599	0.110063852	20.43803217		
III	3	1	C2		3.719958146					10.49433155		
III	3	1	C3		1.807546969				0.014387938	16.4072728		
III	3	2	A1			5.419479646					0.378210462	0.001771812

Hydroperiod	Day	Station	Treatment	*Aphanocapsa*	*Aphanothece*	*Chlorogloea*	*Chroococcus*	*Cyanosarcina*	*Lyngbya*	*Microcoleus*	*Oscillatoria*	*Spirulina*
III	3	2	A3	0.179876044		0.488852919					0.139354751	
III	3	2	B1		0.557959356					12.63787522	0.021212129	
III	3	2	B2		0.479671018					10.03122155	0.010566294	
III	3	2	B3		0.388299382					11.30399788	0.009664021	
III	3	2	C1	0.036056379		0.143679475		0.003395501		6.646215369		
III	3	2	C2		1.823643483	0.014327292			0.092670208	2.346366123	0.150492117	
III	3	2	C3		1.514247104					27.02077358	0.132029392	
III	3	3	A1		0.05801932				0.008508885	21.84132746		
III	3	3	A2	0.198781357	0.248331337	0.278932691			0.030898653	16.70386898		
III	3	3	A3		0.043803682				0.078468895	20.24954831		
III	3	3	B1		1.021391658					16.78279503		
III	3	3	B2		0.133176013					15.63855531		
III	3	3	B3	0.132628898	0.462516225				0.04713708	18.74992356		
III	3	3	C1		0.486614685					15.9094983		
III	3	3	C2		0.068089188		0.011692228			21.01959082		
III	3	3	C3		0.218317041					30.18582112		

Hydroperiod I = dry season of January; Hydroperiod II = dry season of April; and Hydroperiod III = rainy season of November. Day -1: before treatments; Day 1: 24 hrs after treatment; Day 3: 72 hrs. after treatments; Treatment A: distilled water; Treatment B: sterilized seawater; Treatment C: ambient water from the station.

Appendix 5. Statistical Analyses

A. Cyanobacterial Species Richness

A1. Tests for normality for cyanobacterial species richness in the microbial mat

Test	Statistic		P Value	
Shapiro Wilk	W	0.989355	Pr<W	0.0115
Kolmogorov-Smirnov	D	0.157443	Pr>D	<0.0100
Cramer-von Mises	W-Sq	0.226362	Pr>W-Sq	<0.0050
Anderson-Darlin	A-Sq	1.284128	Pr>A-Sq	<0.0050

A2. Descriptive statistics for cyanobacterial species richness among hydroperiods

Hydroperiod	Variable	n	Media	S.D.	Min	Max
Dry, January	Spp. Richness	81	3.46	1.08	1.00	7.00
Dry, April	Spp. Richness	81	4.15	1.30	2.00	7.00
Rainy, November	Spp. Richness	76	2.88	0.99	1.00	6.00

A3. Descriptive statistics for cyanobacterial species richness among stations

Station	Variable	n	Media	S.D.	Min	Max
1	Spp. Richness	81	3.96	1.33	2.00	7.00
2	Spp. Richness	76	3.62	1.26	1.00	7.00
3	Spp. Richness	81	2.95	0.89	2.00	5.00

A4. Descriptive statistics for cyanobacterial species richness among hydroperiods per station.

Hydroperiod	Station	Variable	n	Media	S.D.	Min	Max
Dry, January	1	Spp. Richness	27	4.26	1.16	2.00	7.00
Dry, January	2	Spp. Richness	27	2.96	0.76	1.00	4.00
Dry, January	3	Spp. Richness	27	3.15	0.82	2.00	5.00
Dry, April	1	Spp. Richness	27	4.67	1.18	2.00	7.00
Dry, April	2	Spp. Richness	27	4.67	1.07	3.00	7.00
Dry, April	3	Spp. Richness	27	3.11	1.01	2.00	5.00
Rainy, November	1	Spp. Richness	27	2.96	1.02	2.00	5.00
Rainy, November	2	Spp. Richness	22	3.14	1.17	1.00	6.00
Rainy, November	3	Spp. Richness	27	2.59	0.75	2.00	5.00

A5. Overall species richness of cyanobacteria.

Variable	n	Media	S.D.	Min	Max
Spp. Richness	238	3.51	1.25	1.00	7.00

B. Cyanobacterial Biovolume

B1. Tests for normality for cyanobacterial biovolume in the microbial mat.

Test	Statistic		P Value	
Shapiro Wilk	W	0.961313	Pr<W	0.2359
Kolmogorov-Smirnov	D	0.092241	Pr>D	>0.1500
Cramer-von Mises	W-Sq	0.041692	Pr>W-Sq	>0.2500
Anderson-Darlin	A-Sq	0.394794	Pr>A-Sq	>0.2500

B2. Split Block results for cyanobacterial biovolume under dilution treatments.

Effect	Num Degree of Freedom	Den Degree of Freedom	F Value	Pr > F
Hydroperiod	2	15	5.23	0.0189
Station	2	3	4.69	0.1194
Hydroperiod*Station	4	15	3.06	0.0496
Day	1	15	0.38	0.5456
Hydroperiod*Day	2	15	4.29	0.0336
Station*Day	2	15	0.35	0.7098
Sample*Station*Day	4	15	1.42	0.2751

Note: The asterisk (*) means an interaction within effects

B3. Tukey's test for cyanobacterial biovolume within hydroperiods

Hydroperiod	Average	n		
Dry, January	22.44	27	A	
Rainy, November	34.58	27		B
Dry, April	35.23	27		B

Note: Different letters show significant differences (p<=0.05)

C. Diatom Species Richness

C1. Descriptive statistics for diatom species richness among hydroperiods

Hydroperiod	Variable	n	Media	S.D.	Min	Max
Dry, January	Spp. Richness	9	3.78	0.67	3.00	5.00
Dry, April	Spp. Richness	9	4.78	1.09	3.00	6.00
Rainy, November	Spp. Richness	9	4.00	0.71	3.00	5.00

C2. Descriptive statistics for diatom species richness among stations

Station	Variable	n	Media	S.D.	Min	Max
1	Spp. Richness	9	3.89	0.78	3.00	5.00
2	Spp. Richness	9	4.44	0.88	3.00	6.00
3	Spp. Richness	9	4.22	1.09	3.00	6.00

C3. Descriptive statistics for diatom species richness among hydroperiods per station.

Hydroperiod	Station	Variable	n	Media	S.D.	Min	Max
Dry, January	1	Spp. Richness	3	4.00	1.00	3.00	5.00
Dry, January	2	Spp. Richness	3	3.67	0.58	3.00	4.00
Dry, January	3	Spp. Richness	3	3.67	0.58	3.00	4.00
Dry, April	1	Spp. Richness	3	4.00	1.00	3.00	5.00
Dry, April	2	Spp. Richness	3	5.00	1.00	4.00	6.00
Dry, April	3	Spp. Richness	3	5.33	1.15	4.00	6.00
Rainy, November	1	Spp. Richness	3	3.67	0.58	3.00	4.00
Rainy, November	2	Spp. Richness	3	4.67	0.58	4.00	5.00
Rainy, November	3	Spp. Richness	3	3.67	0.58	3.00	4.00

C4. Overall species richness of diatoms.

Variable	n	Media	S.D.	Min	Max
Spp. Richness	27	4.19	0.92	3.00	6.00

D. Ciliate Species Richness

D1. Descriptive statistics for ciliate species richness among hydroperiods

Hydroperiod	Variable	n	Media	S.D.	Min	Max
Dry, January	Spp. Richness	9	4.22	1.20	3.00	6.00
Dry, April	Spp. Richness	9	5.22	1.20	4.00	7.00
Rainy, November	Spp. Richness	9	3.89	1.90	1.00	7.00

D2. Descriptive statistics for ciliate species richness among stations

Station	Variable	n	Media	S.D.	Min	Max
1	Spp. Richness	9	3.22	1.30	1.00	5.00
2	Spp. Richness	9	5.22	1.64	3.00	7.00
3	Spp. Richness	9	4.89	0.78	4.00	6.00

D3. Descriptive statistics for ciliate species richness among hydroperiods per station.

Hydroperiod	Station	Variable	n	Media	S.D.	Min	Max
Dry, January	1	Spp. Richness	3	3.67	0.58	3.00	4.00
Dry, January	2	Spp. Richness	3	3.33	0.58	3.00	4.00
Dry, January	3	Spp. Richness	3	5.67	0.58	5.00	6.00
Dry, April	1	Spp. Richness	3	4.33	0.58	4.00	5.00
Dry, April	2	Spp. Richness	3	6.67	0.58	6.00	7.00
Dry, April	3	Spp. Richness	3	4.67	0.58	4.00	5.00
Rainy, November	1	Spp. Richness	3	1.67	0.58	1.00	2.00
Rainy, November	2	Spp. Richness	3	5.67	1.15	5.00	7.00
Rainy, November	3	Spp. Richness	3	4.33	0.58	4.00	5.00

D4. Overall species richness of ciliates.

Variable	n	Media	S.D.	Min	Max
Spp. Richness	27	4.44	1.53	1.00	7.00