Net-plankton diatoms of Puerto Rican water reservoirs as potential bioindicators of trophic status

by

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## ABSTRACT

Diatoms are a group of unicellular algae enclosed in a siliceous exoskeleton called the frustule. This ornamented structure allows for taxonomic identification. Diatoms are frequently used as indicators to monitor the integrity of aquatic ecosystems in Europe, Africa and the United States. This study aims to expand the knowledge of these microalgae for the future inclusion of diatom monitoring as part of trophic status monitoring in Puerto Rican Reservoirs. Net-size plankton was sampled from six reservoirs, (Cerrillos, Cidra, Guajataca, La Plata, Patillas and Toa Vaca) which were selected to encompass a wide range of trophic status. A total of six samples were collected from each reservoir between March 2012 and April 2014. Samples were analyzed with light and SEM-microscopies and identified to the lowest taxonomic rank possible. Shannon-Weaver's diversity index, H', ranged between 0.015 and 2.313, Margalef's richness index, R, ranged between 1.071 and 4.969, similarity between samplings measured with Jaccard's index ranged between 25% and 100% index ranged between and Pollution Tolerance Index values were between 2.04 and 3.00. A total of 32 taxa were registered. Dominant taxa in these reservoirs were species of *Achnanthidium* sp., centric diatoms, *Navicula* sp.and *Ulnaria* sp. Mesotrophic reservoirs (Guajataca and Toa Vaca) had the most diverse communities and the highest richness. Some organisms present in the phytoplankton communities, such as *Achnanthidium minuttisimum* and *Ulnaria ulna,* could aid in the differentiation of low nutrient states from high nutrient states. The study showed that net-phytoplankton taxa seem to be useful in the bioindication of the trophic conditions in subtropical reservoirs.

## RESUMEN

Las diatomeas son un grupo de algas unicelulares que poseen un exoesqueleto de sílice conocido como la frústula. Esta estructura ornamentada permite la identificación taxonómica de estos microorganismos. Las diatomeas son utilizadas frecuentemente como indicadoras para monitorear la integridad de sistemas acuáticos en Europa, África y Estados Unidos. Se colectaron muestras de net-plancton en seis embalses seleccionados para representar un amplio espectro de estado trófico. Estos embalses fueron Cerrillos, Cidra, Guajataca, La Plata, Patillas y Toa Vaca. Un total de seis muestras fueron colectadas para cada embalse entre marzo del 2012 y abril del 2014. Los valores para el índice de diversidad Shannon-Weaver, H', estuvieron entre 0.015 y 2.313, el índice de riqueza de especies, Margalef, R, entre 1.071 y 4.696, la similitud entre muestreos estuvo entre 25% y 100%, expresado en el índice de Jaccard y el índice de Tolerancia de Contaminación estuvo entre 2.04 y 3.00. Un total de 32 taxones fueron registrados. Los taxones dominantes fueron *Achnanthidium* sp., diatomeas céntricas, *Navicula* sp.y *Ulnaria* sp.. Embalses mesotróficos (Guajataca y Toa Vaca) presentaron las comunidades más diversas y la mayor riqueza de especies. Algunos organismos presentes en las comunidades de fitoplancton, tales como *Achnanthidium minuttisimum* y *Ulnaria ulna*, pudieran asistir en la diferenciación de estados pobres en nutrientes y estados ricos en nutrientes. Nuestro estudio muestra que las diatomeas del net-plancton pueden ser útiles en la bioindicación de las condiciones tróficas en embalses subtropicales.

**DEDICACIÓN**

A Dios por sus valles, sus manantiales y su creación.

A mis padres Awilda y Rafy, mis abuelos, Lorenzo, Luz y C. Lydia, porque desde que tengo memoria me han enseñado a amar la naturaleza, a dejarla que me asombre, cuidarla y protegerla.

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**Contents**

## List of Tables

## List of Figures

**List of abreviations**

Code for each sampling event per reservoir.

| Code | Reservoir | Sampling event | Sampling Date (M/D/Y) |
|---|---|---|---|
| C1 | Cerrillos | 1 | 3/19/2012 |
| C2 | Cerrillos | 2 | 4/24/2012 |
| C3 | Cerrillos | 3 | 7/2/2012 |
| C4 | Cerrillos | 4 | 1/17/2013 |
| C5 | Cerrillos | 5 | 5/15/2013 |
| C6 | Cerrillos | 6 | 9/26/2013 |
| CI1 | Cidra | 1 | 3/21/2012 |
| CI2 | Cidra | 2 | 5/1/2012 |
| CI3 | Cidra | 3 | 7/12/2012 |
| CI4 | Cidra | 4 | 6/11/2013 |
| CI5 | Cidra | 5 | 9/3/2013 |
| CI6 | Cidra | 6 | 2/5/2014 |
| G1 | Guajataca | 1 | 3/6/2012 |
| G2 | Guajataca | 2 | 4/19/2012 |
| G3 | Guajataca | 3 | 7/10/2012 |
| G4 | Guajataca | 4 | 1/2/2013 |
| G5 | Guajataca | 5 | 5/22/2013 |
| G6 | Guajataca | 6 | 9/17/2013 |
| L1 | La Plata | 1 | 3/15/2012 |
| L2 | La Plata | 2 | 4/26/2012 |
| L3 | La Plata | 3 | 7/11/2012 |
| L4 | La Plata | 4 | 1/24/2013 |

| | | | |
|---|---|---|---|
| L5 | La Plata | 5 | 6/6/2013 |
| L6 | La Plata | 6 | 9/10/2013 |
| P1 | Patillas | 1 | 3/17/2012 |
| P2 | Patillas | 2 | 4/17/2012 |
| P3 | Patillas | 3 | 6/27/2012 |
| P4 | Patillas | 4 | 1/22/2013 |
| P5 | Patillas | 5 | 6/18/2013 |
| P6 | Patillas | 6 | 9/19/2013 |
| T1 | Toa Vaca | 1 | 3/13/2012 |
| T2 | Toa Vaca | 2 | 7/3/2012 |
| T3 | Toa Vaca | 3 | 5/8/2013 |
| T4 | Toa Vaca | 4 | 9/12/2013 |
| T5 | Toa Vaca | 5 | 2/13/2014 |
| T6 | Toa Vaca | 6 | 5/7/2014 |

Species code for each taxa

| **Species code** | **Taxa** |
|---|---|
| D1 | *Achnanthidium minutissimum* |
| D2 | *Amphora angusta* |
| D3 | *Centric diatoms* |
| D4 | *Cocconeis placentula* |
| D5 | *Cymbella helvética* |
| D6 | *Cyclotella meneghiniana* |
| D7 | *Denticula* sp. |
| D8 | *Fragilaria goubardi* |
| D9 | *Geissleria decussis* |

| | |
|---|---|
| D10 | *Gomphonema* sp. |
| D11 | *Gomphonema parvulum* |
| D12 | *Gomphonema gracilis* |
| D13 | *Gomphonema vibrio* var. *pumilum* |
| D14 | *Gyrosigma acuminatum* |
| D15 | *Halamphora coffeaeformis* |
| D16 | *Hantczhia amphioxys* |
| D17 | *Melosira varians* |
| D18 | *Navicula* sp. |
| D19 | *Navicula rhynocephala* |
| D20 | *Nitzchia* sp. |
| D21 | *Nitzchia palea* |
| D22 | *Nitzchia pumilum* |
| D23 | *Nitzchia sigma* |
| D24 | *Pinnularia microstauron* |
| D25 | *Rhopalodia gibba* |
| D26 | *Sellaphora pupula* |
| D27 | *Surirella tenera* var. *nervosa* |
| D28 | *Synedra* sp. |
| D29 | *Synedra rumpens* |
| D30 | *Ulnaria acus* |
| D31 | *Ulnaria biceps* |
| D32 | *Ulnaria ulna* |

## List of Appendices

## OBJECTIVES

To describe the distribution of diatom communities in subtropical reservoirs and their relationship with respect to trophic status of these ecosystems.

Assess the feasibility of using different thresholds of community structure (i.e., taxonomic richness, diversity, composition) to strengthen the regulatory framework.

To test the applicability of diatom assemblages as indicators of their environmental trophic status.

Register a comprehensive and updated inventory of freshwater diatoms of Puerto Rico.

## INTRODUCTION

### Water resources

Water is one of the most important resources for life and economic growth. Freshwater is vital to human life and social well-being. Its use for consumption, irrigation, and transport has taken priority over other commodities and services provided by freshwater ecosystems. However, there is widespread evidence that freshwater ecosystems are amongst the most threatened ecosystems (Ollis et al., 2006). This finite resource experiences accelerating rates of quantitative and qualitative degradation due in part from population growth and expanding utilization. Not considering the value of supporting ecosystem health has led to aquatic ecosystems being severely altered at a greater rate than they are being restored. For this reason, South Africa established their National Water Act in 1998 which states that "…water is (to be) protected, conserved, managed and controlled in a sustainable and equitable manner for the benefit of all persons". Limnological research and adequate monitoring, both chemical and biological, provides information on the quality of resources. This is the first step in ensuring effective management while enabling the optimization of multiple uses and control of chemical composition of water in the desired condition.

## Reservoirs

Reservoirs are engineered systems designed for irrigation, flood prevention, navigation, drinking water supply, fishing, industrial water supply, generation of electrical power and recreation. In Puerto Rico they represent the principal source of potable water. Site specific management practices have impacts on the biological, physical and chemical characteristics of the reservoir system. These water sources can become contaminated by agricultural activity, domestic wastewater discharge, and industrial effluent, which promote eutrophication, and cause the blooming of algae (Chi & Cheng, 2003). In turn, these have a negative effect on the socio-economic functions of the reservoir as well as loss of structural biodiversity (Keke, 2008). Having an adequate management of existing reservoirs can help minimize these adverse effects and to better understand their ecological attributes (Márquez & Guillot, 2001). Due to the lack of natural lakes there are a total of 36 constructed reservoirs to fulfill our needs.

## Cultural eutrophication

Cultural eutrophication in lakes is known as the process by which humans stimulate algal productivity by elevating nutrient inputs. Still today, this continues to rank as one of the most common water quality problems in the world (Smith V. , 2003). Reservoirs may undergo eutrophication by natural processes. However, today it occurs fundamentally through the input of nutrients from anthropogenic sources. This alteration of the water resource interferes with their intended uses, causing substantial economic losses such as high drinking water treatment costs, reduced recreational value and reduced property values (Dodds et al, 2009). Problems associated with eutrophication are the alteration of odor and taste, corrosion of hydroelectric equipment, decreased species diversity, large fluctuations of dissolved oxygen, high decomposition of organic matter, large algal blooms and aquatic vegetation that interferes with light, excesive growth of algal and macrophytic biomass and the redisolution of certain metals from the sediment under anoxic conditions. Europe, North America, and other industrialized countries currently implement active efforts to significantly reduce nutrient loading to lacustrine systems in order to reduce or reverse the effects of cultural eutrophication.

**Algae as bioindicators of ecosystem health**

During the eutrophication process accumulation of nutrients and biomass is accompanied by an increase in the levels of production of the system. Reservoirs phytoplankton constitutes the essential base of the trophic chains; these water bodies can be considered as autotrophic systems, where phytoplankton is capable of accumulating luminic energy in form of energetic chemical components thanks to photosynthesis (Infante, 1988). Excess incorporation of nutrients into aquatic systems has a direct influence on the phytoplankton dynamic, and modifies its specific composition and elevates its productivity (Infante, 1988). Phytoplankton, as the basis of the trophic chain constitutes the biological community in which scientific attention is focused when a management plan is needed or an assessment of the ecosystem health is required (Monbet, 1992; Cloern, 1999; Sin et al., 1999).

Phytoplankton abundance may be influenced by the availability of nutrients, presence of herbivores, bacteria interactions, sinking, temperature, light levels, parasitism, alelopathy and physical parameters in the water column. Variability in the abundance of phytoplankton has been related to the variations of the concentration of phosphorous (TP); a nutrient that often limits their growth (Currie, 1990). Other studies reveal that phytoplankton can be sensitive to Nitrogen: Phosphorous ratio (Suttle & Harrison, 1988) 1988).

Ecological studies of phytoplankton in tropical environments are scarce and recent (Reynolds, 1984; Talling, 1986; Huszar, 1989; Ramírez & Alcaráz, 2002) in comparison to the studies in temperate lakes. Phytoplankton assemblages have been used for some time as water quality bioindicators in the Great Lakes (Stoerrner, 1978). They are used as ecological indicator, since they represent the basis of lake and reservoir food webs and quickly respond to stresses and perturbations (Celik & Ongun, 2007).

**Diatoms**

Biological monitoring provides a direct measure of ecological integrity by using the response of biota to environmental change (Karr, 1991). A bioindicator can be defined as a species or population that provides an integrated record of an ecosystem allowing the early detection of biotic and abiotic modifications (Morin, 2006). Diatoms are one of the most diverse and widely used groups of freshwater organisms in limnological monitoring (Stoermer & Smol, 1999; Battarbee et al., 2001). They inhabit almost any kind of aqueous environment, and can also occur as endosymbionts in dinoflagellates and foraminifers

(Round, Crawford, & Mann, 1990), among others. They have global significance in biogeochemical cycles, and provide 20–25% of globally fixed carbon and atmospheric oxygen (Mann, 1999).

In Europe, as worldwide, there is a long history of using biological indicators to monitor the integrity of lentic ecosystems (Moss et al., 2003). The use of diatom as indicators of environmental quality in reservoirs is important for three basic reasons: their importance in ecosystems, their utility as indicators of environmental state, and their fundamental role in food webs. Diatoms are frequently used as indicators of eutrophication in temperate systems, but little is known about their application in tropical systems. Due to scarce studies in the tropics, managers in these regions often must rely on information from extratropical regions for guidance. Determining if those principles and practices are appropriate for tropical regions is important (Moulton & Wantzen, 2006).

Planktonic diatoms are well adapted to growth in deeply mixed turbulent water, where cells are only intermittently exposed to high light levels (Mitchell and Holm-Hansen 1991; Falkowski and Raven 1997). The sensitivity of diatoms to so many habitat conditions can make them highly valuable indicators, particularly if effects of specific factors can be distinguished.

## Diversity

Eutrophication can lead to the reduction of phytoplankton communities, for example, the diversity, species richness and evenness of diatom communities can change (usually decreasing) in response to organic enrichment (Steinman and McIntire, 1990). On the contrary, species diversity has been linked to ecosystem stability. Highly diverse systems possess species that can compensate for the loss of others should disturbances occur; therefore, species-rich systems are more likely to be considered stable.

This being said, it is important to recognize that ecological systems are inherently complex, composed of many interacting biological and physical components. Predicting the behavior of such complex systems is difficult but management and policy decisions require information on the status, condition, and trends of ecosystems (Anreasen et al., 2001).

Biological diversity is generally considered an indicator of the quality of freshwater systems (Sondergaard & Jeppesen, 2007) and is often assumed to decrease with increasing nutrient levels (Tilman, Kilham & Kilham, 1982; Tilman, Lehman & Thomson, 1997a; Burkholder, 2001; Wassen et al., 2005). Reduced biodiversity may alter the performance and reduce the resilience of the entire ecosystem (resilience being the rate at which a system returns to the steady state following a perturbation).

Diversity typically indicates the number of species present and the pattern of relative abundance in a biological community. Communities appear less diverse if they are dominated by one or a few species. To evaluate the complexity in the ecosystem ecologist have defined various indexes. According to Whittaker, diversity can be described as the number of species per habitat (a) and the turnover of species between these habitats. In simplistic terms, species richness (S) is the total number of species in a sample. The Margalef index (R) measures the number of species present in a given number of individuals. The Shannon-Weaver (H') index expresses how evenly the individuals are distributed within the different species (evenness or equitability). It was generally assumed that an increase in environmental stress was associated with a decrease in diversity and evenness caused by the increased dominance of the most tolerant taxa. Nevertheless, this assumption is probably an oversimplification of the relationship between diversity and environmental stress.

## LITERATURE REVIEW

Potapova and Charles (2005) identified diatom species indicative of river nutrient status, developed and tested diatom metrics for monitoring eutrophication. In this study, diatom-nutrient relationships in regional-scale metrics provided better assessment than similar metrics developed for European inland waters. Benthic and planktonic diatom data were collected from 1993 to 2001 from 1240 river sites throughout the continental U.S. Nutrient data collected were total Phosphorous and total Nitrogen. A total of 1246 taxa were identified and 371 of them were determined to be possible indicators of low or high nutrient concentration.

The aim of the study was to ascertain whether the numerical diatom index developed in Europe has a potential use for indicating general water quality in the North West Province. "Potential for the use of numerical diatom indices as indicators of general water quality and the usefulness of these indices should be verified by further studies that cover a broader geographical area and a broader range of variables" (De la Rey, 2004). The use of a European numerical diatom index, the Specific Pollution sensitivity Index (SPI) reflected water quality status with a high degree of accuracy. The diatom index was sensitive to changes in electrical conductivity, ammonia, chemical oxygen demand, chloride, sulphate and turbidity. It was concluded that there is definite potential for the use of numerical diatom indices as indicators of general water quality and the usefulness of these indices should be verified by further studies that cover a broader geographical area and a broader range of variables.

Diatoms have also been used as indicators of water quality in the recent assessment of the state of the rivers in the Crocodile West and Marico water management area as part of the national River Health Programme in Africa (River Health Programme, 2005). This study evaluated the potential use of diatom based indices by testing it against a macroinvertebrate index (SASS 5) and evaluating the variation in the index scores of the two indices due to changes in chemical water quality and habitat. It was concluded that the diatom monitoring system performs well as bioindicator of water quality.

Surveys of diatoms in the Caribbean were conducted in the 19$^{th}$ and early 20$^{th}$ centuries. Many of these collections were of marine diatoms. Collections of freshwater diatoms have been published from islands such as Guadaloupe (Bourelly and Manguin 1952, Ector 2002), Cuba (Foged 1984), and Jamaica (Podzorski 1985). Navarro has done extensive work on marine diatoms in Puerto Rico. Small works have been done by Foerster (1971), and research with low taxonomic resolution by Candelas Reyes (1956) and Garcia Sais (1999).

Foged collected freshwater samples in 1976 and these were reported in 1984. His research included a total of 209 freshwater diatom taxa, of 29 different genera. The samples were collected from scrapings of water pipes, basins, concrete, water hydrants, stones, moss and leaves. Foged stated that "the Cuban freshwater diatom flora must be considered very rich in species on account of the size, the geological age of the island and of the numerous different biotopes in a favorable climate. But as a matter of fact it seems quite unknown up to now."

Robert Hagelstein published in 1938 a collection of fresh water and marine diatom species of Puerto Rico and the Virgin Islands from samplings collected in 1926, 1928 and 1929. The sampling sites included the Collazo River, Río Grande de Loiza, springs at Santurce and Coamo, Jajome Alto, Carolina, Falls of the Toro Negro River, Hato Rey, Yauco, Quintana springs, Virella springs, Hormigueros, Santa Isabel, Loiza, La Muda, Guaynabo, la Plata River, Villalba, Inabón and Descalabrado River. In his work he reported a total of 230 freshwater taxa from 28 genera including; *Achnanthes, Amphipleura, Amphiprora, Amphora, Biddulphia, Caloneis, Cyclotella, Cymbella, Denticula, Diploneis, Epithemia, Eunotia, Frustulia, Gomphonema, Gyrosigma, Hantzschia, Melosira, Navicula, Neidium, Nitzschia, Pinnularia, Rhopalodia, Stauroneis, Surirella, Synedra* and *Trepsinoï* (Appendix H). From all the genera reported the ones with the most species were *Cymbella, Gomphonema, Navicula, Nitzschia, Pinnularia* and *Synedra.*

The communities along the Río Mameyes, Río Tanamá, Río Espíritu Santo, Río Guyanés, Río Yauco and Río Rosario were surveyed in 2005 by Bryan. The diatom communities were described and a diatom based index was calculated to asses the applicability of this index in Puerto Rican streams. Pollution Tolerance Index (PTI) shifts indicated that diatoms were useful in detecting changes in water chemistry. This study states that the PTI works with tropical diatom communities and more information on local species will make the use of the index stronger. A total of 371 species belonging to 62 genera were reported (Appendix H).

The composition and abundance of benthic algae and relationships with physicochemical parameters were studied during 2006 in five streams minimally impacted by human activity in the central mountainous region (Bosque Olimpia in Adjuntas, San Virón in Jayuya and La Mina in Villalba) and eastern portion of Puerto Rico (Sonadora and Puente Roto Mameyes, both in Luquillo) by Gualtero. In this study, 120 species of algae were identified; diatoms were most abundant and diverse, with 94 reported species. New records for Puerto Rico included: *Achnanthes rupestoides, Adlafia muscora, Gomphonema clavatum, G. dubravicense, G. truncatum, G. pumilum, Navicula tripunctata, Navicula* cf. *recens, Nitzscia dissipata* and *Pinnularia subgibba* (Gualtero, 2007).

The stressor-response relationship between water quality parameters and periphyton biomass on artificial and natural substrates was described by Viggiano (2014). This study included diatoms and surveyed three tropical streams (Río Piedras, Río Mameyes and Río Guanajibo) with contrasting trophic status. A total of 23 genera were reported, five growing on the artificial substrate were identified to species level. The nutrients evaluated were Total Phosphorous and Total Nitrogen. According to Viggiano, "excluding high flood events, the use of benthic algae biomass as biological indicator of trophic status was effectively demonstrated".

The freshwater diatoms in these studies were collected from substrates such as pipes, walls and rocks. Recent studies focused on stream periphyton and evaluated the relationship between diatoms and several parameters. In stream ecosystems diatoms have proven to provide information regarding the parameters present in the water. The previous sampling efforts in these studies had a yield of more than 90 species identified.

## MATERIALS AND METHODS

### Area of study

Site descriptions reproduced with permission from *Nutrient levels associated with ecological thresholds of impairment in reservoirs of Puerto Rico.*

Six reservoirs were selected to encompass a wide range of morphological characteristics (e.g., superficial area, depth), and trophic status. These were Cerrillos, Cidra, Guajataca, La Plata, Patillas and Toa Vaca reservoirs.


**Figure 1. Location of the reservoirs of the six study sites.**

Table 1. Principal characteristics of the six reservoirs sampled.

| | Cerrillos | Cidra | Guajataca | La Plata | Patillas | Toa Vaca |
|---|---|---|---|---|---|---|
| Year built | 1991 | 1946 | 1928 | 1974 | 1914 | 1972 |
| Drainage area ($Km^2$) | 45.33 | 21.50 | 79.77 | 468.80 | 65.26 | 56.72 |
| Superficial area ($Km^2$) | 2.12 | 1.06 | 3.42 | 3.32 | 1.50 | 3.21 |
| Maximum depth (m) | 65.0 | 18.5 | 27.0 | 27.0 | 24.0 | 53.9 |
| Average depth (m) | 27.8 | 5.4 | 12.4 | 10.7 | 9.3 | 19.9 |
| Renewal frequency (times/yr) | 0.9 | 2.6 | 2.5 | 8.2 | 4.5 | 0.3 |

**Cerrillos**

Cerrillos reservoir is located in the municipality of Ponce, in the south region of Puerto Rico (Figure 2). Its maximum pool elevation is 191.84 m above sea level (Soler-López, 2008). The reservoir drains waters from the Río Bucaná watershed which covers an area of 17.5 square miles. Cerrillos is the second largest reservoir of Puerto Rico (behind Toa Vaca). Its dam, finished in 1991, impounds waters of the Río Cerrillos, as well as from an unnamed creek (Soler-López, 2008). A bathymetry study concluded in 2008 reported a water storage loss of 2% since 1991 which amounts to a storage loss rate of 0.12% per year (Soler-López, 2008).

**Figure 2. Aerial photo of Cerrillos reservoir.**

The predominant geological formations (i.e., Anon, Monserrate, and Lago Garzas and Yauco formations) consist of volcaniclastic terranes from the Tertiary and Cretaceous (USGS, 1998). The predominant soil association is Caguabo-Múcara-Morado association (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Survey Geographic (SSURGO) Database for [Ponce Area, Puerto Rico]. Available online at http://soildatamart.nrcs.usda.gov. Accessed [04/23/2012]). The Caguabo series consists of shallow, well drained, moderately permeable soils formed from material that weathered from basalt. These soils and present mainly on very steep slopes. The Mucara series consists of moderately deep, well drained, and moderately permeable soils formed in residuum that weathered from basalt lava and breccia. They are present on summits and side slopes of hills and mountains. The Morado series consists of moderately deep, well drained, moderately permeable soils formed in residuum that weathered from basalt flow and tuff. They are present on summits and side slopes of hills and mountains. The slopes severely limit these soils for cultivation/development which have kept human settling establishments to a minimum. The combination of evergreen forest and shrubland (42.6% of land cover), as well as areas dedicated to coffee and plantains (41.4%) represent the major land-uses at this watershed. Urban establishments (high and low density) cover 1.06% of the area.

**Cidra**

The Cidra reservoir is located in the municipality of Cidra, in the east-central region of Puerto Rico (Figure 3). The spillway crest is at 403 m above sea level (Ortiz et al., 2004). The reservoir drains waters from the Río Bayamón watershed which covers an area of 8.3 square miles. Construction of this reservoir, which impounds the waters of Río Bayamón, Río Sabana and Quebrada Prieta, was finalized in 1946 (Soler-López, 2007). A bathymetry study concluded in 2007 reported a water storage loss of 14% since construction, 0.23% per year (Soler-López, 2007). The predominant geological formation at this watershed consist of submarine basalt and chert terrane (i.e., non-pillowed lava flows, volcanic breccia, sandstone, conglomerate, and minor limestone, siltstone, and tuff (USGS, 1998).

**Figure 3. Aerial photo of Cidra reservoir.**

The predominant soil association is the Daguey-Humatas-Aceitunas association (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Survey Geographic (SSURGO) Database for San Juan Area, Puerto Rico. Available online at http://soildatamart.nrcs.usda.gov . Accessed [04/23/2012]). The Daguey series consists of very deep, well drained, moderately permeable soils formed in fine textured residuum weathered from volcanic rock. They are present on sideslopes, ridgetops and footslopes in volcanic uplands. The Humatas series consists of very deep, well drained, moderately slowly permeable soils formed in clayey and loamy material that weathered from igneous rocks. They are present on side slopes and ridges of strongly dissected uplands. The Aceitunas series consists of very deep, well drained, moderately permeable soils formed in fine-textured alluvial and colluvial sediments. They are present on foot slopes, alluvial fans, and valleys in coastal plains. Land cover is dominated by lands dedicated to pasture (50.1%). This watershed has the highest percent of land cover dedicated to human establishments, covering 8.5% of the area.

## Guajataca

The Guajataca reservoir is located in the north-western region of Puerto Rico about 8 km south of the Quebradillas municipality and 10km northeast of the San Sebastián municipality (Figure 4). The reservoir was completed in 1928. It drains waters from the Río Guajataca watershed which covers an area of 30.8 square miles. The spillway crest is at 196.9 m above sea level (Ortiz, et al., 2004). A bathymetry study concluded in 1999 reported a water storage loss of 13% since construction, 0.18 per year (Soler-López, 2000). The predominant geological formations at this watershed (Lares limestone, and Cibao, Montebello, and Anon formation) consist of limestone and calcareous material, as well as some volcaniclastic breccias and lava from the Tertiary period (USGS, 1998).


**Figure 4. Aerial photo of Guajataca reservoir.**

The predominant soil is the Soller series, which are shallow, well drained, moderately permeable soils formed in materials that weathered from limestone (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil

Survey Geographic (SSURGO) Database for [Mayagüez Area, Puerto Rico]. Available online at http://soildatamart.nrcs.usda.gov. Accessed 04/23/2012). The combination of evergreen forest and shrubland (36.7% of land cover), as well as areas dedicated to pastures (37%), represent the major land-uses at this watershed. Urban establishments (high and low density) cover 5.4% of the area.

**La Plata**

La Plata reservoir is located between the municipalities of Toa Alta and Naranjito (Figure 5) on the north-central region of Puerto Rico (Soler-López, 2008). The reservoir was built in 1974. The normal pool elevation is 52 m above sea level (Ortiz et al., 2004, Table 4). The reservoir drains waters from the Río La Plata watershed which covers an area of 181 square miles. The reservoir impounds the waters of Río La Plata, Río Guadiana and Río Cañas. A bathymetry study concluded in 2006 reported a water storage loss of 22% since construction, 0.69% per year (Soler-López, 2008). Non-pillowed lava flows, and volcanic breccias, sandstone and siltstone from the Cretaceous predominate throughout the watershed (e.g., formations A, B, C, J, as well as Robles formation, and Los Negros formation) (USGS, 1998).

**Figure 5. Aerial photo of La Plata reservoir.**

The predominant soil series are: Caguabo (already described), Humatas (already described), Múcara (already described), Los Guineos, Naranjito, and Maricao (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Survey Geographic (SSURGO) Database for San Juan Area, Puerto Rico. Available online at http://soildatamart.nrcs.usda.gov. Accessed 04/23/2012). The Los Guineos series consists of very deep, well drained soils formed in residuum from sandstone material. The Maricao series consists of very deep, well drained, moderately permeable soils formed in residuum that weathered from basalt bedrock. The Naranjito series consists of moderately deep, well drained, moderately permeable soils formed in material weathered from volcanic rocks. The combination of evergreen forest and shrubland (53.4% of land cover), as well as areas dedicated to pastures (35.6%), represent the major land-uses at this watershed. Urban establishments (high and low density) cover 6.25% of the area, the second largest percent of the reservoirs being studied.

**Patillas**

The Patillas reservoir is located in the municipality of Patillas in the south-east region of Puerto Rico (Soler-López et al., 1997). The reservoir was completed in 1914 (Figure 6). Normal pool elevation is 67.67 m above sea level (Soler-López et al., 1997). The reservoir drains waters from the Río Patillas watershed which covers an area of 25.2 square miles (Ortiz et al., 2004. The reservoir impounds the waters of Río Patillas and Río Marín. A bathymetry study concluded in 2007 reported a water storage loss of 23%, 0.25% per year, since construction (Soler-López, 2010). Two geological formations predominate at this watershed. The A, B, C, J formation consist of submarine basalt and chert terrane (i.e., non-pillowed lava flows, volcanic breccia, sandstone, conglomerate, and minor limestone, siltstone, and tuff). Other predominant material consist of quartz diorite of the Punta Guayanes plutonic complex and quartz diorite facies of the granodiorite of San Lorenzo (USGS, 1998).

**Figure 6. Aerial photo of Patillas reservoir.**

The predominant soil association is Caguabo (already described) - Los Guineos (already described) - Pandura (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Survey Geographic (SSURGO) Database for [Humacao Area, Puerto Rico]. Available online at http://soildatamart.nrcs.usda.gov. Accessed [04/23/2012]). The Pandura series consists of shallow, well drained soils formed in materials weathered from plutonic rocks. The combination of evergreen forest and shrubland (82.4% of land cover constitute the major land-use at this watershed. This is by far the highest percent of any of the reservoirs being studied. Human establishments represent just 0.51% of the area, the lowest of the reservoirs under evaluation.

**Toa Vaca**

Toa Vaca reservoir is located in the municipality of Villalba in the southern region of Puerto Rico (Soler-López, 2002). The reservoir was completed in 1972 (Figure 7). The normal pool elevation is 164.90 m above sea level (Ortiz et al., 2004). The reservoir drains waters from the Río Toa Vaca watershed which covers an area of 21.9 square miles (Ortiz, et al., 2004). The reservoir impounds the waters of Río Toa Vaca. A bathymetry study concluded in 2002 reported a water storage loss of 7% since construction, 0.23% per year (Soler-López, 2002). The Coamo and Maravillas formation, consisting of volcanic breccia, sandstone and siltstone from the Cretaceous, are the predominant geological formations at this watershed (USGS, 1998).

**Figure 7. Aerial photo of Toa Vaca reservoir.**

The predominant soil association is Quebrada-Morado (already described)-Caguabo (already described). (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Soil Survey Geographic (SSURGO) Database for [Ponce Area, Puerto Rico]. Available online at http://soildatamart.nrcs.usda.gov. Accessed [04/23/2012]). The Quebrada series consists of shallow, well drained, moderately permeable soils formed in colluvium and residuum material that weathered from tuffaceous sandstone, siltstone, breccia, and conglomerate, lava, and tuff. The combination of evergreen forest and shrubland (55.4% of land cover), as well as areas dedicated to pastures (38%), represent the major land-uses at this watershed. Urban establishments (high and low density) represents 1.43% of the total watershed area.

## Field collection

Field collection was done with the assistance by technicians from *Nutrient levels associated with ecological thresholds of impairment in reservoirs of Puerto Rico*. A total of 36 sampling events were done, six samplings events for each reservoir. Samples were obtained from the mid (center) section of each reservoir between 9:00 – 11:00am. At each sampling, length and depth-integrated samples of the entire mixed layers (epilimnion) are gathered by longitudinal and oblique tows whithin the first meter using a bongo net system fitted with two metered 64 μm mesh nets. The tow net, with a collecting bottle of at least 1L capacity attached at the bottom, was lowered with a calibrated line to the desired depth, 1m from surface and towed for 5 minutes at 5 mph. The nets were rinsed down gently from the outside with ambient temperature lake water to wash all of the organisms off the net cloth and into the collection bottle. The collection bottle was swirled gently to concentrate the sample. The bottle was detached and the contents transferred to the sample storage bottle. Headspace was left in the storage bottle to accommodate 40mL of preservative. The volume for netplankton diatoms analyses was 500mL. The samples were transferred to 500-mL bottles and preserved with 4% formalin and freshwater solution. Samples were transported to the University of Puerto Rico, Mayagüez; Aquatic Biology Laboratory for further processing.

The pore of the 64 μm mesh nets favours large individuals (>64 μm) and is biased towards smaller diatoms. This method is designed for ease of identification in light microscopes.

**Sample processing**

After the preserved samples arrived at the laboratory from the survey, an additional 10 mL of formalin was added to each sample to enhance the storage life of the sample.

**Cleaning of Diatom Valves (Frustules) and Preparation of Slides for S.E.M. and Light Microscopy**

Cellular contents of diatoms hinder the visualization of the wall markings on which the taxonomy is based; therefore, the organic matter inside the cell was removed (oxidized) prior to identification. The net-plankton samples collected (500mL per sampling) were concentrated by sedimentation to avoid damage to large frustules. The samples were settled for at least 24 hours. In a 50mL centrifuge tube the concentrated sample (approximately 10mL) was oxidized with 10mL of $KMnO_4$ (10%). The sample was placed horizontally in a shaker and homogenized in darkness during 24 hours, then 1mL of concentrated $H_2SO_4$ was added in a chemical hood. Followed by the addition of $H_2O_2$ (30%) until the sample turned colorless. To eliminate excess acid, the sample was cleaned by removing the supernatant and replaced with water, this procedure was done at least six times. This cleaned sample was used to prepare diatom slide for SEM analysis. Final sedimented volume depends on the sample but was frequently between 100µL and 1mL.

Enumeration and identification was done using light microscopy, by scanning parallel strips until at least 300 cells are counted. The number of cells were identified and enumerated to the lowest taxonomic rank possible under oil immersion (1250x).

## Identification by frustule morphology

### Microscopy

Basic identification of diatoms was based on detailed characterization of their silicified cell walls (frustules). The frustules are composed of two valves fitting one over the other (the epivalve and the hypovalve). The valve shape, planes of symmetry and valve ends can be determined in unprocessed cells. For some diatoms, features such as striae patterns, central area and raphe require the cells to be processed in order to remove all organic matter from inside the cell for detailed observation. All these features are considered in order to identify the diatoms to the lowest taxonomic rank with and without cell content in Light Microscopy (LM) and without cell content for Scanning Electronic Microscopy (SEM).

### Sample Analysis

Enumeration and identification of diatom valves was done by scanning parallel strips until at least 300 cells were counted in Light Microscopy for each sample (duplicates), generating a cell count of 600 valves per sampling event. The cells were identified and enumerated to the lowest taxonomic rank possible under oil immersion (1250x).

The cell per milliliter of volume settled was calculated as follows:

Cells/mL =

$$[C*(TA/(A*F))]/V$$

Where:

C = number of organisms counted

TA = total area of the chamber, $mm^2$

A = area of the a field of view, $mm^2$

F = number of fields counted

V = volume of sample settled, mL

The number of scanned fields, the area of the chamber and the area of the field of view were used to calculate the number of cells of each taxon identified per milliliter of volume settled. This calculation was multiplied by the correction factor to adjust for sample dilution. This correction factor depends on the volume settled in relation to the original sample volume (1 L). For example, if ten milliliters were settled that means the correction factor is 100. After that, the number of cells per milliliter was divided by the sample volume (mL) collected by net towing. The sample volume collected by net towing was calculated as follow:

Volume of the bongo net =

$$1/3\ \pi r2h \rightarrow 1/3\pi\ (0.1525m)\ 2(1.7m) = 0.0414m3$$

Where:

$r^2$ = (radio of net)$^2$

h = length of net

Towing distance =

$$Bv \times TNT$$

Where:

Bv = velocity of the boat as miles/minutes

TNT = net towing time in minutes

Number of nets in the distance traveled by the boat = Towing distance/length of net

Sampled volume, mL =

$$(\text{Net volume} \times \#\ \text{nets in the distance traveled})(1{,}000{,}000)$$

**<u>Data analyses</u>**

Several indices were calculated in order to have numerical comparison for previous studies. It is emphasized that our methodology is biased against small individuals and this indices must be evaluated carefully taking into consideration that the number of species in the samples are underrepresented as an effect of the collection efforts, designed to capture large individuals for ease of identification in Light Microscopy.

The community composition was measured with **Shannon Index**, which takes into account both abundance and evenness of species present in the community. It is estimated by the following equation:

$$H = -\sum (P_i * \ln P_i)$$

$P_i$ = is the proportion of individuals found in species i.

Proportion as $p_i = n_i/N$,

$n_i$ = number of individuals in species i

N = total number of individuals in the community

Since by definition the $P_i$ will all be between zero and one, the natural log makes all of the terms of the summation negative, which is why we take the opposite of the sum.

Margalef (R) species richness index is a measure of the number of species or taxa per unit of sample. The reduction of number of species in an ecosystem is a useful measure of stress caused by an aloctonous factor (Muhlhauser, 1987).

$$R= (s-1)/ \ln (n)$$

Where:

s = the number of registered taxons

n = number of total individuals in the sample

Diatom community structure (= diversity or H' value) were estimated based on the taxonomic richness and the relative abundance of taxa, as follows:

$$H = - \Sigma N_i/N \ln (N_i/N,)$$

Where:

H = Shannon-Weaner index; a value that ranges between 1 and 5.

Ni = number of individuals or algal-units (i.e. colonies, biovolume, etc.) per taxon.

N = number of taxa per sample or set of samples.

The community similarity was calculated using Jaccard's index outlined in Klemm et al. 1990.

$$Sj = (a)/(a+b-c)$$

Sj: Jaccard's index

a: numbers of species in reservoir A

b: numbers of species in reservoir B

c: numbers of species in both reservoirs

The Pollution Tolerance Index (PTI) was developed by the Kentucky Department of Natural Resources Division of Water (KDOW) (Brumley, 2002), and used in Puerto Rico by Bryan, 2010. This index was selected by Bryan over other available diatom based indexes for it extensive species list with over 380 species. In our study the PTI was calculated in an effort to present the applicability of this index in Puerto Rican reservoirs.

Pollution Tolerance Index

$$PTI = (\Sigma n_i * t_i)/N$$

Where

$n_{i:}$ the number of cells of species i

$t_i$: the tolerance value assigned to that species

N: the total number of cells in a sample

Tolerance values range between 1 for the most tolerant species to 4 for the most sensitive species.

Three trophic state indexes were determined: Secchi Depth [TSI (SD)], Trophic state index based on Chlorophyll-a [TSI (Chl-a)] and that based on phosphorus [TSI (TP)]. These indexes were calculated after Carlson (Akpan & Offem, 1993)] using the following equations:

$$\text{TSI (Secchi Disk)} = 60 - 14.1 \ln (\text{SD})$$

Where:

SD=Secchi Disk in meters (m)

$$\text{TSI (Chlorophyll a)} = 9.81(\ln \text{Chl-a}) + 30.6$$

Where:

Chl a = mean chlorophyll a in $\mu g \cdot l^{-1}$

$$\text{TSI (Total Phosphorus)} = 14.41 \ln (\text{TP}) + 4.15$$

Where:

TP = mean total phosphorus in $\mu g \cdot l^{-1}$.

**Nutrient Status and Ancillary Water Column Parameters:**

Results from six sampling events performed on each reservoir are included in this report. Samples were obtained from the mid (center) section of each reservoir between 9:00 – 11:00 A.M. Sampling criteria required a minimum of 5 days without a 2yr - 24 hour rain event or greater. In the case of nutrients and Chl-a, samples were obtained in triplicates, one at each of three points located along a spatially defined transect on the center portion of the reservoir. Samples were obtained with a horizontal-type 2-L Van Dorn sampler at a depth of 1m. Water samples for nutrient analyses were transferred to pre-labeled polypropylene bottles and preserved on-site by acidification. Separate aliquots were used for chlorophyll-*a* analyses. Samples for Chl-*a* analyses were filtered on site through glass fiber filter (Whatman GF/F). Measurements of pH, temperature ($^{0}$C), and dissolved oxygen (mgL-1) were performed with a YSI 6600 multiparameter sonde (YSI Inc.) Water transparency was determined with a 20-cm Secchi disk (SD) and the photic zone determined from 2.7*SD. *In situ* depth profiles (1m resolution) of pH, electrical conductivity, dissolved oxygen, water temperature, turbidity, oxidation-reduction potential were obtained at one of the sampling stations to document the effect of stratification at each reservoir. Samples (including chlorophyll-*a* filters) were transported at 4°C to the Soil and Water Chemistry Laboratory at the University of Puerto Rico within 6 hours of collection. Algal biomass was estimated by means of the chlorophyll-*a* (Chl-*a)* acetone extraction method and quantification of Chl-*a* using a model 10-AU fluorometer (Turner Designs Inc., Sunnyvale, CA). Nutrient analyses included dissolved and total reactive P (EPA method 365.2), total Kjeldhal nitrogen (EPA method 351.2), and nitrate (EPA method 353.1). Samples for nitrate, and dissolved phosphorus were filtered through a 0.45 μm Gelman acrodisc filters before analysis.

Detailed information of nutrient status available in "*Nutrient levels associated with ecological thresholds of impairment in reservoirs of Puerto Rico"* report by Gustavo Martinez, et al. 2014.

## RESULTS

Analysis of phytoplankton diatom community composition present in netplankton was conducted at each reservoir to assess the feasibility of using different thresholds of community structure (i.e., taxonomic richness, diversity, pollution tolerance). Taxonomic richness was evaluated with Margalef's species richness index. This index ranged from 1 to 3 in most sampling events with the exception of the second sampling in Toa Vaca (July, 2012) which was close to five (Figure 10). Lowest values were registered for Guajataca, La Plata and Toa Vaca. Highest values were registered for La Plata, Patillas and Toa Vaca.

Shannon Weaver's H' was calculated as a diversity index (Figure 9). This index takes into account total individuals in a sample (abundance) and the proportion of each species (evenness). A low diversity would be represented by a value close to zero. Values were all lower than 2.4, most values were close to 1. The lowest value was reported in the fifth sampling event for Cerrillos (May, 2013), the highest value was reported for Toa Vaca's first sampling event (March, 2012). Values were between 0.015 and 2.31.

Pollution Tolerance Index was calculated to assess the applicability of this index in Puerto Rican reservoirs. This index was tested by Bryan in 2008 in lotic systems in Puerto Rico. Our data presented values that ranged from 2 to 3 which indicates moderately tolerant species or indifferent species. A value of zero was assigned to sampling events where no presence of species with pollution tolerance value was available (Figure 12). With the exception of sampling events were values where not registered (were equal to zero) lowest values were registered for Cidra, July, 2012, Patillas, March, 2012, and Toa Vaca, February

2014. Highest values were registered for Cerrillos, May, 2013, Guajataca, May, 2013 and La Plata, April, 2012.

Jaccard's similarity index was calculated to compare the diatom community composition within each reservoir, throught all sampling events (Figure 11). Values were highest for sampling events at La Plata, Patillas and Toa Vaca. The lowest values were registered for Guajataca, La Plata and Toa Vaca. Higher values indicate communities that share the same taxa and lowest values for communities that have different composition. The reservoirs that had the most similar communities were Cerrillos and Guajataca (90.5%) of similarity, the reservoirs with the communities with less taxa in common were Guajataca and Toa Vaca (Appendix F). The similarities between communities in each reservoir were not associated with trophic status considering that Guajataca and Toa Vaca were both mesotrophic reservoirs and were the least similirar while Cerrillos, oligotrophic, had more taxa in common with Guajataca, mesotrophic, instead of Patillas.

Cell densities were calculated as average cells per liter of water in each reservoir (Figure 13). The reservoirs with the highest density of cells per liter of reservoir water were Cerrillos, Guajataca and Patillas, with values that ranged between 20,000 and 60,000 cells per liter of water. Lowest values were registered for Cidra, La Plata and Toa Vaca.

## **Reservoir trophic status**

Reservoirs were selected to encompass a wide range of trophic status based on previous records listed for each reservoir. The combined Carlson's trophic status index indicated oligotrophic nutrient state for Cerrillos, Guajataca, La Plata, Patillas and Toa Vaca. Mesotrophic conditions were present in Guajataca, La Plata and Toa Vaca. Eutrophic conditions were present in Cidra, La Plata and Patillas (Figure 8). La Plata exhibited oligotrophic conditions in March, 2012, eutrophic conditions in April and July of 2012 and mesotrophic conditions in the remaining sampling events (2013).

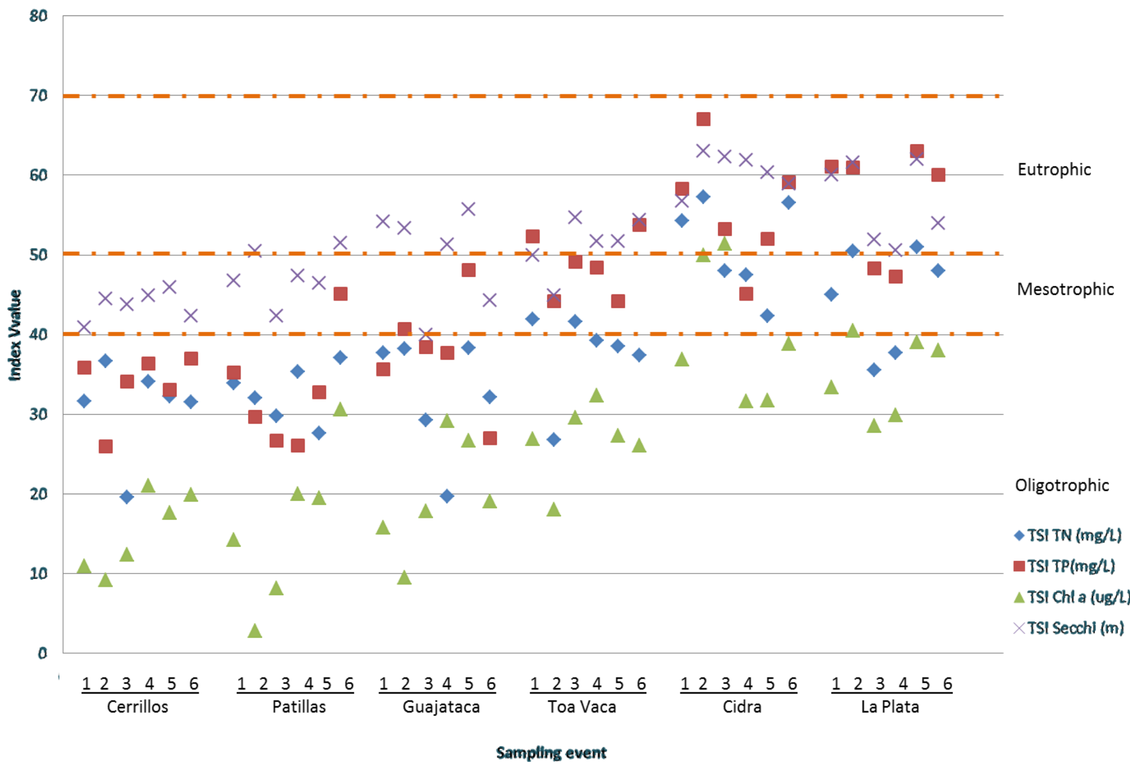


Figure 8. Carlson’s Trophic State Indexes for each sampling event.

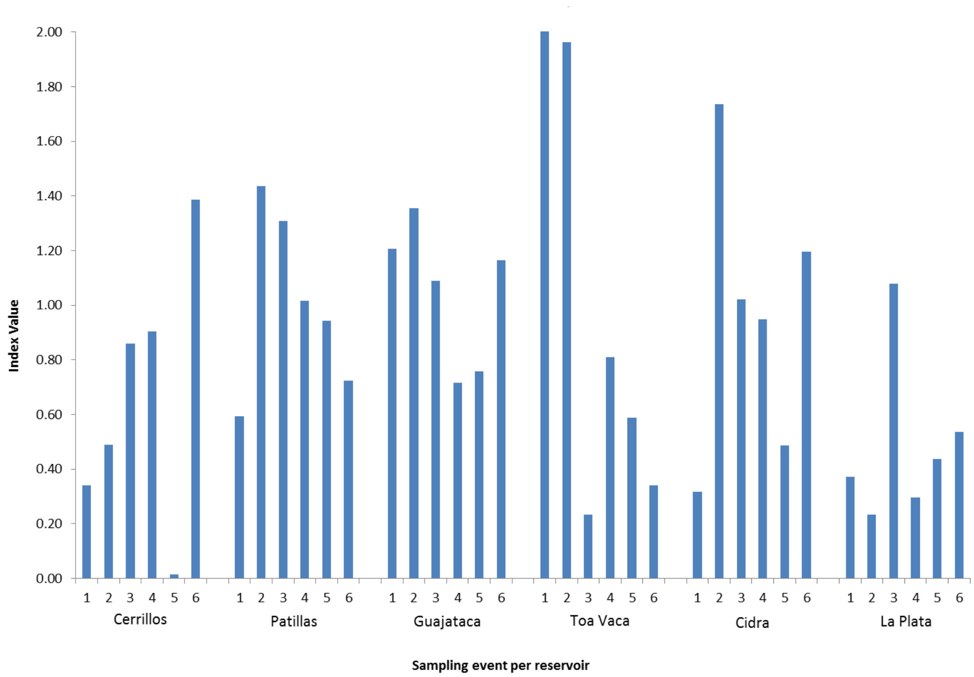


Figure 9. Shannon-Weiner's (H') diversity index for each sampling event.

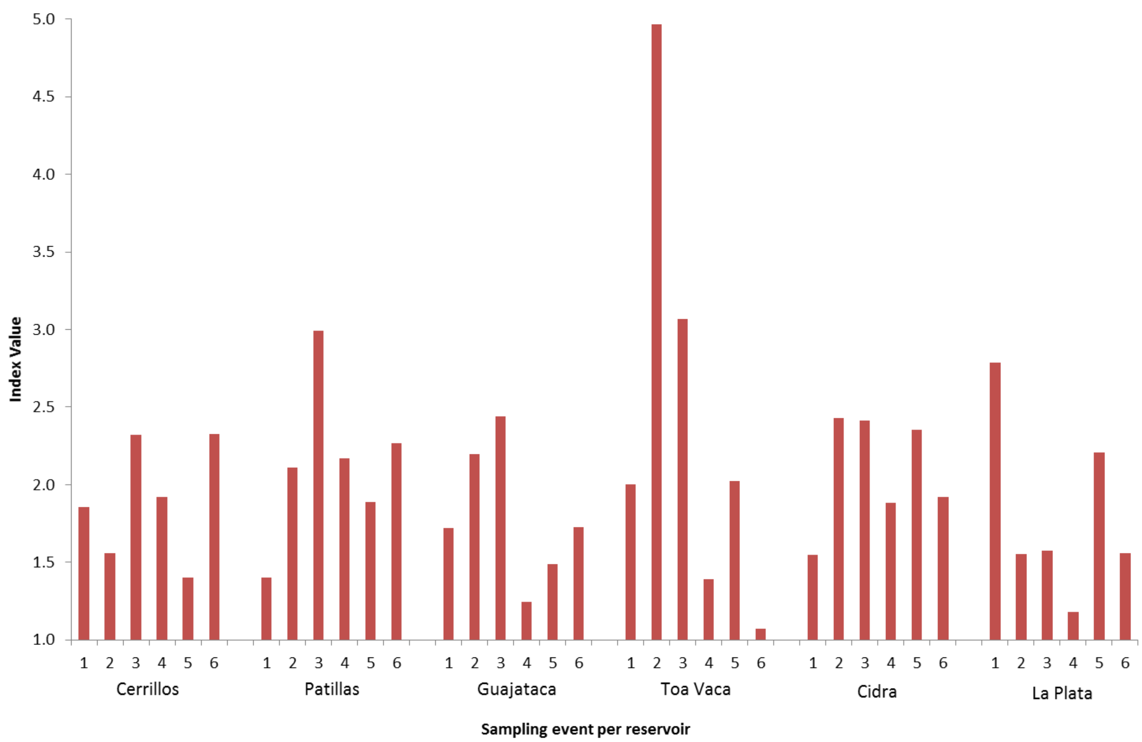


Figure 10. Margalef's (R) index for each sampling event.

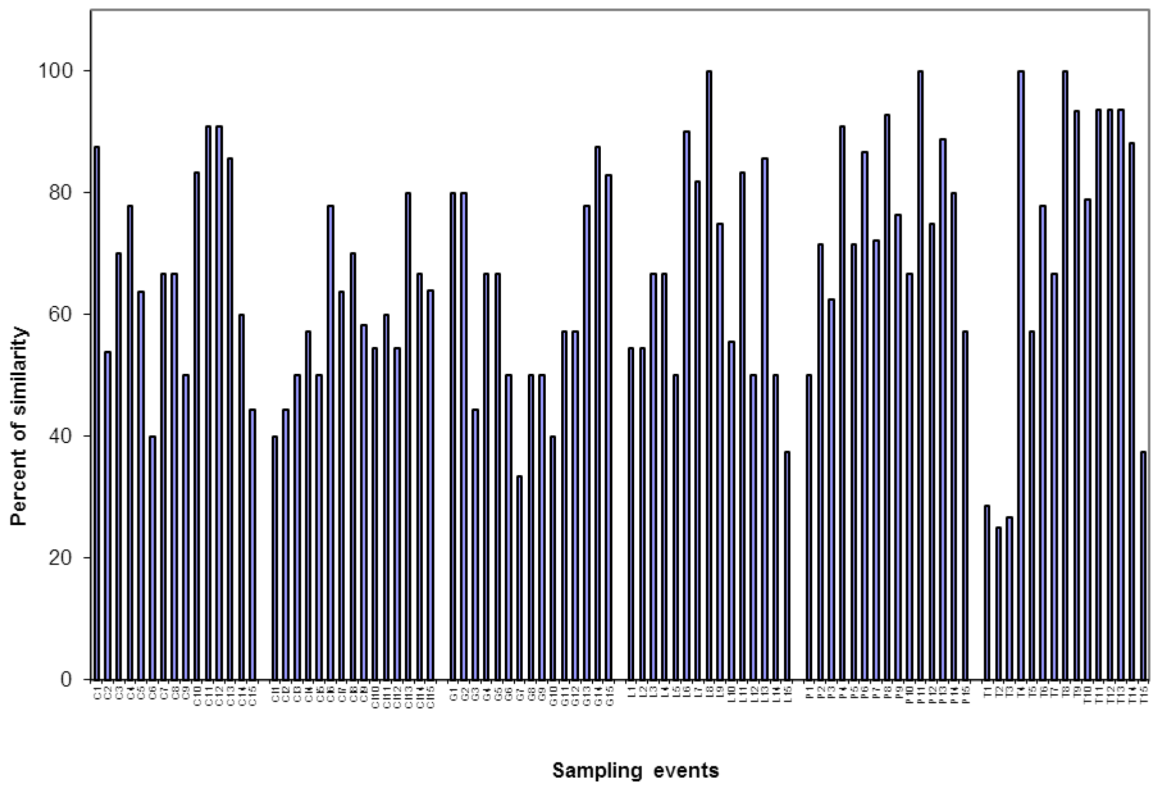


Figure 11. Jaccard's percent of similarity between sampling events in each reservoir

Legend:

| Code | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling event combination (a,b) | 1,2 | 1,3 | 1,4 | 1,5 | 1,6 | 2,3 | 2,4 | 2,5 | 2,6 | 3,4 | 3,5 | 3,6 | 4,5 | 4,6 | 5,6 |

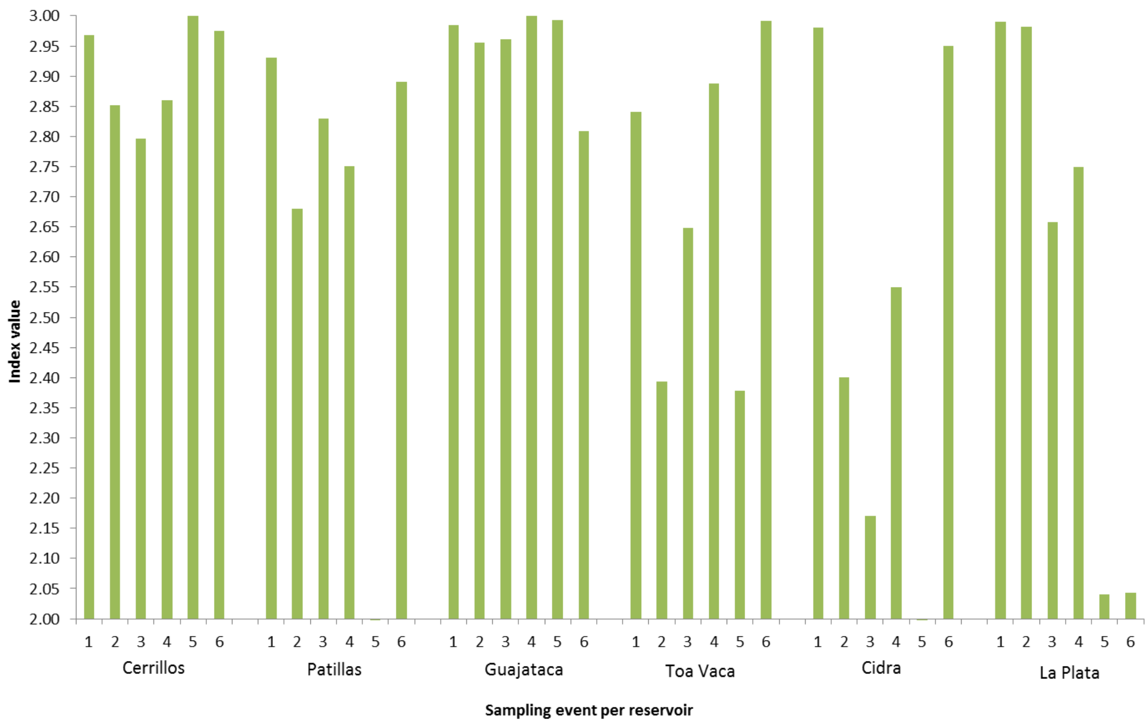


Figure 12. Pollution Tolerance Index for each sampling event.

## CERRILLOS

Six sampling events were performed at each reservoir. According to Carlson's Trophic State Index all sampling events were oligotrohic (Figure 8). Abundant species were *Achnanthidium minutissimum*, *Ulnaria acus* and *Ulnaria ulna* (Table 2). A total of 19 taxa were registered from 11 genera. Minimum and maximum values for the calculated indices were: Margalef's- 1.4 and 2.3 (Figure 10), Pollution Tolerance Index- 2.8 and 3 (Figure 12), Jaccard- 40% - 91% (Figure 14) and Shannon-Weaver-0 .015 and 1.390 (Figure 9).

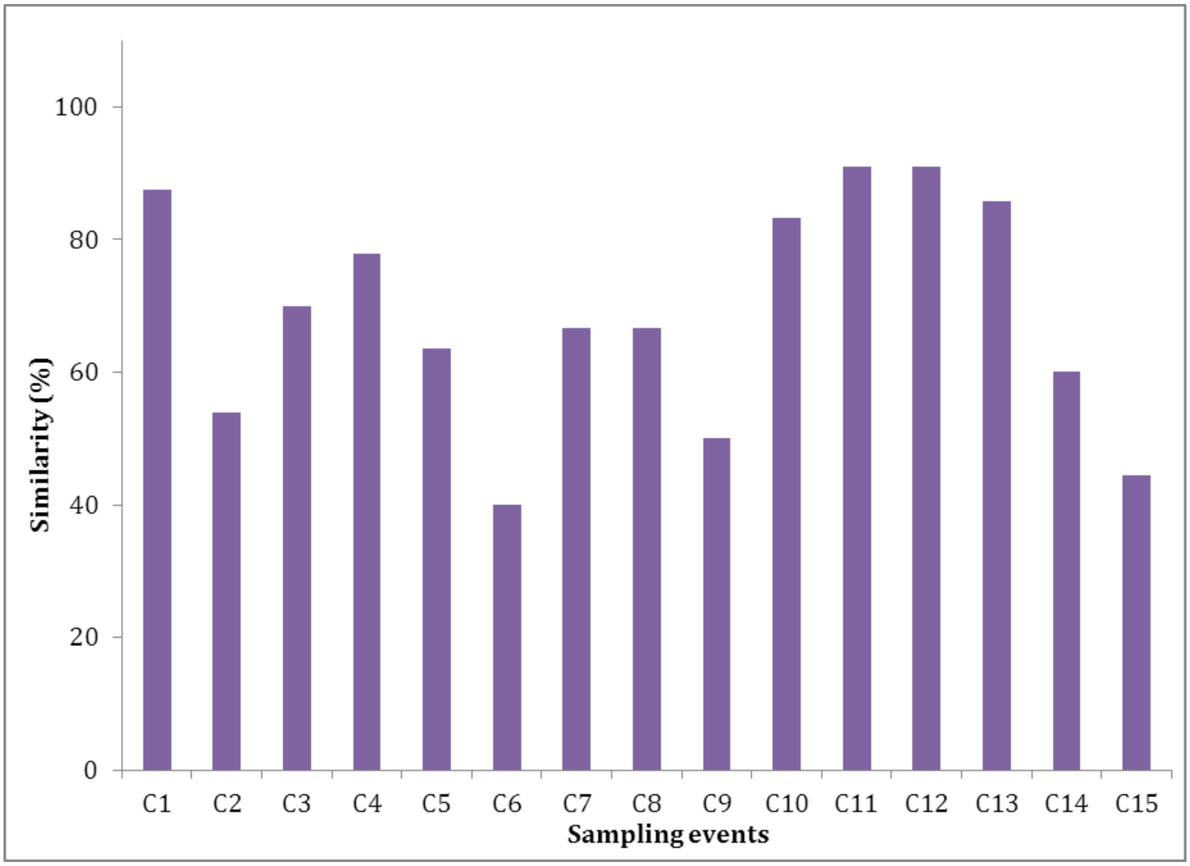


Figure 14. Jaccard's percent of similarity between samplings in Cerrillos reservoir

CIDRA

Taxa present in 25% of abundance or more were: *Achnanthidium minutissimum*, centric diatoms, *Navicula* sp., *Ulnaria acus* and *Ulnaria ulna* (Table 2). A total of 14 taxa were registered from 9 genera. Margalef's index ranged between 1.2 and 2.4 (Figure 10), while the Pollution Tolerance Index ranged between 1.2 and 2.8 (Figure 12), Jaccard's similarity index ranged between: 40% and 90% (Figure 15), and Shannon-Weaver's index ranged between 0.317 and 1.735 (Figure 9). Trophic status was oligotrophic in all six sampling events (Figure 8).

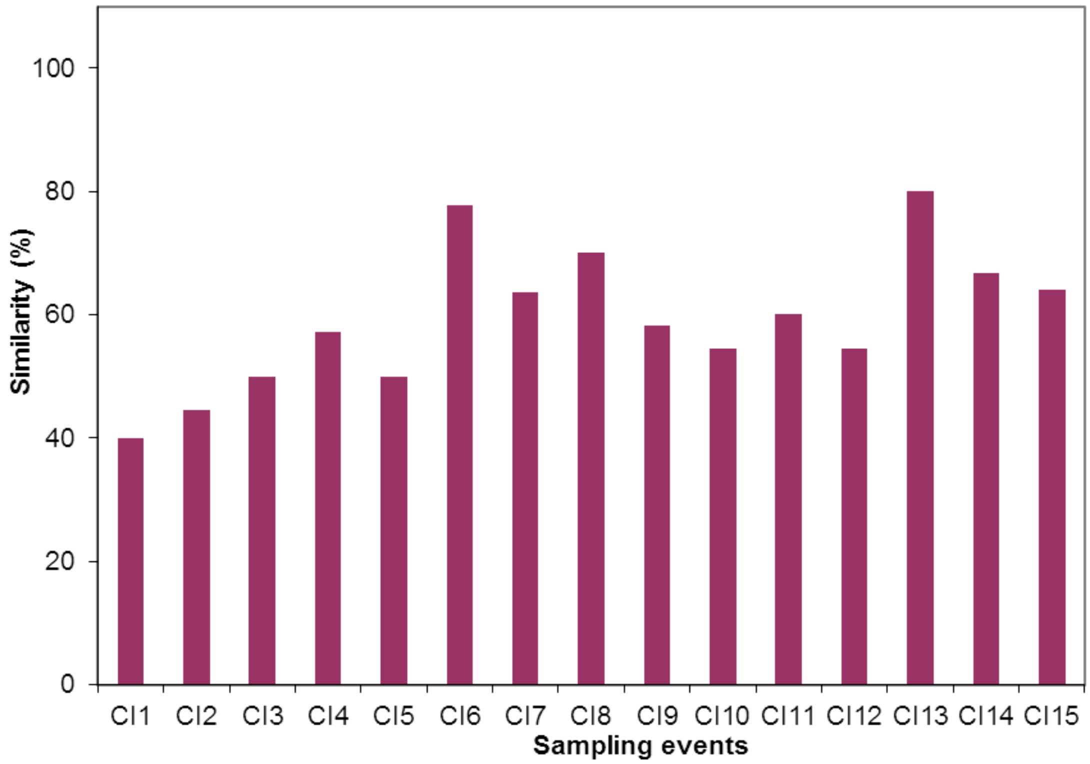


Figure 15. Jaccard's percent of similarity between samplings in Cidra.

GUAJATACA

Taxa present in 25% of abundance or more were *Achnanthidium minutissimum, Navicula* sp., *Ulnaria acus* and *Ulnaria ulna* (Table 2). A total of 10 taxa were registered from 9 genera. Margalef's index ranged between 1.2 and 2.4 (Figure 10), while the Pollution Tolerance Index ranged between 2.7 and 3 (Figure 12), Jaccard's similarity index ranged between 50% and 80% (Figure 16) and Shannon-Weaver's index ranged between 0.716 and 1.207 (Figure 9). Trophic status was eutophic in all six sampling events (Figure 8).

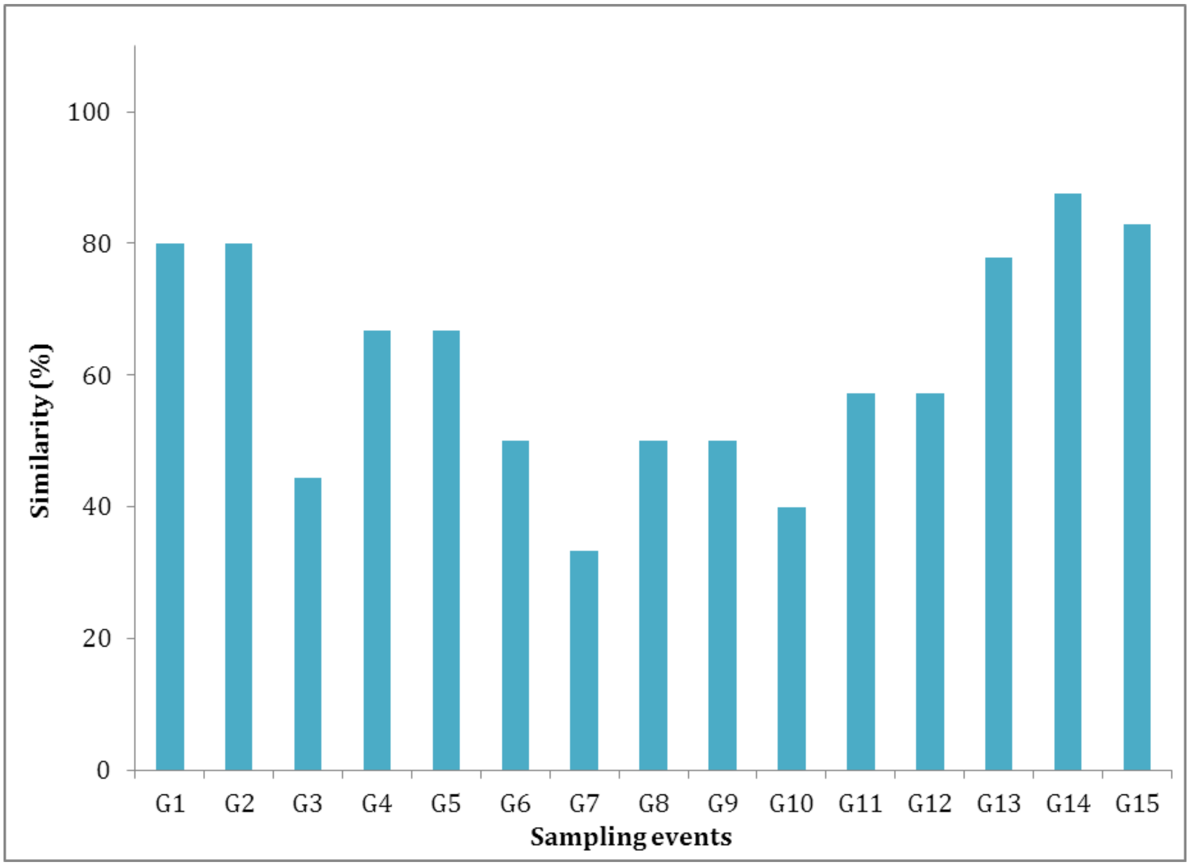


Figure 16. Jaccard's percent of similarity between samplings in Guajataca.

LA PLATA

Taxa present in 25% of abundance or more were: *Ulnaria acus* and *Ulnaria ulna* (Table 2). A total of 16 taxa were registered from 11 genera. Margalef's index ranged between 1.2 and 2.8 (Figure 10), while the Pollution Tolerance Index ranged between 2.7 and 3 (Figure 12), Jaccard's similarity index ranged between 0.232 and 0.078 (Figure 17) and Shannon-Weaver's index ranged between 0.232-1.07 (Figure 9). Trophic status was eutrophic for the first, second and fifth sampling events and mesotrophic for the remaining sampling events (Figure 8).

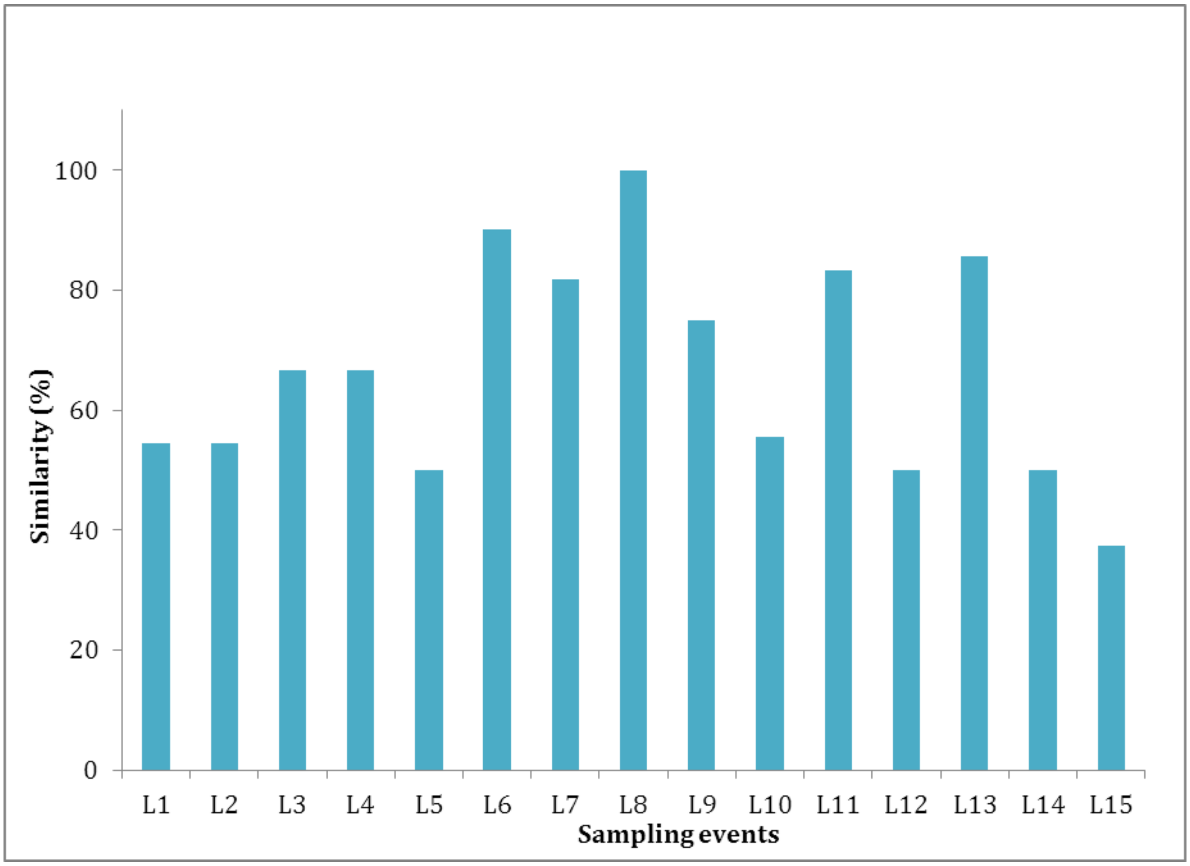


Figure 17. Jaccard's percent of similarity between samplings in La Plata.

PATILLAS

Taxa present in 25% of abundance or more were: *Navicula* sp., *Ulnaria acus* and *Ulnaria ulna* (Table 2). A total of 19 taxa were registered from 12 genera. Margalef's index ranged between 1.4 and 3.0 (Figure 10), while the Pollution Tolerance Index ranged between 0 and 3 (Figure 12), Jaccard's similarity index ranged between: 38% and 100% (Figure 18); Shannon-Weaver's index ranged between 0.594 and 1.437 (Figure 9). Trophic status was oligotrophic for the first five sampling events and mesotrophic for the last (Figure 8).

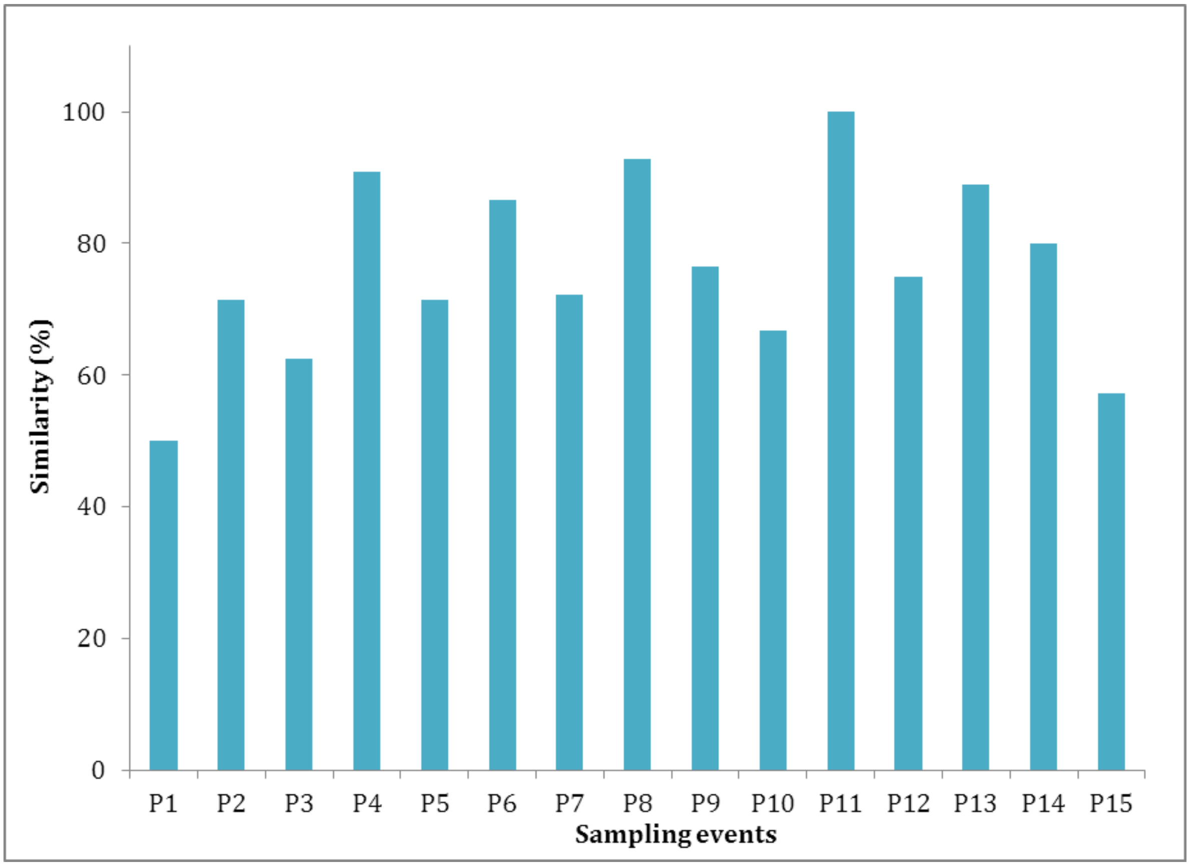


Figure 18. Jaccard's percent of similarity between samplings in Patillas.

TOA VACA

Taxa present in 25% of abundance or more were: *Ulnaria acus* and *Ulnaria ulna* (Table 2). A total of 22 taxa were registered from 14 genera. Margalef's index ranged between 1.1 and 5.0 (Figure 10), while the Pollution Tolerance Index ranged between 2.3 and 3.0 (Figure 12), Jaccard's similarity index ranged between: 25% and 100% (Figure 19); Shannon-Weaver's index ranged between 0.234 and 2.313 (Figure 9). Trophic status was oligotrophic for the second sampling event and remaining sampling events were mesotrophic (Figure 8).

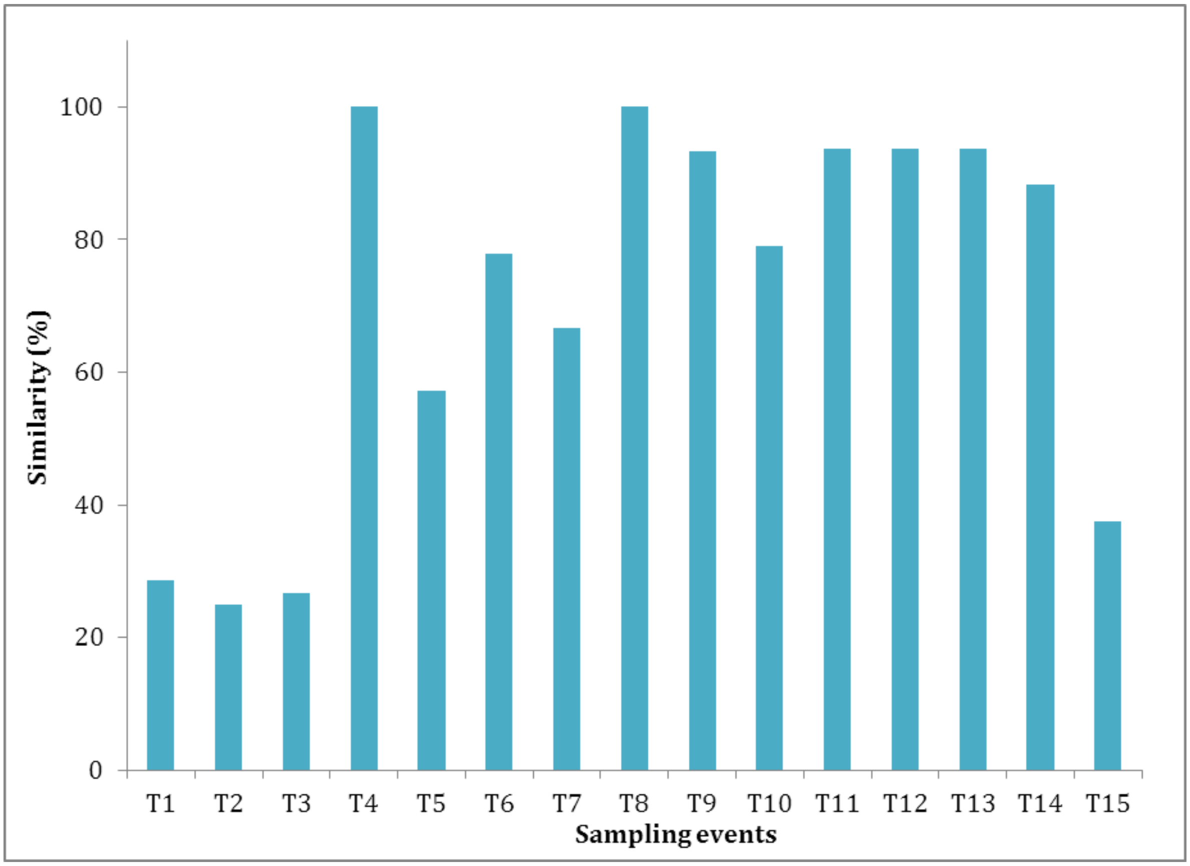


Figure 19. Jaccard's percent of similarity between samplings in Toa Vaca.

## **Diatoms**

A total of 32 taxa were identified, belonging to 20 genera (Table 6). Of all the documented taxa, 22 were identified to species level.  Most common taxa with 25% of abundance or higher were *Achnanthidium minutissimum*, centric diatoms, *Navicula* sp., *Ulnaria acus* and *Ulnaria ulna* (Table 2). Dominant taxa for oligotrophic sampling events were represented by four taxons: *Achnanthidium minutissimum*, *Navicula* sp., *Ulnaria acus* and *Ulnaria ulna* (Table 3). In mesotrophic sampling events there were five dominant taxa *Achnanthidium minutissimum*, *Navicula* sp., *Synedra*, *Ulnaria acus* and *Ulnaria ulna* (Table 4). In eutrophic sampling events dominant taxa were centric diatoms, *Navicula* sp., *Synedra*, *Ulnaria acus* and *Ulnaria ulna* (Table 5). The reservoirs with the most dominance were Cerrillos and La Plata. The reservoirs with less dominance were Guajataca and Toa Vaca (Appendix B, C and D). *Ulnaria ulna* was the most common and abundant species, present in all six reservoirs with more than 25% of abundance (Appendix B, C and D ).

Table 2. Most common taxa present in 25% relative abundance or more in each reservoir.

| | Cerrillos | Cidra | Guajataca | La Plata | Patillas | Toa Vaca |
|---|---|---|---|---|---|---|
| *A. minitissimum* | 67.9 | 59.7 | | | | |
| Centric diatoms | | 26.5 | | | | |
| *Navicula* sp. | | 52.4 | 33.6 | 44.0 | | |
| *Ulnaria acus* | 87.2 | 39.3 | 29.6 | 93.3 | 94.9 | 54.5 |
| *Ulnaria ulna* | 92.7 | 92.3 | | 56.7 | 79.0 | 32.6 |

Table 3. Taxa present in 25% of relative abundance or more in oligotrophic sampling events

| | Sampling events | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C1 | C2 | C3 | C4 | C5 | C6 | G4 | G5 | L1 | P2 | P3 | P4 | P5 | T2 |
| *A. minutissimum* | | | 69.9 | | | 44.0 | | | | | | | | |
| *Navicula* sp. | | | | | | | | | | | 44.4 | 47.6 | 69.6 | |
| *Ulnaria acus* | | 87.2 | | | | | 40.1 | | 93.6 | 54.5 | | | | |
| *Ulnaria ulna* | 92.6 | | | 62.3 | 99.5 | 35.6 | 59.1 | 74.7 | | | 32.9 | 42.6 | | 30.4 |

Table 4. Taxa present in 25% of relative abundance or more in mesotrophic sampling events.

| | Sampling events | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G2 | G3 | G6 | L4 | L6 | P6 | T1 | T3 | T4 | T5 | T6 |
| *A. minutissimum* | | 59.4 | | | | | | | | | |
| *Navicula* sp. | 33.6 | | 32.1 | | | 69.6 | | | | | |
| *Synedra* sp. | | | | | | | | | 95.0 | | |
| *Ulnaria acus* | 39.3 | 29.5 | | | | | | 20.4 | | 51.8 | 72.5 |
| *Ulnaria ulna* | | | 52.1 | 91.5 | 79.3 | | 84.3 | 34.0 | | 25.7 | 27.2 |

Table 5. Taxa present in 25% of relative abundance or more in eutrophic sampling events

| | Sampling Events | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | CI1 | CI2 | CI3 | CI4 | CI5 | CI6 | G1 | L2 | L3 | P1 |
| Centric diatoms | | | 26.5 | | | | | | | |
| *Navicula* sp. | | 33.3 | 22.0 | | | | 52.3 | | | |
| *Synedra* sp. | | | | | | 33.2 | | | | |
| *Ulnaria acus* | | | | | | | | 94.9 | | |
| *Ulnaria ulna* | 92.3 | | | 65.9 | 86.7 | 41.7 | | | 63.5 | 79.0 |

## ***<u>Species presence and abundance</u>***

*Achnanthidium minutissimum*

Present in 20 sampling events (56%). This species was abundant in the third sampling of the Cerrillos and Guajataca reservoir with 68 and 60% of abundance, respectively.

Centric diatoms

The genera of the centric diatoms were not identified in light microscopy (where valve count was performed) due to the low resolution of internal patterns. *Cyclotella* *meneghiniana* was identified in SEM. The centric diatoms were present in 31 sampling events (86%). This group was abundant (present in 25% or more) in the third sampling for Cidra, fifth sampling in La Plata.

*Navicula* sp.

Present in 29 sampling events (81%). This genus was abundant in the first and second sampling events in the Guajataca reservoir 52 and 34% and in the third sampling event in Patillas, 44%.

*Ulnaria acus*

Present in 28 of the sampling events (78%). This species was present in 24% of abundance or higher in 7 sampling events; the first sampling event in La Plata, 93%, the second sampling of Cerrillos, 87% , Cidra, 24%, Guajataca, 39%, Patillas, 55%, and La Plata, 95%, the third sampling event in Guajataca 30%.

*Ulnaria ulna*

Present in 34 of the sampling events (94%). This species was also present in 25% of abundance or higher in 7 sampling events. These were the first sampling events for Cerrillos, 93%, Cidra, 92%, Patillas, 79% and Toa Vaca, 82%, the second sampling event for Toa Vaca 36% and the third sampling event for La Plata, 57%, and Toa Vaca 34%.

*Gomphonema* sp.

*Gomphonema* species were not abundant in any sampling event. This genus was present in 20 of the sampling events (56%).

Table 6. Taxa present in each reservoir.

| **Taxa** | Code | Cerrillos | Cidra | Guajataca | La Plata | Patillas | Toa Vaca |
|---|---|---|---|---|---|---|---|
| *Achnanthidium minutissimum* | D1 | X | X | X | X | X | X |
| *Amphora angusta* | D2 | X | X | X | X |  | X |
| Centric diatoms | D3 | X | X |  | X | X | X |
| *Cocconeis* cf. *placentula* | D4 | X |  |  | X | X | X |
| *Cymbella helvetica* | D5 | X | X | X | X | X | X |
| *Cyclotella meneghiniana* | D6 |  |  | X | X |  |  |
| *Denticula* sp. | D7 |  |  |  | X |  | X |
| *Fragilaria goubardi* | D8 | X |  |  |  |  |  |
| *Geissleria decussis* | D9 |  | X |  | X | X | X |
| *Gomphonema sp.* | D10 | X | X | X | X | X | X |
| *Gomphonema parvulum* | D11 | X | X |  |  | X | X |
| *Gomphonema gracile* | D12 | X |  |  | X | X | X |
| *Gomphonema vibrio* var. *pumilum* | D13 |  |  |  |  | X |  |
| *Gyrosigma acuminatum* | D14 |  |  | X |  | X | X |
| *Hamphora coffeaformis* | D15 |  |  |  |  | X |  |
| *Hantzscia amphioxys* | D16 |  |  |  |  |  | X |
| *Melosira varians* | D17 | X |  |  |  |  |  |
| *Navicula* sp. | D18 | X | X | X | X | X | X |
| *Navicula rhynchocephala* | D19 |  |  |  |  |  | X |
| *Nitzchia* sp. | D20 | X | X |  | X |  | X |
| *Nitzchia palea* | D21 | X | X |  | X | X | X |
| *Navicula pumilum* | D22 |  |  | X | X |  |  |
| *Nitzchia sigma* | D23 | X |  |  |  |  | X |
| *Nitzchia pumilum* | D24 |  |  |  |  | X |  |
| *Rhopalodia gibba* | D25 | X |  |  |  |  |  |
| *Sellaphora pupula* | D26 |  |  |  |  | X |  |
| *Surirella tenera* var. *nervosa* | D27 | X |  | X |  | X | X |
| *Synedra* sp. | D28 |  | X |  |  |  | X |
| *Ulnaria acus* | D30 | X | X | X | X | X | X |
| *Ulnaria biceps* | D31 | X | X | X |  | X | X |
| *Ulnaria ulna* | D32 | X | X |  | X | X | X |

## DISCUSSION

### Reservoir Trophic Status

Carlson's trophic state index was calculated for nitrogen, phosphorous, chlorophyll a, and Secchi Disk and a generalized index was also calculated averaging nitrogen, Chl A and Secchi Disk. For a total of 36 sampling events evaluated (six for each reservoir) each trophic status (oligotrophic, mesotrophic and eutrophic) was registered no less than ten times. This confirmed our aim of selecting reservoirs that exhibited a range of trophic status. Cerrillos (oligotrophic) and Cidra (eutrophic) showed no variability in trophic states along all sampling events. Guajataca shifted from eutrophic to mesotrophic in the second sampling event (2012), in the fourth sampling event trophic state shifted from mesotrophic to oligotrophic and in the last sampling event trophic status shifted back from oligotrophic to mesotrophic. La Plata presented shifts between all three trophic states; in the second sampling event from oligotrophic to eutrophic, in the fourth sampling event from eutrophic to mesotrophic. Patillas was eutrophic only in the first sampling event; for the remaining sampling events the trophic status was oligotrophic. Toa Vaca was oligotrophic for the second sampling event and mesotrophic for the remaining five sampling events. The least stable environments in terms of trophic state were Guajataca and La Plata. Guajataca was reported as mesotrophic and La Plata as eutrophic by Martinez et al. (2005).

Carlson's trophic state indices were calculated to evaluate relationships between freshwater diatoms and nutrient concentration in these reservoirs. Although netplankton was collected with bongo nets with 63μm pores expecting to collect large frustules for ease of identification with light microscopy and feasibility of implementation in future monitoring, these pores are reduced during sampling due to clogging of the net, allowing for the collection of smaller species such as *Cyclotella meneghiniana* (~10μm). To evaluate the limitations of the sampling method, samples were collected with bottles and no net. There was no difference in the species present in the samples collected with the net and the bottles (C. Santos-Flores, pers. comm.).

In a study of freshwater diatoms in South Africa species evenness did not exhibit a strong linear association with water quality so that a high level of dominance was not equal to polluted or less favorable conditions (De la Rey,2008). In our study, diatoms from the *Ulnaria* genus were dominant in all reservoirs independent of trophic status. The low diversity registered due to the bias against smaller individuals does not account for poor water quality or unfavourable conditions.

The diatom flora in Toa Vaca exhibits the principle established by Stevenson and Lowe (1986) "minimum dominance should occur at intermediate levels of nutrients when reproduction rates of low nutrient taxa equal those of high nutrient taxa; and maximum richness should occur at intermediate levels of enrichment/productivity because richness in a 600 valve count is highly related the dominance (or eveness) of species abundances in the assemblage".

## Indexes and cell densities

Highest diversity, H', values were observed in Toa Vaca where trophic status was mesotrophic for most sampling events. Based on Carlson's trophic index there were no other reservoirs with constant mesotrophic conditions. The average diatom species richness, R, for Toa Vaca was the highest of all reservoirs. Average cells per liter of water in Toa Vaca was lower than 5,000 cells/L. Similarity, $S_j$, was on average 70.7% between sampling events

Diversity was generally low in all reservoirs, including sampling events where just three taxa were registered. In oligotrophic reservoirs , Cerrillos and Cidra, diversity, H', was lower than Toa Vaca (mesotrophic), but not Guajataca (mesotrophic). Diversity within a phytoplankton community is thought to increase with higher concentrations of nutrients (Hutchinson, 1961). The highest diversity was found in Toa Vaca, which was mesotrophic, the second and third reservoirs with the highest diversity were Cerrillos and Patillas which were oligotrophic.

Despite being classified as "eutrophic" using Carlson's index Cidra and La Plata showed cell densities lower than 5,000 cells/L. These densities were considerably lower than Cerrillos and Patillas, both oligotrophic reservoirs. These high cell densities in the absence of high concentrations of nutrients could be the product of low grazing.

During pollution increase events the pollution intolerant species decline in abundance and the pollution tolerant species can grow rapidly without competition for space, nutrients, or other resources. These results in community abundance patterns of heavy dominance and fewer species (Van Dam, 1982). The PTI indicates that the taxa present in all reservoirs are moderately tolerant to pollution (2-3).

The reservoirs with the lowest Jaccard's index were Guajataca and La Plata with 43.5%. Cerrillos and Guajataca presented the highest similarity with 90.5%. Similarity between sampling events was evaluated showing that the lowest similarities ocurred in between sampling from the same reservoir: Toa Vaca's 1st and 3rd (25%); Guajataca's 2nd and 4th (33%) and La Plata's 5th and 6th. This variability in each reservoir (between sampling events) prevents us from observing differences between reservoirs.

No strong relation was found between the variables which influence community composition . Diatoms could be dependent on the concentration of ions which were not evaluated in the study, for example calcium and silicon (Reynolds, 2006).

**Species Ecology**

*Achnanthidium minuttisimum*

*A. minutissimum* is found to be sensitive to trophic conditions (oligo-mesotrophic to mesotrophic in indices Trophy D, TDI and Rott), pH, oxygen (van Dam et al., 1994), physical disturbance or toxic substances (Barbour et al., 1999; Charles et al., 2006) and is also known as an early coloniser (Biggs et al., 1998; Rimet et al., 2007). The indices Trophy D and Rott describe this taxon as oligo-mesotrophic, TDI places it in mesotrophic class, whereas indices GM B&O, GM Seen, Hofmann and van Dam mark place it as indifferent, and thus exclude it from further analysis. In experimental studies, the absolute abundance of *A. minutissimum* responded positively to nutrient enrichment, in particular to nitrogen additions (e.g. Carrick et al., 1988; Fairchild et al., 1985), while in many observational studies it decreased (Kelly and Whitton, 1995; Pan et al., 1996; Potapova and Charles, 2007; Soininen and Niemela, 2002). Potapova and Charles (2007) noticed that Stoermer's (1980) characterization of *A. minutissimum* as 'apparently tolerant to nutrient addition, but also quite abundant in more oligotrophic regions' effectively summarized our current knowledge about the relation of this taxon to nutrients. A trophic scaling of indifferent might thus be the best.

*Achnanthidium minutissimum* complex is an R-strategist with the ability to maintain high growth rate in advanced stages (Stevenson et al., 1991) and also has high tolerance of several environmental factors (Ponader and Potapova, 2007). The genus *Achnanthidium*

(*Achnanthidium minutissimum* complex) occurs under a wide range of ecological conditions, being found in oligo- to hypereutrophic systems (Luttenton and Lowe, 2006).

Centric diatoms

*Cyclotella meneghiniana*

*Cyclotella meneghiniana* is regarded as the most common species of global diatom diversity and occupies a wide range of habitat types (Håkansson, 2002). According to Denys (1991), *C. meneghiniana* is a tychoplanktonic species, occurring in brackish and freshwater, eutraphentic, α-meso- to polysaprobic environments (Van Dam *et al.*, 1994). According to Krammer & Lange-Bertalot (1991a), it is common in ditches and puddles and in eutrophic lakes and rivers. Cells of *C. meneghiniana* can grow in a wide variety of habitats but not in highly competitive situations (Patrick and Roberts, 1979). In strongly eutrophic and polluted waters, which are presumably free of interspecies competition, *C. meneghiniana* may develop large populations (Wojtal & Kwandrans, 2006). According to regional classifications (Lobo *et al.*, 2004a), this species has a high tolerance for organic pollution and an average tolerance for eutrophication. Lobo *et al*. (2002) classified *C. meneghiniana* as highly tolerant to organic pollution in the lower reaches of the Rio Pardo hydrographical basin, southern Brazil.

*Ulnaria* sp.

*Ulnaria acus* has been reported as eutrophication tolerant and has been associated with eutrophication in tropical streams (Lobo et al., 2010). *Ulnaria ulna* has been accepted as an indicator for eutrophic lakes by Van Dam and Mertens (1993).

Desirable reservoir water quality depends upon the specific use for that water. Many governments establish near-natural conditions as management objective. Understanding threshold responses in diatom communities along trophic status is valuable for management strategies. Advancement in monitoring techniques and implementation, and a more complete understanding of the ecology of diatoms remains as an alternative to establish environmental assessments and proper management.

This study indicates that several species: *Ulnaria ulna, Ulnaria acus* could tolerate a wide range of water quality.

## CONCLUSIONS

Diatom relative abundance was related to trophic status for *Achnanthidium minutissimum, Synedra* sp., and Centric diatoms. Our methodology is biased toward diatoms smaller than 60um. For this reason bioindicator values is not determinant of trophic status. Reservoirs with stable trophic status exhibit higher diversity indexes. Constant mesotrophic contitions were found in Toa Vaca, coincident with the highest diversity among all reservoirs.

The Pollution Tolerance Index is not well suited for Puerto Rican reservoirs. A comprehensive and updated database of Puerto Rican diatoms is now available with approximately 700 taxa.

Puerto Rican water reservoirs are singularly characterized by the presence of the genus *Ulnaria.* Most abundant taxa in lentic systems in Puerto Rico are *Achnanthidium minitussimum,* centric diatoms, *Navicula* sp., *Ulnaria acus* and *Ulnaria ulna.*

Development of a diatom based index that takes into account local species will aid in the integration of effective monitoring techniques for water quality. Species reported are cosmopolitan and aid in the construction of the freshwater diatom flora in Puerto Rico.

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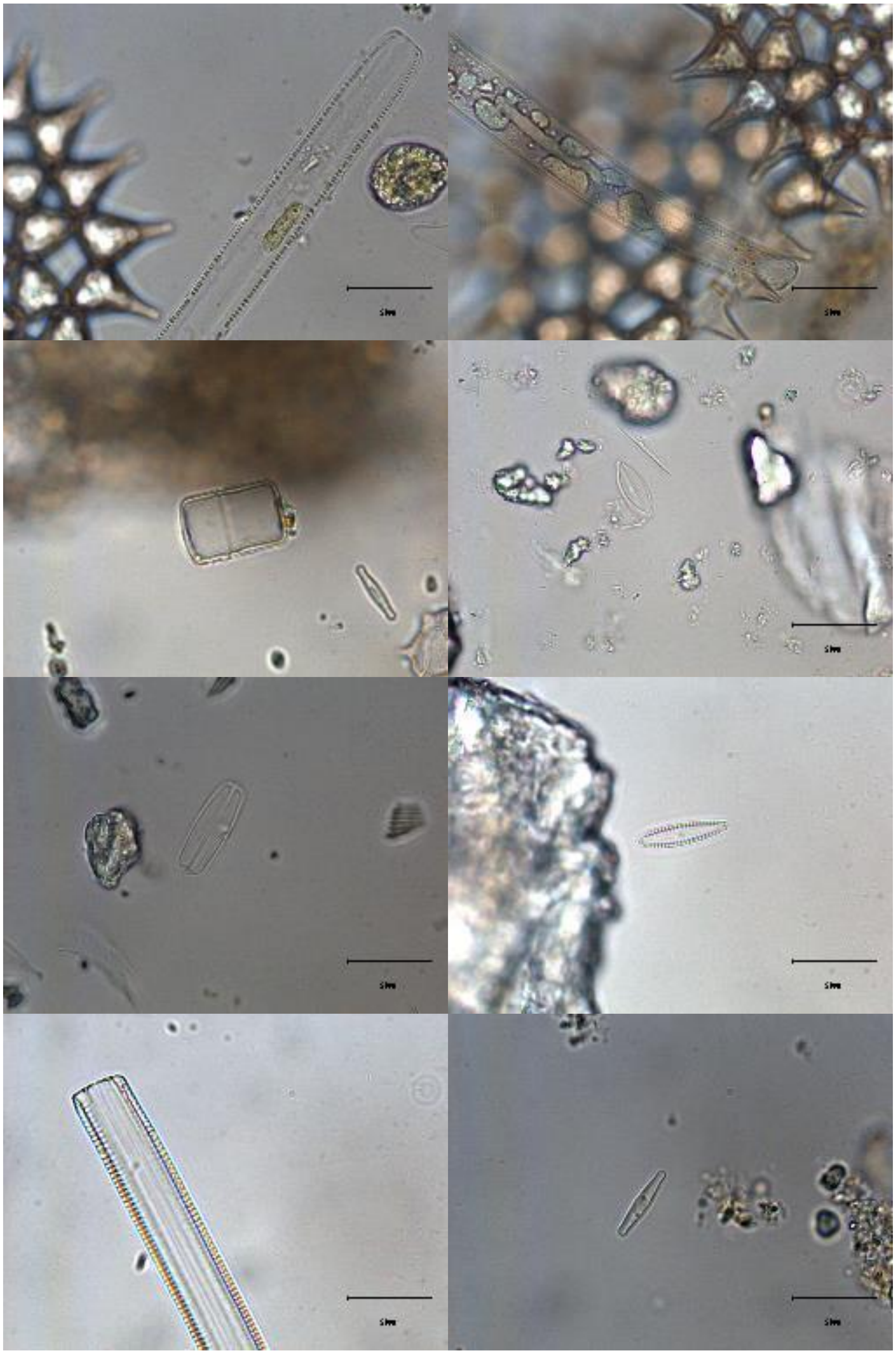
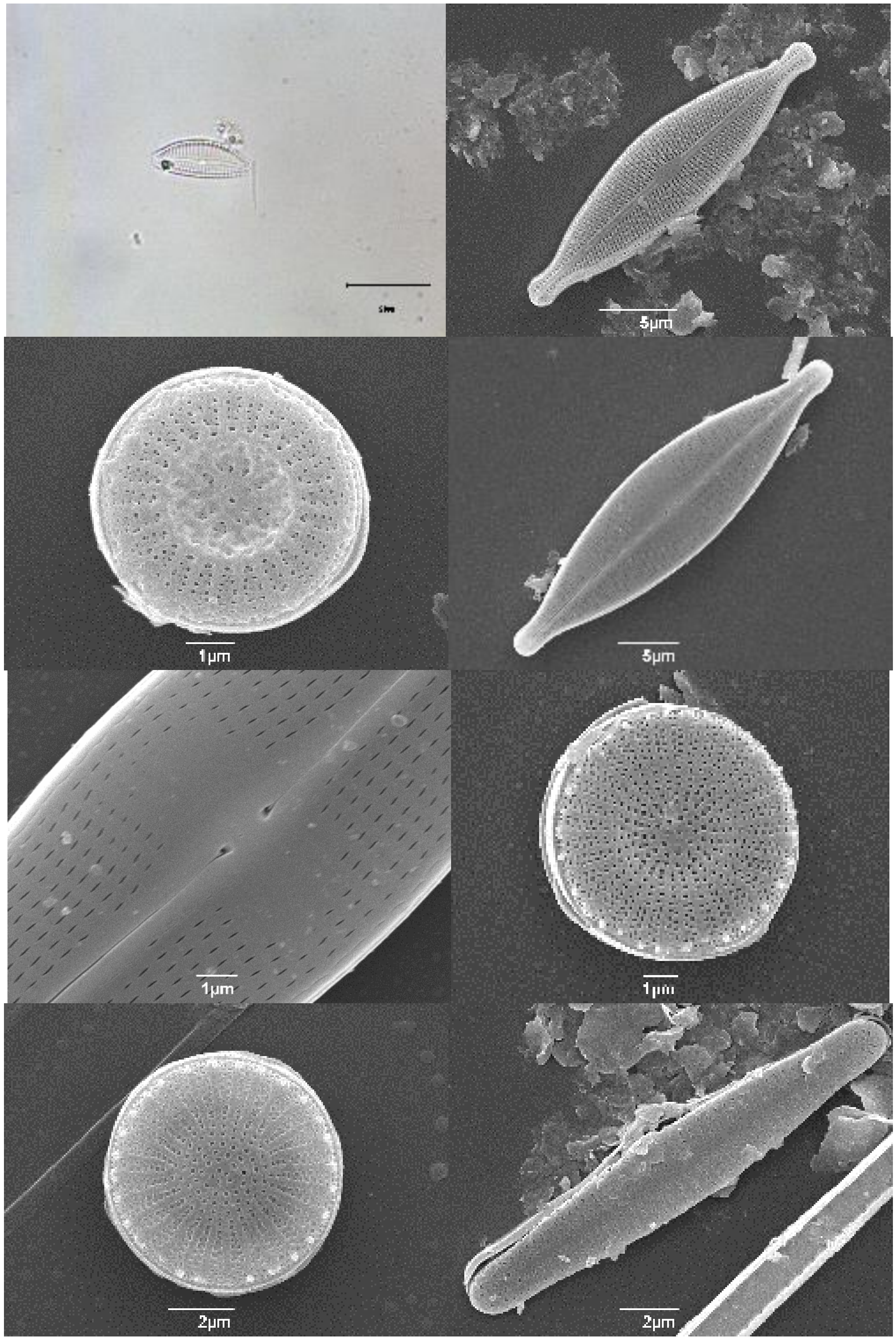
# APPENDIX

# APPENDIX A: Photos of Light and Scanning Electronic Microscopy.

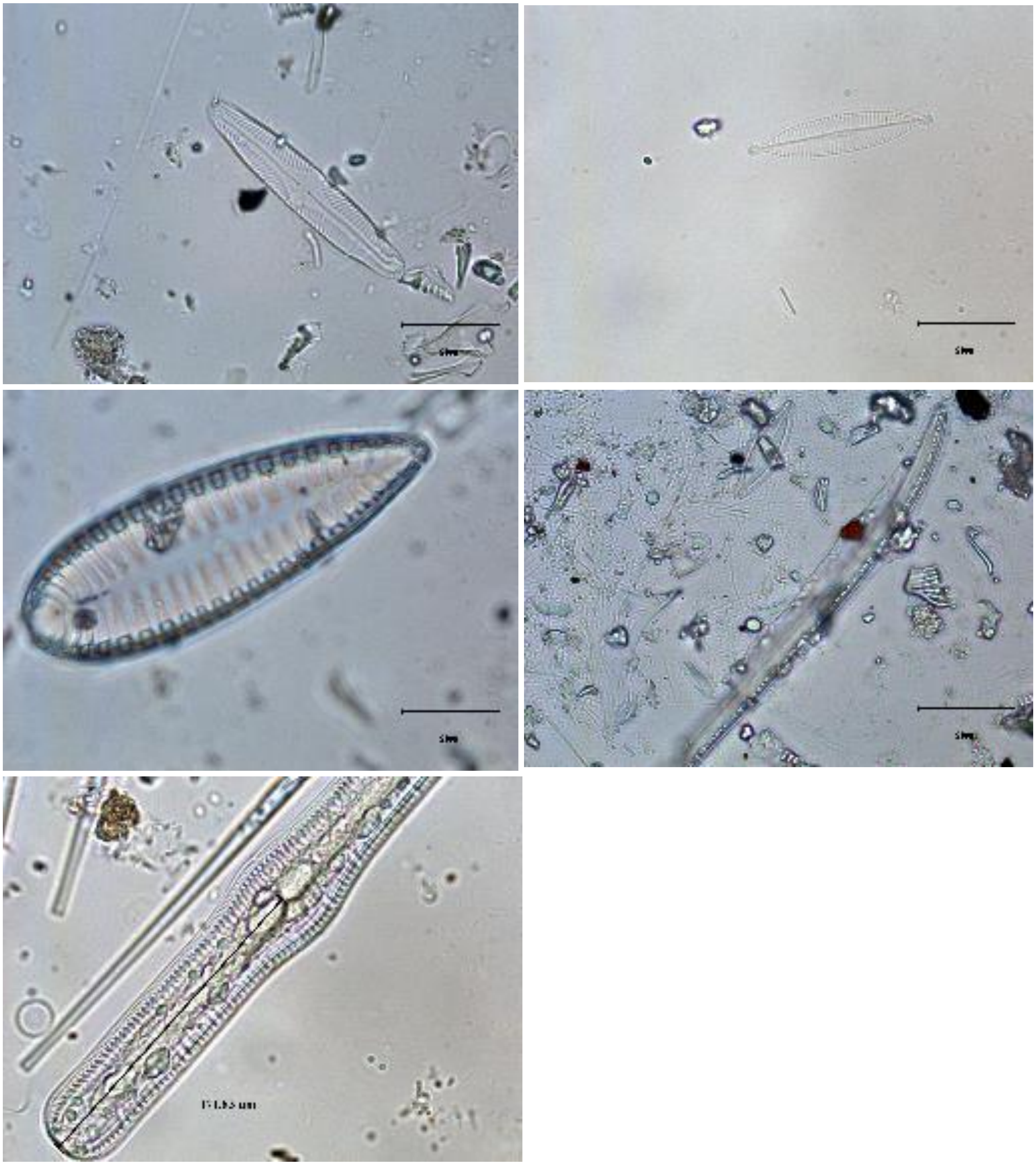


1a.*Nitzschia sigma,* 1b.*Nitzschia sigmoidea,* 1c.*Melosira varians,*
1d.*Geissleria decussis,* 1e.*Sellaphora pupula,* 1f.*Denticula* sp.
1g.*Ulnaria biceps,* 1h.*Achnanthidium minutissimum*

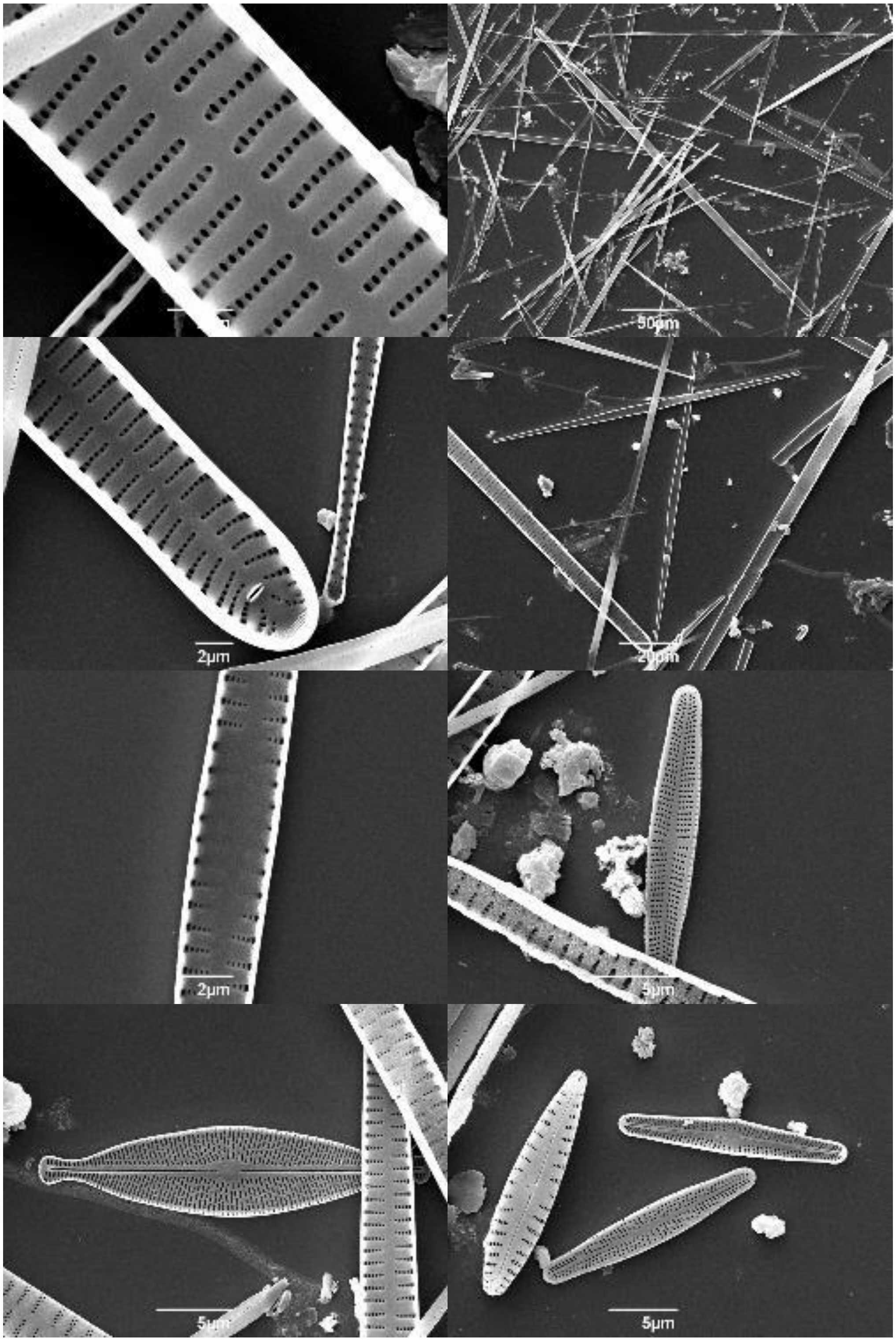
2a.*Denticula* sp., 2b.*Cymbella helvetica,* 2c.*Gomphonema gracilis,*
2d. *Hantzchia amphioxys,* 2e.*Melosira varians,* 2f.*Gyrosigma acuminatum,*
2g.Centric diatom, 2h.*Pinnularia* sp.



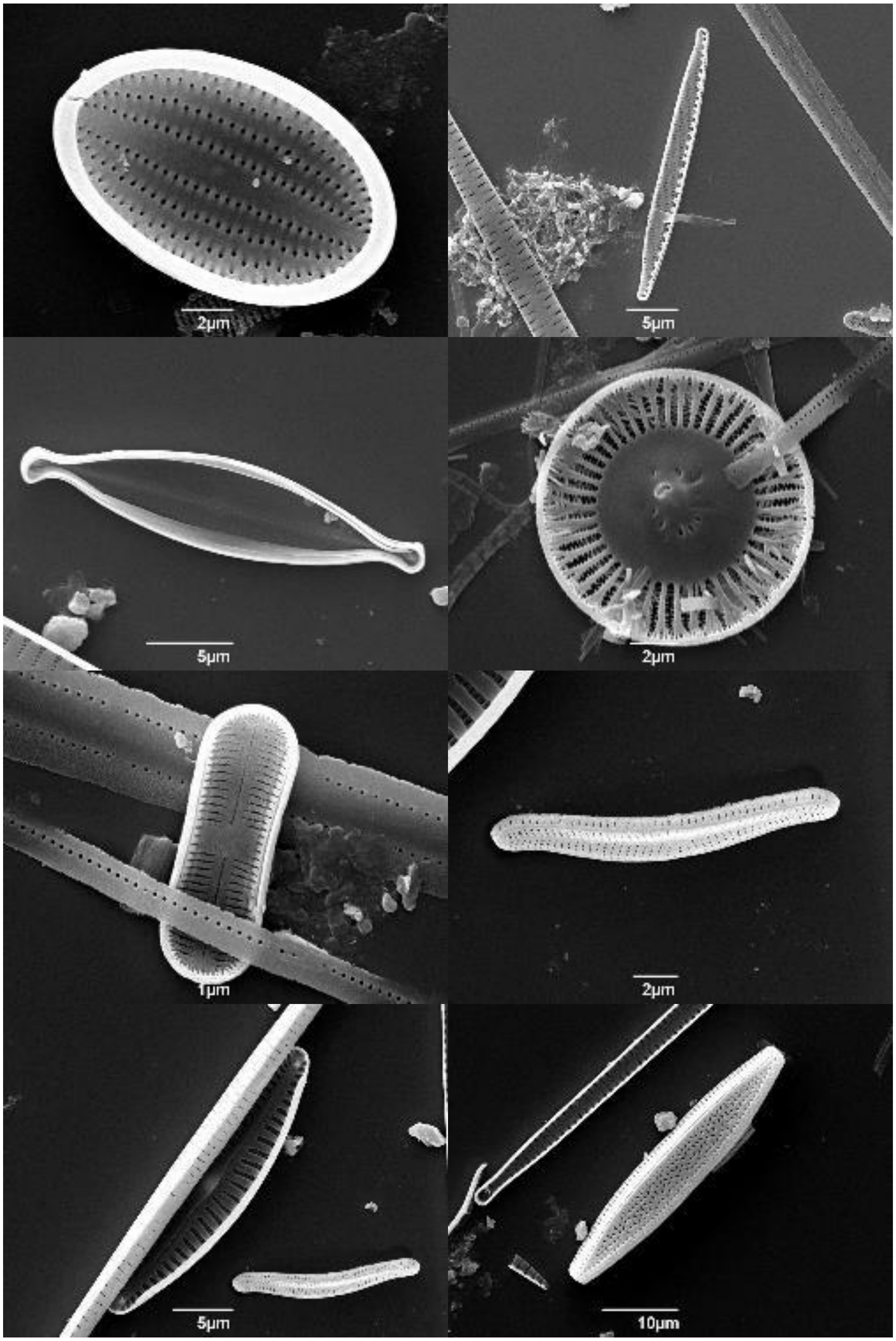

3a.*Amphora angustatum*, 3b, 3d & 3e. *Navicula* sp.; 3c., 3f. & 3g, *Cyclotella meneghiniana*, 3h.*Achnanthidium minutissimum*



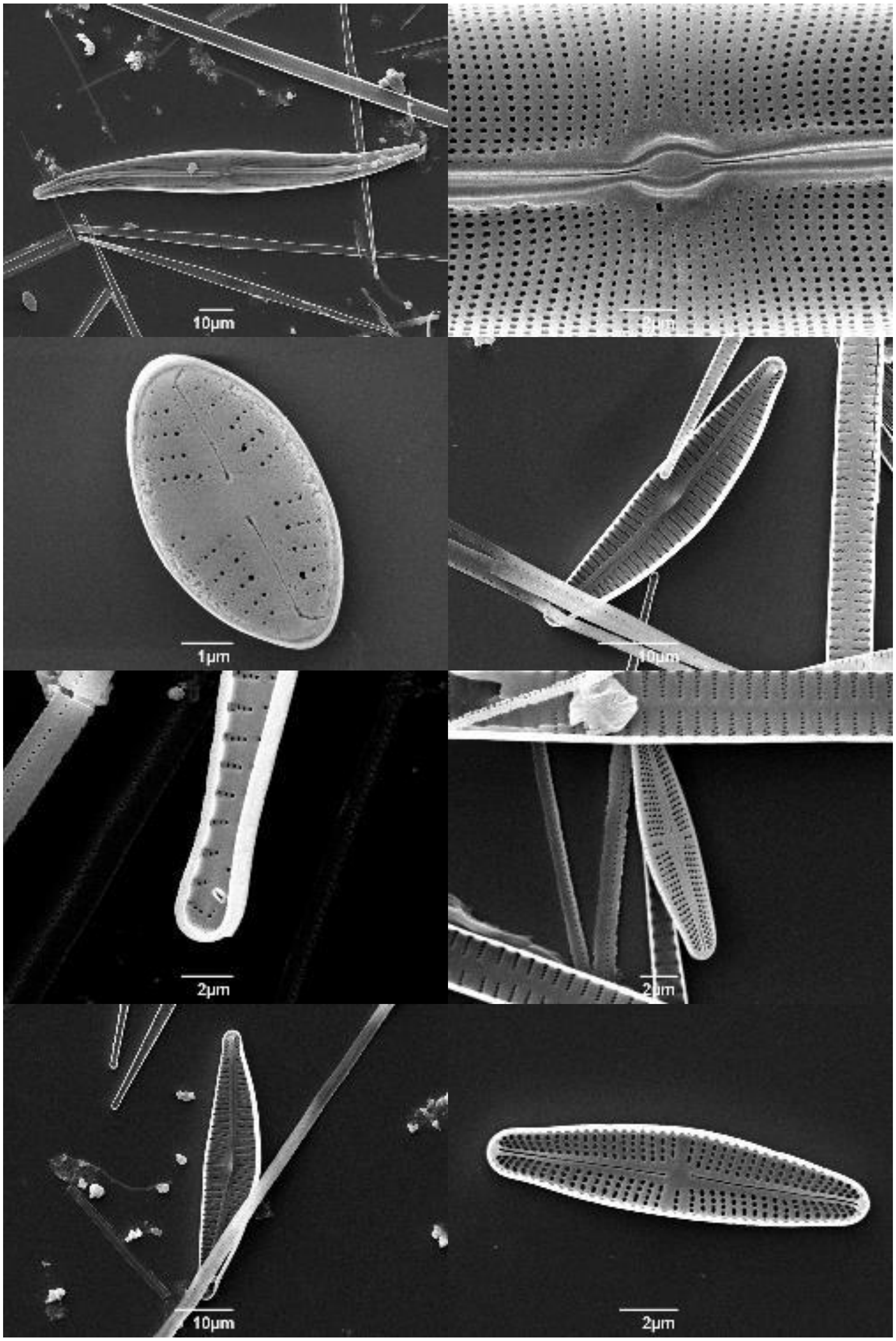
4a.*Pinnularia microstauron*, 4b.*Navicula rhynocephala*, 4c.*Surirella tenera*, 4d.*Nitzchia* sp., 4e.*Rhopalodia gibba*




5a & 5b.*Ulnaria ulna*, 5c.*Ulnaria* sp., 5d & 5e.*Ulnaria acus*,
5f.*Achnanthidium minutissimum*, 5g.*Navicula* sp.,
5h.*Gomphonema parvulum* & *Achnanthidium minutissimum*




6a.*Cocconeis* sp., 6b.*Nitzchia palea*, 6c.*Navicula* sp., 6d.Centric diatom, 6e.Unknown 6f.*Achnanthidium minutissimum*, 6g.*Cymbella* sp., 6h.Unknown




7a & 7b.*Gyrosigma acuminatum,* 7c.Unknown, 7d & 7g.*Cymbella helvetica,*
7e.*Ulnaria ulna,* 7f & 7h.*Achnanthidium minutissimum*

Appendix B. Minimum and maximum registered for each taxa for TKN-N, NO3-N3, TN, TN/TP, Chl a, DO, Secchi disk, PH of netplankton diatoms in samples from March 2012 to April 2014

| Species code | TKN-N (mg/L) | | NO3-N (mg/L) | | TN (mg/L) | | TN/TP | | TP(mg/L) | | Chl a (ug/L) | | DO (% sat) | | DO (mg/L) | | Sechi (m) | | pH (std units) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** | **Min** | **Max** |
| D1 | 0.14 | 1.41 | 0.00 | 0.07 | 0.14 | 1.43 | 6.94 | 58.41 | 0.00 | 0.07 | 0.85 | 174.80 | 57.1 | 148.5 | 4.75 | 11.50 | 0.8 | 3.8 | 7.15 | 9.09 |
| D2 | 1.32 | 1.32 | 0.01 | 0.01 | 1.33 | 1.33 | 21.31 | 21.31 | 0.07 | 0.07 | 110.25 | 110.25 | 106.7 | 106.7 | 8.43 | 8.43 | 2.8 | 2.8 | 8.50 | 8.50 |
| D3 | 1.78 | 1.78 | 0.06 | 0.06 | 1.78 | 1.78 | 78.24 | 78.24 | 0.09 | 0.09 | 174.80 | 174.80 | 151.5 | 151.5 | 11.76 | 11.76 | 3.5 | 3.5 | 9.09 | 9.09 |
| D4 | 0.11 | 1.41 | 0.00 | 0.04 | 0.11 | 1.43 | 9.82 | 60.33 | 0.00 | 0.06 | 1.94 | 174.80 | 79.8 | 109.7 | 6.50 | 8.82 | 1.0 | 2.8 | 7.64 | 8.39 |
| D5 | 0.11 | 0.84 | 0.00 | 0.05 | 0.11 | 0.85 | 9.31 | 60.33 | 0.00 | 0.05 | 1.93 | 32.37 | 59.9 | 137.0 | 4.89 | 10.83 | 0.9 | 3.2 | 7.15 | 9.09 |
| D7 | 0.43 | 0.84 | 0.00 | 0.01 | 0.44 | 0.85 | 16.07 | 17.73 | 0.02 | 0.05 | 15.30 | 32.37 | 106.7 | 137.0 | 8.43 | 10.83 | 0.9 | 1.5 | 8.27 | 9.09 |
| D8 | 0.22 | 0.22 | 0.00 | 0.00 | 0.22 | 0.22 | 19.82 | 19.82 | 0.01 | 0.01 | 2.45 | 2.45 | 100.0 | 100.0 | 8.32 | 8.32 | 3.5 | 3.5 | 8.21 | 8.21 |
| D9 | 0.33 | 1.27 | 0.00 | 0.01 | 0.33 | 1.28 | 17.73 | 78.24 | 0.00 | 0.07 | 1.24 | 106.82 | 91.7 | 148.5 | 6.95 | 11.50 | 0.9 | 3.3 | 7.64 | 8.82 |
| D12 | 0.15 | 1.27 | 0.00 | 0.01 | 0.16 | 1.28 | 9.31 | 19.17 | 0.01 | 0.07 | 3.66 | 40.57 | 90.5 | 148.5 | 6.97 | 11.50 | 0.9 | 3.2 | 7.67 | 8.82 |
| D10 | 0.01 | 1.41 | 0.00 | 0.06 | 0.02 | 1.43 | 3.53 | 78.24 | 0.00 | 0.07 | 0.85 | 174.80 | 79.8 | 137.0 | 6.50 | 10.83 | 0.8 | 3.8 | 7.44 | 9.09 |
| D13 | 0.33 | 0.33 | 0.01 | 0.01 | 0.33 | 0.33 | 78.24 | 78.24 | 0.00 | 0.00 | 1.24 | 1.24 | 91.7 | 91.7 | 6.95 | 6.95 | 3.3 | 3.3 | 7.64 | 7.64 |
| D14 | 0.11 | 0.33 | 0.00 | 0.04 | 0.11 | 0.33 | 9.31 | 78.24 | 0.00 | 0.02 | 1.24 | 14.03 | 88.4 | 124.7 | 6.95 | 9.80 | 1.2 | 3.3 | 7.64 | 8.82 |
| D15 | 0.33 | 0.33 | 0.01 | 0.01 | 0.33 | 0.33 | 78.24 | 78.24 | 0.00 | 0.00 | 1.24 | 1.24 | 91.7 | 91.7 | 6.95 | 6.95 | 3.3 | 3.3 | 7.64 | 7.64 |
| D16 | 0.15 | 0.43 | 0.00 | 0.00 | 0.16 | 0.44 | 9.31 | 17.73 | 0.02 | 0.02 | 3.66 | 15.30 | 90.5 | 106.7 | 6.97 | 8.43 | 1.5 | 2.8 | 8.27 | 8.40 |
| D17 | 0.14 | 0.14 | 0.01 | 0.01 | 0.14 | 0.14 | 15.89 | 15.89 | 0.01 | 0.01 | 1.94 | 1.94 | 90.1 | 90.1 | 7.02 | 7.02 | 2.8 | 2.8 | 8.29 | 8.29 |
| D19 | 0.15 | 0.43 | 0.00 | 0.01 | 0.16 | 0.44 | 9.31 | 78.24 | 0.00 | 0.02 | 1.24 | 15.30 | 90.5 | 106.7 | 6.95 | 8.43 | 1.5 | 3.3 | 7.64 | 8.50 |
| D18 | 0.01 | 1.41 | 0.00 | 0.07 | 0.02 | 1.43 | 3.53 | 78.24 | 0.00 | 0.09 | 0.85 | 174.80 | 57.1 | 148.5 | 4.75 | 11.50 | 0.8 | 3.8 | 7.15 | 8.84 |
| D21 | 0.15 | 1.32 | 0.00 | 0.05 | 0.16 | 1.33 | 9.31 | 78.24 | 0.00 | 0.09 | 1.24 | 110.25 | 59.9 | 151.5 | 4.89 | 11.76 | 0.8 | 3.3 | 7.15 | 9.09 |
| D22 | 0.85 | 0.85 | 0.05 | 0.05 | 0.90 | 0.90 | 10.29 | 10.29 | 0.09 | 0.09 | 54.22 | 54.22 | 151.5 | 151.5 | 11.76 | 11.76 | 0.8 | 0.8 | 9.06 | 9.06 |
| D23 | 0.16 | 0.32 | 0.00 | 0.06 | 0.16 | 0.38 | 11.26 | 23.87 | 0.01 | 0.02 | 11.29 | 15.92 | 86.9 | 97.2 | 7.15 | 8.07 | 1.8 | 2.4 | 7.96 | 7.99 |
| D20 | 0.01 | 1.32 | 0.00 | 0.06 | 0.02 | 1.33 | 3.53 | 44.56 | 0.01 | 0.07 | 3.90 | 110.25 | 59.9 | 148.5 | 4.89 | 11.50 | 0.8 | 2.8 | 7.15 | 8.82 |
| D25 | 0.01 | 0.22 | 0.00 | 0.02 | 0.02 | 0.22 | 3.53 | 19.82 | 0.01 | 0.01 | 2.45 | 3.90 | 100.0 | 109.5 | 8.32 | 8.80 | 2.8 | 3.5 | 8.21 | 8.34 |
| D26 | 0.33 | 0.33 | 0.01 | 0.01 | 0.33 | 0.33 | 78.24 | 78.24 | 0.00 | 0.00 | 1.24 | 1.24 | 91.7 | 91.7 | 6.95 | 6.95 | 3.3 | 3.3 | 7.64 | 7.64 |
| D27 | 0.14 | 0.33 | 0.00 | 0.07 | 0.16 | 0.38 | 11.26 | 78.24 | 0.00 | 0.02 | 1.24 | 15.92 | 57.1 | 103.9 | 4.75 | 8.48 | 1.2 | 3.3 | 7.63 | 8.50 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D30 | 0.01 | 1.41 | 0.00 | 0.07 | 0.02 | 1.43 | 3.53 | 78.24 | 0.00 | 0.09 | 0.85 | 174.80 | 57.1 | 151.5 | 4.75 | 11.76 | 0.8 | 3.4 | 7.15 | 9.09 |
| D31 | 0.11 | 1.78 | 0.00 | 0.07 | 0.11 | 1.78 | 9.31 | 34.25 | 0.01 | 0.08 | 2.93 | 47.01 | 57.1 | 110.0 | 4.75 | 8.62 | 0.9 | 2.8 | 7.15 | 8.50 |
| D32 | 0.01 | 1.78 | 0.00 | 0.07 | 0.02 | 1.78 | 3.53 | 58.41 | 0.00 | 0.09 | 0.85 | 174.80 | 57.1 | 151.5 | 4.75 | 11.76 | 0.8 | 3.5 | 7.15 | 9.09 |

Appendix C. Relative abundance (%) of all of the netplankton diatoms present for 2012.

| 2012 | C1 | C2 | C3 | CI1 | CI2 | CI3 | G1 | G2 | G3 | L1 | L2 | L3 | P1 | P2 | P3 | T1 | T2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D1 | 0.5 | 0.2 | 69.9 | | 4.8 | 11.4 | 0.8 | 2.5 | 59.4 | 0.9 | | | | 1.5 | | | 1.8 |
| D2 | | | 0.2 | | 0.2 | | | | | 0.2 | | | | | | | 7.1 |
| D3 | 0.8 | 4.9 | 6.8 | | 17.0 | 26.5 | 0.5 | 0.8 | | | 0.5 | 7.4 | 2.3 | 3.8 | 4.4 | 5.3 | 8.9 |
| D4 | | | 0.2 | | | | | | | 0.3 | | | | | | | |
| D5 | | | 0.3 | | | | | 0.2 | | 0.5 | | | | | | | 5.4 |
| D6 | | | | | | | | | 1.9 | 0.3 | | | | | | | |
| D7 | | | | | | | | | 0.5 | 0.5 | | | | | | | |
| D8 | 0.2 | | | | | | | | | | | | | | | | |
| D9 | | | | | | 0.4 | | | | | | | | | 0.2 | | |
| D10 | | | 0.2 | | 4.2 | 1.8 | 20.7 | 3.1 | 1.0 | 0.3 | 0.2 | | | 4.0 | 0.9 | | 3.6 |
| D11 | 0.5 | | | | | 0.2 | | 0.8 | | | | | | 0.8 | | | 1.8 |
| D12 | | | | | | | | | | | | | | | | | 3.6 |
| D13 | | | | | | | | | | | | | | | 0.2 | | |
| D14 | | | | | | | | | | | | | | | 0.1 | | 5.4 |
| D15 | | | | | | | | | | | | | | | 0.4 | | |
| D16 | | | | | | | | | | | | | | | | | 1.8 |
| D17 | | | 0.2 | | | | | | | | | | | | | | |
| D18 | 5.2 | 0.3 | 0.5 | 0.5 | 33.3 | 22.0 | 52.3 | 33.6 | 3.9 | 0.8 | | | 18.5 | 14.1 | 44.4 | 9.0 | 5.4 |
| D19 | | | | | | | | | 1.0 | | | | | | 1.9 | 0.2 | 1.8 |
| D20 | | | | | 1.5 | | | | | 0.2 | | | | | | 0.2 | |
| D21 | | | | 0.2 | 0.6 | 0.8 | | 0.2 | | 0.2 | 0.2 | 3.2 | 0.2 | 9.5 | 0.2 | | 1.8 |
| D22 | | | | | | | | | | | | 5.8 | | | | | |
| D23 | | | | | | | | | | 0.2 | | | | | | | |
| D24 | | | | | | | | | | | | | | | 0.4 | | |
| D25 | 0.3 | | | | | | | | | | | | | | | | |
| D26 | | | 0.2 | | | | | | | | | | | | 1.6 | 0.2 | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D27 | | | | | | | | | | | | | | | 0.6 | 0.2 | |
| D30 | | 87.2 | 21.7 | 6.4 | 23.7 | 21.4 | 20.6 | 39.3 | 29.5 | 93.6 | 94.9 | 20.2 | | 54.5 | 11.2 | 0.8 | 10.7 |
| D31 | | | | 0.6 | 2.1 | 0.4 | | | | | | | | | 0.5 | | 10.7 |
| D32 | 92.6 | 7.4 | | 92.3 | 12.7 | 15.2 | 5.0 | 19.5 | 2.9 | 2.2 | 4.3 | 63.5 | 79.0 | 11.8 | 32.9 | 84.3 | 30.4 |

Appendix D. Relative abundance (%) of all of the netplankton diatoms present for 2013

| 2013 | C4 | C5 | C6 | CI4 | CI5 | G4 | G5 | G6 | L4 | L5 | L6 | P4 | P5 | T3 | T4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D1 | 7.2 | 0.2 | 44.0 | 0.9 | | | 14.0 | 5.8 | | 0.9 | 0.8 | | | 0.6 | |
| D2 | | | | | | | | | | | | | | 2.8 | |
| D3 | 0.6 | | 0.9 | 14.8 | 6.7 | | 0.2 | 6.4 | 8.4 | 31.3 | 1.1 | 8.0 | 11.6 | 7.9 | 3.8 |
| D4 | | | | | | | | | | | | 0.5 | 0.3 | | |
| D5 | | 0.2 | 6.0 | | | | | | | | | 0.3 | 0.2 | | |
| D7 | | | 0.5 | | | | | | | | | | | | |
| D9 | | | | | | | | | | 0.2 | | | | | |
| D10 | | | 1.6 | 0.2 | | | | | | | | 0.3 | | 5.1 | |
| D12 | | | 0.5 | | | | | | | 0.2 | | | | | |
| D13 | | | | | | | | | | | | | | 0.8 | |
| D14 | | | | | | | 0.1 | | | | | 0.3 | 0.2 | | |
| D18 | 4.3 | 0.2 | 1.3 | 17.8 | | 0.8 | 10.9 | 32.1 | 0.1 | 2.8 | 6.2 | 47.6 | 69.6 | 14.4 | 0.3 |
| D19 | | | | | | | | | | | | | | 5.1 | |
| D20 | | | | | | | | | | 1.4 | | | | | |
| D21 | | | | | | | | | | 0.2 | | | | 2.0 | |
| D27 | 0.2 | | | | | | | | | | | | | 4.0 | |
| D28 | | | | | | | | | | | | | | | 95.0 |
| D29 | | | | | | | | | | | | | | 0.3 | |
| D30 | 21.3 | | 7.7 | 0.3 | | 40.1 | | 3.6 | | 54.7 | 12.5 | | | 20.4 | |
| D31 | 4.1 | | 1.9 | 0.2 | 6.7 | | | | | | | 0.5 | 3.6 | 2.5 | |
| D32 | 62.3 | 99.5 | 35.6 | 65.9 | 86.7 | 59.1 | 74.7 | 52.1 | 91.5 | 8.3 | 79.3 | 42.6 | 14.5 | 34.0 | 0.9 |

Appendix E. Relative abundance (%) of all of the netplankton diatoms present for 2014

| 2014 | C7 | C8 | C9 | CI6 | CI7 | CI8 | G7 | G8 | L7 | L8 | P7 | P8 | T5 | T6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D1 | | | 0.3 | | 0.6 | 10.4 | 3.4 | 46.1 | 0.8 | 0.2 | | | | |
| D2 | | | | | | | | | | | | | | |
| D3 | 0.3 | 0.2 | 0.3 | 1.6 | 1.7 | 7.8 | | 0.4 | 1.1 | 3.5 | 0.3 | 2.0 | 19.5 | 0.3 |
| D4 | | | | | 1.2 | | | | | | | | | |
| D10 | | 0.3 | | | 1.2 | | | | | | | | 0.2 | |
| D12 | | | | | | | | | | | 0.2 | | | |
| D13 | | | | | | | | | | | | | | |
| D14 | | | | | | | | 2.6 | | | 0.2 | | | |
| D18 | | 0.9 | 0.3 | 22.1 | 14.5 | 74.8 | 79.8 | 10.8 | 6.2 | 2.0 | 49.1 | 6.2 | 0.7 | |
| D20 | | 0.6 | | | | | | 0.6 | | 0.5 | | | | |
| D21 | | | | | | | | | | | | | 0.8 | |
| D23 | | | | | | | | | | | | | 1.0 | |
| D25 | | 0.3 | | 0.3 | | | | | | | | | | |
| D27 | | | | | | | 1.3 | 3.4 | | | 0.2 | | 0.3 | |
| D28 | 34.8 | | | 33.2 | | | | | | | | | | |
| D29 | | | | | | | | | | | | | | |
| D30 | | 92.0 | 98.7 | 1.0 | 4.0 | | 12.1 | 2.5 | 12.5 | 1.7 | 43.1 | 71.2 | 51.8 | 72.5 |
| D31 | | | | | | | | | | | | | | |
| D32 | 64.8 | 5.6 | 0.4 | 41.7 | 76.9 | 7.0 | 3.4 | 33.6 | 79.3 | 92.1 | 7.1 | 20.6 | 25.7 | 27.2 |

Appendix F. Shannon-Weineer (H'), Margalef (R), Carlson's Trophic State Indexes and trophic state.

| | H' | R | CTSI | PTI | TS | **TSI TN (mg/L)** | **TSI TP(mg/L)** | **TSI Chl a (ug/L)** | **TSI Secchi (m)** |
|---|---|---|---|---|---|---|---|---|---|
| C1 | 0.341 | 1.854 | 36 | 2.97 | O | 32 | 36 | 11 | 41 |
| C2 | 0.490 | 1.560 | 36 | 2.85 | O | 37 | 26 | 9 | 45 |
| C3 | 0.859 | 2.319 | 33 | 2.80 | O | 20 | 34 | 12 | 44 |
| C4 | 0.903 | 1.924 | 38 | 2.86 | O | 34 | 36 | 21 | 45 |
| C5 | 0.015 | 1.404 | 37 | 3.00 | O | 32 | 33 | 18 | 46 |
| C6 | 1.386 | 2.325 | 37 | 2.97 | O | 32 | 37 | 20 | 42 |
| CI1 | 0.317 | 1.548 | 56 | 2.98 | E | 54 | 58 | 37 | 57 |
| CI2 | 1.735 | 2.429 | 62 | 2.40 | E | 57 | 67 | 50 | 63 |
| CI3 | 1.022 | 2.413 | 55 | 2.17 | E | 48 | 53 | 51 | 62 |
| CI4 | 0.947 | 1.884 | 52 | 2.55 | E | 47 | 45 | 32 | 62 |
| CI5 | 0.485 | 2.352 | 52 | 0.00 | E | 42 | 52 | 32 | 60 |
| CI6 | 1.196 | 1.921 | 58 | 2.95 | E | 57 | 59 | 39 | 59 |
| G1 | 1.207 | 1.720 | 55 | 2.95 | E | 52 | 54 | 45 | 59 |
| G2 | 1.354 | 2.194 | 43 | 2.98 | M | 38 | 36 | 16 | 54 |
| G3 | 1.089 | 2.438 | 44 | 2.96 | M | 38 | 41 | 10 | 53 |
| G4 | 0.716 | 1.243 | 36 | 2.96 | O | 29 | 38 | 18 | 40 |
| G5 | 0.758 | 1.487 | 36 | 3.00 | O | 20 | 38 | 29 | 51 |
| G6 | 1.165 | 1.724 | 47 | 2.99 | M | 38 | 48 | 27 | 56 |
| L1 | 0.372 | 2.786 | 34 | 2.81 | O | 32 | 27 | 19 | 44 |
| L2 | 0.232 | 1.553 | 55 | 2.99 | E | 45 | 61 | 33 | 60 |
| L3 | 1.078 | 1.576 | 58 | 2.98 | E | 51 | 61 | 40 | 62 |
| L4 | 0.296 | 1.179 | 45 | 2.66 | M | 36 | 48 | 29 | 52 |
| L6 | 0.535 | 2.209 | 45 | 2.75 | M | 38 | 47 | 30 | 51 |
| P1 | 0.594 | 1.400 | 59 | 2.04 | E | 51 | 63 | 39 | 62 |
| P2 | 1.437 | 2.109 | 39 | 2.93 | O | 34 | 35 | 14 | 47 |
| P3 | 1.307 | 2.991 | 37 | 2.68 | O | 32 | 30 | 3 | 50 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| P4 | 1.017 | 2.171 | 33 | 2.83 | O | 30 | 27 | 8 | 42 |
| P5 | 0.944 | 1.886 | 36 | 2.75 | O | 35 | 26 | 20 | 47 |
| P6 | 0.723 | 2.266 | 36 | 0.00 | O | 28 | 33 | 19 | 47 |
| T1 | 2.313 | 2.001 | 48 | 2.84 | M | 42 | 52 | 27 | 50 |
| T2 | 1.962 | 4.969 | 39 | 2.39 | O | 27 | 44 | 18 | 45 |
| T3 | 0.234 | 3.068 | 48 | 2.65 | M | 42 | 49 | 30 | 55 |
| T4 | 0.809 | 1.393 | 46 | 2.89 | M | 39 | 48 | 32 | 52 |
| T5 | 0.588 | 2.025 | 45 | 2.38 | M | 39 | 44 | 27 | 52 |
| T6 | 0.341 | 1.071 | 49 | 2.99 | M | 37 | 54 | 26 | 54 |

H': Shannon-Weaver; R: Margalef; CTSI: Carlson's trophic state index; TSI: Trophic state index; TS: Trophic state; O: Oligotrophic; M: Mesotrophic, E: Eutrophic

Appendix G. Jaccard's similarity between reservoirs

| Sample a | Sample b | Jaccard | Similarity (%) |
|---|---|---|---|
| C | CI | 0.613 | 61.3 |
| C | G | 0.905 | 90.5 |
| C | L | 0.826 | 82.6 |
| C | P | 0.704 | 70.4 |
| C | T | 0.76 | 76 |
| CI | G | 0.824 | 82.4 |
| CI | L | 0.636 | 63.6 |
| CI | P | 0.609 | 60.9 |
| CI | T | 0.609 | 60.9 |
| G | L | 0.556 | 55.6 |
| G | P | 0.476 | 47.6 |
| G | T | 0.435 | 43.5 |
| L | P | 0.64 | 64 |
| L | T | 0.667 | 66.7 |
| P | T | 0.833 | 83.3 |

Appendix H. Jaccard's similarity index between sampling events for each reservoir

| Sample a | Sample b | Jaccard |
|---|---|---|
| C1 | C2 | 0.875 |
| C1 | C3 | 0.538 |
| C1 | C4 | 0.700 |
| C1 | C5 | 0.778 |
| C1 | C6 | 0.636 |
| C2 | C3 | 0.400 |
| C2 | C4 | 0.667 |
| C2 | C5 | 0.667 |
| C2 | C6 | 0.500 |
| C3 | C4 | 0.833 |
| C3 | C5 | 0.909 |
| C3 | C6 | 0.909 |
| C4 | C5 | 0.857 |
| C4 | C6 | 0.600 |
| C5 | C6 | 0.444 |
| CI1 | CI2 | 0.400 |
| CI1 | CI3 | 0.444 |
| CI1 | CI4 | 0.500 |
| CI1 | CI5 | 0.571 |
| CI1 | CI6 | 0.500 |
| CI2 | CI3 | 0.778 |
| CI2 | CI4 | 0.636 |
| CI2 | CI5 | 0.700 |

| | | |
|---|---|---|
| CI2 | CI6 | 0.583 |
| CI3 | CI4 | 0.545 |
| CI3 | CI5 | 0.600 |
| CI3 | CI6 | 0.545 |
| CI4 | CI5 | 0.800 |
| CI4 | CI6 | 0.667 |
| G1 | G2 | 0.800 |
| G1 | G3 | 0.800 |
| G1 | G4 | 0.444 |
| G1 | G5 | 0.667 |
| G1 | G6 | 0.667 |
| G2 | G3 | 0.500 |
| G2 | G4 | 0.333 |
| G2 | G5 | 0.500 |
| G2 | G6 | 0.500 |
| G3 | G4 | 0.400 |
| G3 | G5 | 0.571 |
| G3 | G6 | 0.571 |
| G4 | G5 | 0.778 |
| G4 | G6 | 0.875 |
| L1 | L2 | 0.545 |
| L1 | L3 | 0.545 |
| L1 | L4 | 0.667 |
| L1 | L5 | 0.667 |
| L1 | L6 | 0.500 |
| L2 | L3 | 0.900 |
| L2 | L4 | 0.818 |

| | | |
|---|---|---|
| L2 | L5 | 1.000 |
| L2 | L6 | 0.750 |
| L3 | L4 | 0.556 |
| L3 | L5 | 0.833 |
| L3 | L6 | 0.500 |
| L4 | L5 | 0.857 |
| L4 | L6 | 0.500 |
| L5 | L6 | 0.375 |
| P1 | P2 | 0.500 |
| P1 | P3 | 0.714 |
| P1 | P4 | 0.625 |
| P1 | P5 | 0.909 |
| P1 | P6 | 0.714 |
| P2 | P3 | 0.867 |
| P2 | P4 | 0.722 |
| P2 | P5 | 0.929 |
| P2 | P6 | 0.765 |
| P3 | P4 | 0.667 |
| P3 | P5 | 1.000 |
| P3 | P6 | 0.750 |
| P4 | P5 | 0.889 |
| P4 | P6 | 0.800 |
| P5 | P6 | 0.571 |
| T1 | T2 | 0.286 |
| T1 | T3 | 0.250 |
| T1 | T4 | 0.267 |
| T1 | T5 | 1.000 |

| | | |
|---|---|---|
| T1 | T6 | 0.571 |
| T2 | T3 | 0.778 |
| T2 | T4 | 0.667 |
| T2 | T5 | 1.000 |
| T2 | T6 | 0.933 |
| T3 | T4 | 0.789 |
| T3 | T5 | 0.938 |
| T3 | T6 | 0.938 |
| T4 | T5 | 0.938 |
| T4 | T6 | 0.882 |
| T5 | T6 | 0.375 |

## Appendix I. Freshwater diatoms registered for Puerto Rico.

Table 1. Freshwater diatoms registered for Puerto Rico.

| | Hagelstein 1938 | Foged 1984 | Bryan 2001 | Bryan 2008 | Gualtero 2010 | Viggiano 2014 | Rodríguez 2014 |
|---|---|---|---|---|---|---|---|
| *Achnanthidium affine* (Grunow) Czarnecki | X | | | | | | |
| *Achnanthes biasolettiana* var. *subatomus* Lange-Bertalot | X | | X | | X | | |
| *Achnanthes brevipes* Agardh | | | | X | | | |
| *Achnanthes brevipes* var. *intermedia* (Kützing) Cleve | | X | | | | | |
| *Karayevia clevei* (Grunow) Bukhtiyarova | | | X | | | | |
| *Planothidium conspicuum* (Mayer) E.A.Morales | | | | X | X | | |
| <u>*Psammothidium*</u> *daonense* (Lange-Bertalot) Lange-Bertalot | | | X | X | | | |
| *Achnanthes exigua* Grunow | X | X | | | X | | |
| *Achnanthidium exiguum* var. *heterovalvum* (G.Krasske) D.B.Czarnecki | | X | | | | | |
| *Achnantes holsatica Hustedt* | | | X | | X | | |
| *Achnanthes inflata* (Kutzing) Grunow | X | X | | X | | | |
| *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot | X | X | X | | | | |
| *Planothidium haynaldii* (Schaarschmidt) Lange-Bertalot | X | | | | | | |
| *Achnanthidium biasolettianum* (Grunow) Round & Bukhtiyarova | X | X | | | | | |
| *Achnanthes marginulata* Grunow | | | | | X | | |
| *Achnanthidium minutissimum* (Kützing) Czarnecki | X | X | X | | | | X |
| *Kolbesia ploenensis* (Hustedt) J.C.Kingston | | | | X | X | | |
| *Achnanthes pseudoswazi* J.R. Carter | | | | X | X | | |
| *Achnanthes rupestoides* Holn | | | | X | X | | |
| *Platessa rupestris* (Krasske) Lange-Bertalot | | | | X | | | |
| *Achnanthes* sp. | | | | X | | X | |
| *Planothidium salvadorianum* (Hustedt) Lange-Bertalot | | | X | | X | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Achnanthes subhudsonis* Hustedt | | | | X | | | |
| *Karayevia submarina* (Hustedt) Bukhtiyarova | | | | X | | | |
| *Achnanthes suchlandtii* Hustedt | | | | X | | | |
| *Achnanthes trinodis* (Smith) Grunow | | | | X | | | |
| *Achnanthes* cf *kryptophila** | | | | X | | | |
| *Achnanthes brevipes* var. *intermedia* (Kützing) Cleve | | X | | | | | |
| *Achnanthidium* sp. Kützing | | | | | X | | |
| Achnanthidium pyrenaicum (Hustedt) H.Kobayasi | | | | | X | | |
| *Achnanthidium deflexum* (C.W.Reimer) J.C.Kingstom | | | | X | | | |
| *Achnanthes exigua* Grunow | | | | X | X | | |
| *Achnanthidium minutissimum (*Kutzing) Czarnecki | | | | X | X | | X |
| *Achnanthidium saxonica** | | | | | X | | |
| *Adlafia* sp. Gerd Moser, Lange-Bertalot & Metzeltin | | | | | X | | |
| *Adlafia muscora* (Kociolek & Reviers) Moser, Lange-Bertalot & Metzelin | | | | X | X | | |
| *Amphipleura lindheimeri* (Grunow) | | X | | | | | |
| *Berkeleya rutilans* (Trentepohl ex Roth) Grunow | | | | X | | | |
| *Amphipleura pellucida* Kützing | X | X | | | | | |
| *Entomoneis alata* (Ehrenberg) Ehrenberg | X | | | | | | |
| *Amphora angusta* Gregory | | | | | | | X |
| *Amphora* sp. Ehrenberg | | | | X | X | X | |
| *Halamphora acutiuscula* (Kützing) Levkov | | | | X | | | |
| *Halamphora bullatoides* (Hohn & Hellerman) Levkov | | | | X | | | |
| *Amphora coffeaeformis* (C. Agardh) Kutzing | | | X | X | X | | |
| *Halamphora exigua* (Gregory) Levkov **Syn.** *Amphora exigua* | | | | | | X | |
| *Halamphora fontinalis* (Hustedt) Levkov **Syn.** *Amphora fontinalis* Hustedt | | X | | | | | |
| *Halamphora holsatica* (Hustedt) Levkov **Syn.** *Amphora holsatica* Hustedt | | X | | | | | |
| *Halamphora normanii* (Rabenhorst) Levkov **Syn.** | X | | | | X | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Amphora* cf. *normanii* | | | | | | | |
| *Halamphora montana* (Krasske) Levkov **Syn.** *Amphora montana* Krasske | | | | X | | | |
| *Amphora ovalis* Kützing | X | | | X | | | |
| *Amphora libyca* Ehrenberg | X | X | | | | | |
| *Amphora pediculus* (Kutzing) Grunow ex. A. Schmidt | X | X | | X | X | | |
| *Halamphora sabiniana* (Reimer) Levkov | | | | X | X | | |
| *Halamphora obscura* (Krasske) Levkov | | | | X | | | |
| *Halamphora turgida* (Gregory) Levkov **Syn.** *Amphora turgida* Gregory | | X | | | | | |
| *Halamphora veneta* (Kützing) Levkov **Syn.** *Amphora veneta* Kützing | X | X | | X | X | | |
| *Brachysira brebissonii* R.Ross **Syn.** *Anomoeoneis brachysira* (Brébisson) Cleve | | | | X | X | | |
| *Navicula exilis* Kützing **Syn.** *Anomoeoneis exilis* (Kützing) Cleve | X | X | | | | | |
| *Anomoeoneis exilis* var. *lanceolata* A. Meyer | X | X | | | | | |
| *Brachysira zellensis* (Grunow) Round & Mann | X | X | | | | | |
| *Asterionella formosa* Hassall | X | | | X | X | | |
| *Aulacoseira distans* (Ehrenberg) Simonsen | X | | | X | | | |
| *Aulacoseira granulata* (Ehrenberg) Simonsen | | | | X | | | |
| *Aulacoseira tenuior* (Grunow) Krammer | X | | | X | | | |
| *Bacillaria paxillifera* (O.F.Müller) T. Marsson | X | X | | X | | | |
| *Pleurosira laevis* (Ehrenberg) Compère | X | | | | | | |
| *Caloneis aerophila* Bock | X | | | X | | | |
| *Caloneis aequatorialis* Hustedt | X | X | | | | | |
| *Caloneis* sp. | | | | | X | X | |
| *Caloneis bacillaris* (Gregory) Cleve | X | | | | | | |
| *Caloneis bacillum* (Grunow) Cleve | X | | | X | X | | |
| *Caloneis beccariana* Grunow Cleve | | X | | | | | |
| *Caloneis holstii* Cleve | X | | | | | | |

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|---|---|---|---|---|---|---|---|
| *Caloneis hyalina* Hustedt | X | | | X | | | |
| *Caloneis incognita* Hustedt | X | X | | | | | |
| *Caloneis latiuscula* Kützing | | | | | | | |
| *Caloneis lauta* Carter & Bailey-Watts | X | | | X | | | |
| *Caloneis molaris* (Grunow) Krammer | X | | | X | | | |
| *Caloneis schumanniana* (Grunow) Cleve var. *biconstricta* Grunow | X | X | | | | | |
| *Caloneis limosa* (Kützing) R.M.Patrick | X | X | | | | | |
| *Caloneis silicula* var. *tumida* Hustedtt | | X | | | | | |
| *Caloneis silicula ventricosa* Ehrenberg | X | | | | | | |
| *Caloneis tenuis* (Gregory) Krammer | X | X | | X | | | |
| *Caloneis westii* (W.Smith) Hendey | X | | | | | | |
| Campylodiscus clypeus (Ehrenberg) Ehrenberg ex Kützing | X | | | | | | |
| *Cavinula cocconeiformis* (Gregory ex Greville) Mann & Stickle | X | X | | X | | | |
| *Cavinula lapidosa* (Krasske) Lange-Bertalot | X | X | | X | | | |
| *Cavinula variostriata* (Krasske) Mann & Stickle | X | X | | X | | | |
| *Cocconeis apiculata* (Greville) A.W.F.Schmidt | | | | | | X | |
| *Cocconeis disculus* (Schumann) Cleve | X | X | | X | | | |
| *Cocconeis fluviatilis* Wallace | X | X | | X | | | |
| *Cocconeis tenuissima* var. *laevis* Kützing | X | | | | | | |
| *Cocconeis lagerheimii* Cleve | X | | | | | | |
| *Cocconeis pediculus* Ehrenberg | X | | | X | | | |
| *Cocconeis placentula* Ehrenberg | X | X | X | | X | | X |
| *Cocconeis placentula lineata* (Ehrenberg) Van Heurck | X | | | X | | | |
| *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow | X | X | | X | | | |
| *Cocconeis placentula* var. *intermedia* (Héribaud & Peragallo) Cleve | X | | | X | | | |
| *Cocconeis placentula* var. *ineada* Van Heurck | X | X | | X | X | | |
| *Cocconeis placentula* var. *placentula* Ehrenberg | X | | | X | | | |

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|---|---|---|---|---|---|---|---|
| *Cocconeis pseudolineata* (Geitler) Lange-Bertalot | X | | | X | | | |
| *Cocconeis rugosa* Sovereign | X | | | X | | | |
| *Cocconeis scutellum* var. *parva* (Grunow) Cleve | | | | X | | | |
| *Cocconeis cingulata* F. Hustedt | | | | | | X | |
| *Cocconeis* sp. Ehrenberg | | | | | | X | |
| *Coscionodiscus lacustris* Grunow | | | | X | | | |
| *Craticula accomoda* (Hustedt) Mann | | | | X | | | |
| *Craticula halophila* (Grunow ex Van Heurck) Mann | | | | X | | | |
| *Craticula halophilioides* Hustedt Lange Bertalot | | | | X | | | |
| *Cyclostephanos novae-zeelandiae* (Cleve) Round | | | | X | | | |
| *Cyclotella kuetzingiana* Thwaites | X | | | | | | |
| *Cyclotella meneghiniana* Kützing | X | X | | X | X | | X |
| *Cyclotella* sp. (Kützing) Brébisson | | | | | | X | |
| *Cyclotella striata* (Kützing) Grunow | | X | | | | | |
| *Cymbella aequalis* (W. Smith) | X | | | | | | |
| *Cymbella affinis* Kützing | X | x | | | | | |
| *Cymbella affinis obesa* Cleve | X | | | | | | |
| *Gomphocymbellopsis ancyli* (Cleve) K.Krammer | | | | X | | | |
| *Cymbella* cf. *tropica* Krammer | | | | X | | | |
| *Cymbella cistula* (Hemprich & Ehrenberg) Kirchner | | | | X | | | |
| *Cymbella cistula maculata* (Kützing) Van Heruck | X | | | | | | |
| *Cymbella cymbiformis* Aghard | | | | X | | | |
| *Cymbella coamoensis* Hagelstein | X | | | | | | |
| *Delicata delicatula* (Kützing) K.Krammer | | X | | | | | |
| *Cymbella excise* Kützing | X | | | | | | |
| *Encyonema gracile* Rabenhorst | | X | | | | | |
| *Cymbella helvetiva* Kutzing | X | | | X | | | X |
| *Cymbella Hustedtti* Krasske | | X | | | | | |
| *Cymbella inelegans* Cleve | | | | X | | | |

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|---|---|---|---|---|---|---|---|
| *Cymbella kolbei* var. *angusta* Krammer | | | | X | | | |
| *Cymbella laevis* Naegeli ex Kützing | | X | X | | X | | |
| *Cymbella leptoceros* (Ehrenberg) Kutzing | | | | X | | | |
| *Encyonopsis microcephala* (Grunow) Krammer | | X | | | | | |
| *Cymbella mülleri Hustedt* | | X | | | | | |
| *Cymbella parva* (Smith) Cleve | | | | X | | | |
| *Navicymbula pusilla* (Grunow) K.Krammer | | | | X | | | |
| *Cymbella reinhardtii (*Grunow) K. Krammer | | | | X | | | |
| *Cymbella rupicola* (Grunow) Krammer | | X | | | | | |
| *Cymbella sinuate* Gregory | | | | X | | | |
| *Cymbella* sp. C. Agardh | | | | | | X | |
| *Cymbella subaequalis* Grunow fo. *Krasskei* (Foged) Reimer | | X | | X | | | |
| *Cymbella tumida* (Brébisson ex Kutzing) Van Heruck | X | | X | X | X | | |
| *Cymbella turgida* Gregory | X | X | | | | | |
| *Cymbella turgidula* Grunow | X | | | X | | | |
| *Cymbella ventricosaovata* Kützing | X | X | | | | | |
| *Denticula* sp. Kützing | | | | | X | X | X |
| *Denticula elegans* Kützing | | | | X | | | |
| *Denticula kuetzingii* Grunow | | | X | X | X | | |
| *Denticula occidentalis Østrup* | X | | | | | X | |
| *Denticula subtilis* Grunow | | | | X | | | |
| *Denticula tenuis inflate* (W. Smith) Grunow, Van Heruck | X | | | | | | |
| *Diadesmis brekkaensis* (Krasske) Mann | X | | | X | | | |
| *Diadesmis confervacea* Kützing | X | | X | X | | | |
| *Diadesmis contenta* var. *biceps* (Grunow) Hamilton | X | | | X | | | |
| *Diadesmis contenta* (Grunow ex Van Heurck) D.G.Mann | X | | X | | X | | |
| *Humidophila pantropica* (Lange-Bertalot) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & | X | | | X | | | |

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| Kopalová | | | | | | | |
| *Diadesmis paracontenta* Lange-Bertalor & Werum | X | | | X | | | |
| *Diatoma hyemails* (Roth) Heiberg | X | | | X | | | |
| *Diatoma vulgaris* Bory de Saint-Vincent | X | | | X | | | |
| *Diploneis boldtiana* P. Cleve | X | | | | | | |
| *Diploneis elliptica* Kützing (Cleve) | X | | | | | | |
| *Diploneis fusca* (Gregory) Cleve | | X | | | | | |
| *Diploneis modica* Hustedt | X | | | X | | | |
| *Diploneis oculata* (Brébisson) Cleve | X | X | | X | | | |
| *Diploneis ovalis* (Hilse) Cleve | X | X | | X | | | |
| *Diploneis ovalis oblongella* (Naegeli) Cleve | | X | | | | | |
| *Diploneis pseudovalis* (Hilse) Cleve | X | | | X | | | |
| *Diploneis smithii* (Brébisson) Cleve | X | X | | X | | | |
| *Diploneis smithii* var. *pumila* (Grunow) Hustedtt | | X | | X | | | |
| *Diploneis smithii* var. *recta* M. Perag | | X | | | | | |
| *Diploneis* sp. Ehrenberg ex Cleve | | | | | | X | |
| *Diploneis subovalis* Cleve | X | | | | | | |
| *Discostella stelligera* (Cleve & Grunow) Houk & Klee | X | | | X | | | |
| *Encyonema brehmii* Hustedt Mann | X | | | X | | | |
| *Encyonema elginense* (Krammer) Mann | X | | | X | | | |
| *Encyonema gracile* Rabenhorst | X | | | X | | | |
| *Encyonema muelleri* (Hustedt) Mann | X | | | X | | | |
| *Encyonema perpusillum* (A. Cleve) Mann | X | | | X | | | |
| *Encyonema silesiacum* (Bleisch) Mann | X | | | X | | | |
| *Encyonema* sp. | | | | | X | | |
| *Epithemia muelleri* Fricke | | X | | | | | |
| *Epithemia* sp. Kützing | | | | | X | X | |
| *Epithemia turgida* (Ehrenberg) Kützing | X | | | | | | |
| *Epithemia Zebra Porcellus* (Kützing) | X | | | | | | |

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|---|---|---|---|---|---|---|---|
| *Eucocconeis flexella* (Kutzing) Meister | X | | | X | | | |
| *Eunotia arcus* Ehrenberg | X | X | | X | | | |
| *Eunotia bilunaris* var. *linearis* (Okuno) Lange-Bertalot & Nörpel | X | | | X | | | |
| *Eunotia didyma* Grunow | X | | | | | | |
| *Eunotia exigua* (Brébisson ex Kutzing) Rabenhorst | X | | | X | | | |
| *Eunotia fallax* A. Cleve | X | | | X | | | |
| *Eunotia flexuosa* (Brébisson) Kutzing | X | | | X | | | |
| *Eunotia implicata* Norpel, Lange-Bertalos & Alles | X | | | X | | | |
| *Eunotia incisa* Smith ex Gregory | X | | | X | | | |
| *Eunotia indica* Grunow | X | | | | | | |
| *Eunotia indica* var. *undulata* R.Hagelstein | X | | | | | | |
| *Eunotia intermedia* (Krasske ex Hustedt) Noerpel Lange-Bertalot | | | | X | | | |
| *Eunotia bilunaris* (Ehrenberg) Schaarschmidt **Syn.** *Eunotia lunaris* (Ehrenberg) Grunow | X | | | | | | |
| *Eunotia lunaris* var. *duolineata* R.Hagelstein | X | | | | | | |
| *Eunotia lunaris excisa* (Grunow) Van Heruck | X | | | | | | |
| *Eunotia lunaris* (Ehrenberg) Grunow *subarcuta* (Neag.) Grunow | | X | | | | | |
| *Eunotia minor* (Kutzing) Grunow | | | | X | | | |
| *Eunotia monodon* Ehrenberg | X | | | X | | | |
| *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst **Syn.** *Eunotia paludosa* Grunow | | | | X | | | |
| *Eunotia parallela* Ehrenberg | X | | | | | | |
| *Eunotia parallela ventrales* (Ehrenberg) Grunow | X | | | | | | |
| *Eunotia pectinalis* (Kutzing) Rabenhorst | X | X | | X | | | |
| *Eunotia pectinalis* var. *minor* (Kutzing) Rabenhorst | | | | X | | | |
| *Eunotia pectinalis* var. *ventricosa* (Ehrenberg) Grunow<br><br>**Syn.** *Eunotia pectinalis* var. *ventralis* (Ehrenberg) Hustedtt | | X | | | | | |
| *Eunotia pectinalis undulaae* (Ralfs) | X | | | | | | |

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|---|---|---|---|---|---|---|---|
| *Eunotia pectinalis ventricosa* (Grunow) | X | | | | | | |
| *Eunotia praerupta* Ehrenberg | | X | | | | | |
| *Eunotia rhomboidea* Hustedt | | | | X | | | |
| *Eunotia* sp. Ehrenberg | | | | | X | | |
| *Eunotia steineckii* Petersen | | | | X | | | |
| *Eunotia sudetica* O.F. Muler | | X | | X | | | |
| *Eunotia tenella* (Grunow) Hustedtt | | | | X | | | |
| *Eunotia paludosa* Grunow | | | | X | | | |
| *Karayevia oblongella* (Østrup) M.Aboal | | | | | X | | |
| *Nitzschia capitellata* Hustedt | | | | X | | | |
| *Nitzschia clausii* Hantzsch | X | | X | | X | | |
| *Nitzschia communis* Rabenhorst | | | | X | | | |
| *Nitzschia cummutata* Grunow; Cleve & Grunow | X | | | | | | |
| *Nitzschia dissipata* (Kützing) Grunow | | X | | X | X | | |
| *Nitzschia dissipata* var. *media* (Hantzsch) Grunow | | | | X | | | |
| *Grunowia sinuata* (Thwaites) Rabenhorst | | | | X | | | |
| *Nitzschia filiformis* (W. Smith) Hustedt | | X | | | | | |
| *Nitzschia filiformis* var. *conferta* (Richt) Lange-Bertalot | | | | X | | | |
| *Nitzschia flexa* Schumann | | | | X | | | |
| *Nitzschia flexoides* Geitler | | | | X | | | |
| *Nitzchia fonticola* Grunow | | | | X | X | | |
| *Nitzschia frauenfeldi* (Grunow) Cleve & Grunow | X | | | | | | |
| *Nitzschia frustulum* (Kützing) Grunow | | X | | X | X | | |
| *Nitzschia gandersheimiensis* Krasske sensu Lange-Bertalot & Simonsen | | X | | | | | |
| *Nitzschia hantzschiana* Rabenhorst | | | | X | | | |
| *Nitzschia nana* Grunow **Syn.** *Nitzschia ignorata* Krasske | X | X | | | | | |
| *Nitzschia inconspicua* Grunow | | | X | X | X | | |
| *Nitzschia intermedia* Hantzsch | | | | X | | | |

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|---|---|---|---|---|---|---|---|
| *Nitzschia kitonni* H.L. Smith | X | | | | | | |
| *Nitzschia iebethruthii* Rabenhorst | | | | X | | | |
| *Nitzschia linearis* (Agardh) W. Smith | X | X | | | X | | |
| *Nitzschia linearis tenuis* (W. Smith) Brun | X | | | | | | |
| *Nitzschia incerta* (Grunow) M.Peragallo<br><br>**Syn.** *Nitzchialorenziana* var. i*ncerta* Grunow | X | | | | | | |
| *Nitzchia microcephala* Grunow | | | | X | X | | |
| *Nitzschia modesta* Hustedt | | | | X | | | |
| *Nitzschia nana* Grunow | | | | X | | | |
| *Nitzschia obtusa brevisima* (Grunow) Van Heurck | X | | | | | | |
| *Nitzschia pinuat* var. *scalpelliformis* Grunow | | X | | | | | |
| *Nitzschia palea* (Kützing) | X | X | X | X | X | | X |
| *Nitzschia palea* var. *debilis* (Kützing) Smith | | | | X | | | |
| *Nitzschia fonticola* (Grunow) Grunow **Syn.** *Nitzschia palea fonticola* Grunow; Cleve & Grunow | X | | | | | | |
| *Nitzchia palea* var. *minuta* (Bleisch) | | | | | | | |
| *Nitzschia paleacea* Grunow | | | X | X | X | | |
| *Nitzschia parvula* Lewis | | X | | | | | |
| *Nitzschia perminuta* (Grunow) M.Peragallo **Syn.** *Nitzschia perminutum* (Grunow) M. Peragallo | X | | | | | | |
| *Nitzschia pseudosigma* Hustedt | | X | | | | | |
| *Nitzschia pura* Hustedt | | | | X | | | |
| *Nitzschia pusila* Grunow | | | | X | | | |
| *Nitzschia recta* Hantzsch | X | | | X | | | |
| *Nitzschia robusta* Hustedtt | | X | | | | | |
| *Nitzschia scalpeliformis* (Grunow) Grunow | | | | X | | | |
| *Nitzschia sigma* (Kützing) W. Smith | | X | | X | | | |
| *Nitzschia sigma rigidula* (Grunow) Van Heruck | X | | | | | | |
| *Nitzschia sigmoidea armoricana* (Kützing) Grunow; Cleve & Grunow | X | | | | | | |

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|---|---|---|---|---|---|---|---|
| *Nitzschia sociabilis* Hustedt | | | | X | | | |
| *Nitzschia solita* Hustedt | | | | X | | | |
| *Nitzschia* sp. Hassall | | | | | | X | |
| *Nitzschia subacicularis* Hustedt | | | | X | | | |
| *Nitzschia subcohaerens* var. *scotica* (Grunow) Van Heruck | | | | X | | | |
| *Nitzschia vitrea var. subvitrea* (Hustedt) E.J.F.Wood<br>**Syn.** *Nitzschia subvitrea* Hustedt | | X | | | | | |
| *Nitzschia suchlandi* Hustedt | | | | X | | | |
| *Nitzschia supralitorea* Lange-Bertalot | | | | X | | | |
| *Nitzchia terrestris* (Peterson) Hustedtt | | | | | X | | |
| *Nitzschia thermalis* (Kützing) Auersw. | X | | | | | | |
| *Nitzschia thermalis minor* (Hilse) Rabenhorst | X | | | | | | |
| *Nitzscia tropica* Hustedt | | | | X | | | |
| *Nitzschia tryblionella* Hantzsch; Rabenhorst | X | | | | | | |
| *Nitzschia umbonata* (Ehrenberg) Lange-Bertalot **Syn.** *Nitzschia stagnorum* Rabenhorst | | | | X | | | |
| *Nitzschia valdecostata* Lange-Bertalot | | | | X | | | |
| *Nitzschia vermicularis* (Kützing) Hantzsch | | | | X | | | |
| *Nitzschia vitrea* Norman | X | | | | | | |
| *Nitzschia vitrea* var. *salinarum* Grunow | X | X | | | | | |
| *Nupela* sp. Vyverman & Compere | | | | | X | | |
| *Orthoseira* sp. Thwaites | | | | X | | | |
| *Pinnularia acrosphaeria* (Brébisson) Cleve | X | X | | X | | | |
| *Pinnularia acuminate* Smith | | | | X | | | |
| *Pinnularia appendiculata* (Agardh) Cleve | X | | | X | | | |
| *Pinnularia appendiculata budensis* (Grunow) Cleve | X | | | | | | |
| *Pinnularia borealis* Ehrenbergh | X | X | | | | | |
| *Pinnularia borealis scalaris* (Ehrenberg) Cleve | X | | | | | | |
| *Pinnularia brauniana* (Gruno ex Schmidt) Cleve | | | | X | | | |

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| *Pinnularia brevicostata* Cleve | | X | | | | | |
| *Pinnularia* cf. *marchica* | | | | X | | | |
| *Pinnularia distinguenda* Cleve | X | | | | | | |
| *Pinnularia divergens* W. Smith | | X | | X | | | |
| *Pinnularia divergentissima* (Grunow) Cleve | | | | X | | | |
| *Pinnularia subrostrata* (A.Cleve) Cleve-Euler<br>**Syn.** *Pinnularia divergentissima* var. *subrostrata* A. Cleve | | | | X | | | |
| *Pinnularia dubitabilis* Hustedt | | | | X | | | |
| *Pinnularia clevei* var. *minor* (Hustedt) K.Krammer<br>**Syn.** *Pinnularia esox* Cleve | X | | | | | | |
| *Pinnularia floridae* Brun | X | | | | | | |
| *Pinnularia gentilis* (Donk.) Cleve | | X | | | | | |
| *Pinnularia gibba* Ehrenberg | X | X | | X | | | |
| *Pinnularia gracillima* Gregory | | X | | | | | |
| *Pinnularia graciloides Hustedt* | | X | | | | | |
| *Pinnularia* cf. *globiceps* | | | | | X | | |
| *Pinnularia isostauron* (Ehrenberg) Cleve<br>**Syn.** *Pinnularia micostauron* (Grunow) Cleve | X | | | | | | |
| *Pinnularia infirma* Krammer | X | | | | | | |
| *Pinnularia intermedia* (Lagerstedt) Cleve | | | | X | | | |
| *Pinnularia interrupta* fo. *minutissima* Hustedt | | X | | | | | |
| *Pinnularia joculata* (Manguin) Krammer | | | | X | | | |
| *Pinnularia krockii* (Grunow) Cleve | | X | | | | | |
| *Pinnularia latarea* Krammer | | | | X | | | |
| *Pinnularia latevittata domingensis* Cleve | X | | | | | | |
| *Pinnularia latevittata minor* | X | | | | | | |
| *Pinnularia legumen* Ehrenberg | | X | | | | | |
| *Pinnularia lundii* Hustedt | | | | X | | | |
| *Pinnularia marchica* Schonfelder | | | | X | | | |

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| *Pinnularia mayeri* K. Krammer **Syn.** *Pinnularia braunii* (Grunow) Cleve var. *amphicephala* (A. Meyer) Hustedt | | X | | | | | |
| *Pinnularia microstauron* (Ehrenberg) Cleve<br><br>**Syn.** *Pinnularia gibba* var. *parva* (Ehrenbergh) Grunow | | | | | | | X |
| *Pinnularia molaris* (Grunow) Cleve | X | | | | | | |
| *Pinnularia neomajor* var. *intermedia* (Cleve) K.Krammer<br><br>**Syn.** *Pinnularia viridis intermedia* Cleve | X | | | | | | |
| *Pinnularia nodosa* (Ehrenberg) Smith | | | | X | | | |
| *Pinnularia obscura* Krasske | | | | X | | | |
| *Pinnularia ovata* K.Krammer<br><br>**Syn.** *Pinnularia divergens elliptica* (Grunow) Cleve | | X | | | | | |
| *Pinnularia parva*(Gregory) | X | | | | | | |
| *Pinnularia procera* | X | | | | | | |
| *Pinnularia pseudoparva* K.Krammer & Lange-Bertalot<br><br>**Syn.** *Navicula parvula* Ralfs | | | X | | | | |
| *Pinnularia rangoonensis* (Grunow) Cleve | X | | | | | | |
| *Pinnularia salinarum* | | X | | | | | |
| *Pinnularia salinarum* var. *boyeri* | | X | | | | | |
| *Pinnularia schroederi* (Hustedt) Krammer | | | | X | | | |
| *Pinnularia septentrionalis* K.Krammer **Syn.** *Pinnularia mesolepta stauroneiformis* (Grunow) Cleve | X | | | | | | |
| *Pinnularia sinistra* K. Krammer | | | | X | | | |
| *Pinnularia* sp. | | | | | | X | |
| *Pinnularia stauroptera interrupta* (Grunow) Cleve | X | | | | | | |
| *Pinnularia stomatophara* (Grunow) Cleve | | X | | X | | | |
| *Pinnularia subcapitata* Gregory | X | X | | X | | | |
| *Pinnularia subgibba* | | | | | X | | |
| *Pinnularia subrostrata* (A. Cleve) Cleve-Euler | | | | X | | | |
| *Pinnularia sudetica* (Hilse) Hilse | | | | X | | | |
| *Pinnularia titusiana* | X | | | | | | |

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|---|---|---|---|---|---|---|---|
| *Pinnularia tropica Hustedt* | | X | | | | | |
| *Pinnularia viridis* (Nitzsch) Ehrenbergh | X | X | | X | X | | |
| *Pinnularia viridis subconstricta* | X | | | | | | |
| *Placoneis* sp. | | | | | X | | |
| Placoneis elginensis (Gregory) E.J.Cox<br><br>**Syn.** *Navicula elginensis* (Gregory) Ralfs | | | | | | X | |
| *Placoneis gastrum* (Ehrenberg) Mereschkovsky<br><br>**Syn.** *Navicula gastrum* (Ehrenbergh) Kützing | X | | | | | | |
| *Planothidium conspicuum* (Mayer) E.A.Morales<br><br>**Syn.** *Achnanthes conspicua* Mayer | | | X | X | | | |
| *Planothidium frecuentissimum* (Lange Bertalot) Round & Bukhtiyarova | | | | X | | | |
| *Planothidium lanceolata* (Brébisson) Lange-Bertalot | | | | | X | | |
| *Planothidium robustis* Lange-Bertalot | | | | X | | | |
| *Planothidium salvadorianum Hustedt*t Lange-Bertalot<br><br>**Syn.** *Achnantes salvadoriana* | | | | X | | | |
| *Platessa rupestris* (Krasske) Lange-Bertalot<br><br>**Syn.** *Achnanthes rupestris* Krasske | | | | | X | | |
| *Pleurosigma salinarum* Grunow (Grunow) | | X | | X | | | |
| *Pleurosigma salinarum* var. *boyeri* (Keely) Reimer | | X | | | | | |
| *Pleurosigma subsalsum* Wilslouch & Kolbe | | X | | | | | |
| *Pleurosigma* sp. | | | | | | X | |
| *Pleurosira laevis* (Ehrenberg) Compere | | | | X | | | |
| *Punctastriata* sp. | | | | X | | | |
| *Rhoicosphenia* sp. | | | | | X | | |
| *Rhopalodia acuminata* Krammer | | | | X | | | |
| *Rhopalodia brebissonii* Krammer | | | | X | | | |
| *Rhopalodia curvata* (Kutzing) Grunow ex Rabenhorst | | | | X | | | |
| *Rhopalodia gibba* (Ehrenberg) O. Muller | X | X | | X | | | X |

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|---|---|---|---|---|---|---|---|
| *Rhopalodia gibba var. parallela* (Grunow) H.Peragallo & M.Peragallo **Syn.** *Rhopalodia parallela* (Grunow) O. Muller | X | X | | | | | |
| *Rhopalodia gibba var. ventricosa* (Kützing) H.Peragallo & M.Peragallo **Syn.** *Rhopalodia gibba* var. *ventricosa* (Ehrenberg) Grunow | X | X | | | | | |
| *Rhopalodia gibberula* (Ehrenbergh) O. Muller | | X | | X | | | |
| *Rhopalodia gibberula argentina* | X | | | | | | |
| *Rhopalodia gibberula producta* (Grunow) Fricke | X | | | | | | |
| *Rhopalodia musculus* (Kutzing) O Muller | | X | | | | | |
| *Rhopalodia operculata* (Agardh) Hak | | | | X | | | |
| *Rhopalodia ventricosa* (Kützing) O. Muller | X | | | | | | |
| *Rhaphoneis* sp. | | | | | | X | |
| *Rossithidium pusilum* (Grunow) Round & Bukhtiyarova | | | | X | | | |
| *Schizonema domingense* Kuntze **Syn.** *Pinnularia latevittata* Cleve | X | | X | | | | |
| *Sellaphora bacillum* (Ehrenberg) Mann | | | | X | | | |
| *Sellaphora laterostrata* Metzeltin & Lange-Bertalot | | | | X | | | |
| *Sellaphora pupula* (Kutzing) Mereschkovsky<br><br>**Syn.** *Navicula pupula* Kützing | | | | X | | | |
| *Sellaphora pupula* var. *capitata* (Skvortzov & K.I.Meyer) Poulin **Syn.** *Navicula pupula* fo. *Capitata* Skvortzow & Meyer | | X | | | | | X |
| *Sellaphora rectangularis* (Gregory) Lange-Bertalot & Metzeltin<br><br>**Syn.** *Navicula pupula* fo. *rectangularis* (Gregory) Grunow | | X | | | | | |
| *Sellaphora seminulum* (Grunow) Mann<br><br>**Syn.** *Navicula seminulum* Grunow | | | | X | | | |
| *Sellaphora wittrockii* (Lagerstedt) Lange-Bertalot & D.Metzeltin **Syn.** *Navicula wittrockii* (Lagerst) Cleve Euler | | | | | | | |
| *Seminavis* sp. | | | | X | | | |
| *Stauroneis agrestis* Petersen | | X | | | | | |

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|---|---|---|---|---|---|---|---|
| *Stauroneis anceps* Ehrenberg **Syn.** *Stauroneis anceps* Ehrnbergh fo. *gracilis* Rabenhorst | X | X | | X | | | |
| *Stauroneis anceps* fo. *robusta* | | X | | | | | |
| *Stauroneis hannae* Patrick and Freese | | X | | | | | |
| *Stauroneis lundii* Hustedt | | | | X | | | |
| *Sellaphora nana* (Hustedt) Lange-Bertalot, Cavacini, Tagliaventi & Alfinito **Syn.** *Stauroneis nana* Hustedt | | | | X | | | |
| *Stauroneis pachycephala* Cleve | | X | | | | | |
| *Stauroneis phoenicentron* (Nitzsch) Ehrenbergh | | X | | | | | |
| *Stauroneis phoenicentron amphilepta* (Ehrenbergh) Cleve | X | | | | | | |
| *Stauroneis smithii* Grunow | | | | X | | | |
| *Stauroneis* sp. | | | | X | X | X | |
| *Stenopterobia delicatissima* (F.W.Lewis) Brébisson ex van Heurck **Syn.** *Surirella delicatissima* Lewis | | X | | | | | |
| *Surirella amphioxys* W. Smith var. *alaskaensis* Foged | | X | | | | | |
| *Surirella brebissonii* Krammer & Lange-Bertalot | | | | X | | | |
| *Surirella* cf. *angusta* Kützing | | | | | X | | |
| *Surirella inducta* (A. Schmidt) Atlas | X | | | | | | |
| *Surirella linearis* Smith | | | | X | | | |
| *Surirella linearis* Smith var. *constricta* | | X | | | | | |
| *Surirella* sp. | | | | | X | | |
| *Surirella splendida* (Ehrenberg) Kützing | X | | | | | | |
| *Surirella splendida constricta Hustedt*t A. Schmidt | X | | | | | | |
| *Surirella splendida minima* Ostrup, Meddel. Gronl. | X | | | | | | |
| *Surirella tenera* W.Gregory<br><br>**Syn.** *Surirella robusta* Ehrenberg | X | X | X | | | | X |
| *Synedra amphiryncus* Ehrenberg | | | | X | | | |
| *Synedra delicatissima mesoleia* Grunow | X | | | | | | |
| *Synedra famelica* Kützing | | | | X | | | |
| *Synedra filiformis* Carter & Denny | | | | X | | | |

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|---|---|---|---|---|---|---|---|
| *Synedra goulardi* (Brébissoni) Cleve & Grunow | X | | | | | | |
| *Synedra goulardi elongate* M. Perag | X | | | | | | |
| *Synedra* cf. *inequalis* | | | | | X | | |
| *Synedra minuscule* Grunow | | | | X | | | |
| *Synedra oxyrhynchus* | X | | | | | | |
| *Synedra oxyrhynchus undulata* | X | | | | | | |
| *Synedra tabulata* var. *Fasciculata* Kützing Grunow | | X | | | | | |
| *Synedra rumpens* Grunow | | | | | X | | |
| *Synedra rumpens* var *fragilaroides* | | | X | | | | |
| *Sunedra* sp. | | | | | | X | X |
| *Synedra socia* Wallace | | | | X | | | |
| *Synedra ulna* (Nitzsch) Ehrenberg | | | X | | X | | |
| *Synedra ulna lanceolata* | X | | | | | | |
| *Synedra ulna subaequalis* Grunow | X | | | X | | | |
| *Synedra ulna* var. *aequalis* (Kützing) Hustedt | | X | | | | | |
| *Synedra ulna* var. *biceps* (Kützing) von Schonfeldt | | X | | X | | | |
| *Synedra ulna* var. *danica* (Kützing) Grunow | | | | X | | | |
| *Synedra ulna* var. *lanceolata* Grunow | | X | | | | | |
| *Synedra vitrea* | X | | | | | | |
| *Tabellaria flocculosa* (Rabh) Kützing | | X | | | | | |
| *Tabularia tabulata* (Agardh) Snoeijs | | | | X | | | |
| *Terpsinoë musica* Ehrenbergh | X | X | | X | | | |
| *Thalasiosira weissflogii* (Grunow) Fryxell & Hasle | | | | X | | | |
| *Trybionella* sp. | | | | | X | | |
| *Trybionella acuminatum* Smith | | | | X | | | |
| *Trybionella apiculata* Gregory<br>**Syn.** *Nitzschia apiculata* (Gregory) Grunow | | | | X | | | |
| *Trybionella coarctata* (Grunow) Mann | | | | X | | | |
| *Trybionella compressa* (Bailey) Poulin | | | | X | | | |

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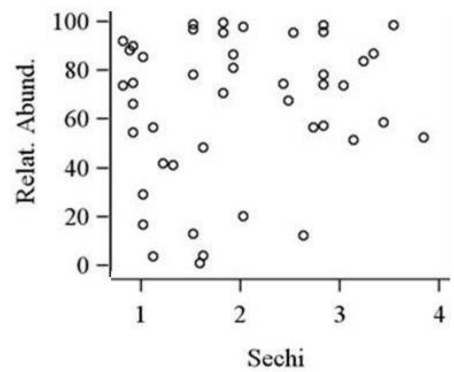
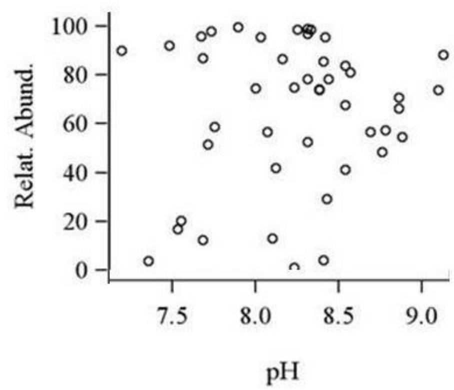
|---|---|---|---|---|---|---|---|
| *Trybionella constricta* Gregory | | | | X | | | |
| *Trybionella debilis* Arnott<br><br>**Syn.** *Nitzschia debilis* (Arnott) Grunow; Cleve & Grunow | | | | X | | | |
| *Tryblionella gracilis* var. *subsalina* (O'Meara) M.Aboal<br><br>**Syn.** *Nitzschia tryblionella subsalina* (O'Meara) Grunow; Cleve & Grunow | X | | | | | | |
| *Tryblionella hungarica* (Grunow) Frenguelli<br><br>**Syn.** *Nitzschia hungarica* Grunow | X | X | | | | | |
| *Tryblionella levidensis* W.Smith<br><br>**Syn.** *Nitzschia tryblionella levidensis* (W. Smith) Grunow, Cleve & Grunow | X | | | | | | |
| *Tryblionella littoralis* (Grunow) D.G.Mann<br><br>**Syn.** *Nitzschia littoralis* Grunow; Cleve & Grunow | X | | | | | | |
| *Tryblionella scalaris* (Ehrenberg) P.Siver & P.B.Hamilton<br><br>**Syn.** *Nitzschia scalaris* (Ehrenberg) W. Smith | X | X | | | | | |
| *Tryblionella victoriae* Grunow<br><br>**Syn.** *Nitzschia levidensis* (W. Smith) Grunow | | X | | | | | |
| *Ulnaria* sp. | | | | | | X | X |
| *Ulnaria acus* (Kützing) M.Aboal<br><br>**Syn.** *Synedra acus* Kützing | X | X | | X | | | X |
| *Ulnaria biceps* (Kützing) P.Compère<br><br>**Syn.** *Fragilaria biceps* | | | X | | X | | X |
| *Ulnaria danica* (Kützing) Compère & Bukhtiyarova<br><br>**Syn.** *Synedra danica* Kützing | X | | | | | | |

*Taxa not found in databases: WoRMs or Algaebase

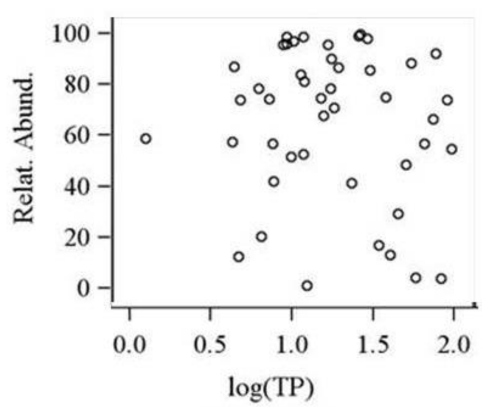
Appendix J. Correlation between diatom abundance in each sampling event and Secchi depth(m).



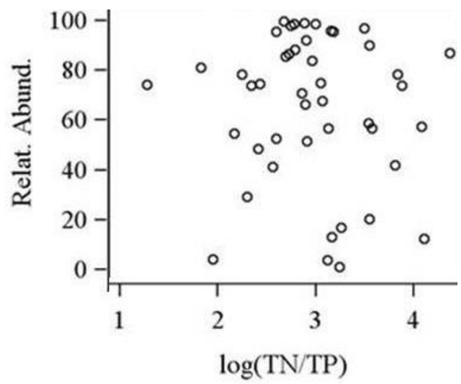

Appendix K. Correlation between diatom abundance in each sampling event and pH.



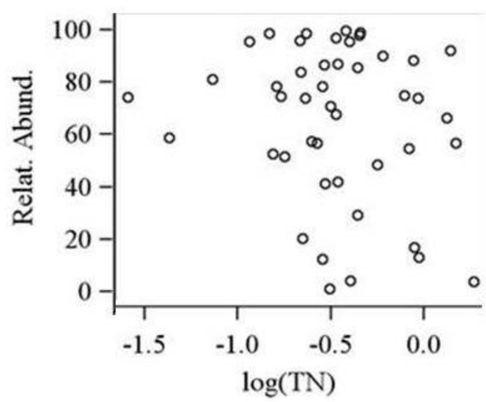

Appendix L. Correlation between diatom abundance in each sampling event and log(TP).



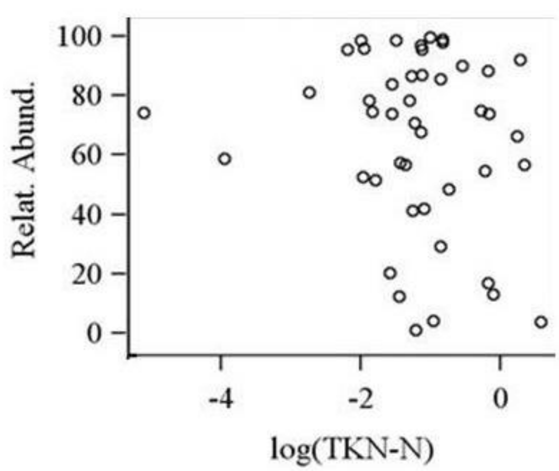
Appendix M. Correlation between diatom abundance in each sampling event and log (TN/TP).



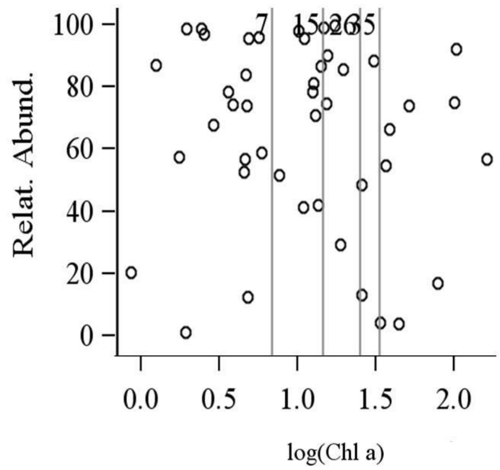

Appendix N. Correlation between diatom abundance in each sampling event and log(TN).



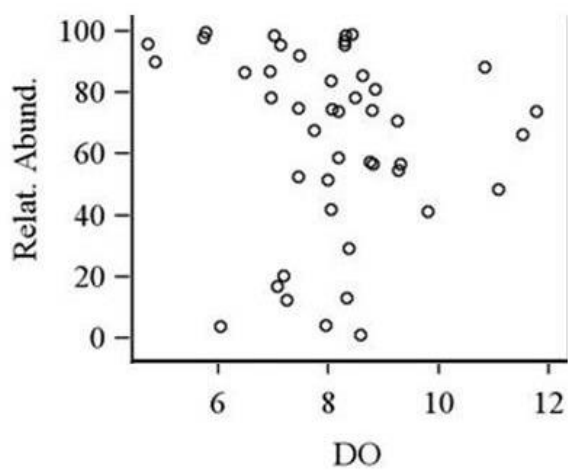

Appendix O. Correlation between diatom abundance in each sampling event and log(TKN-N)



Appendix P. Correlation between diatom abundance in each sampling event and log(CHl a).

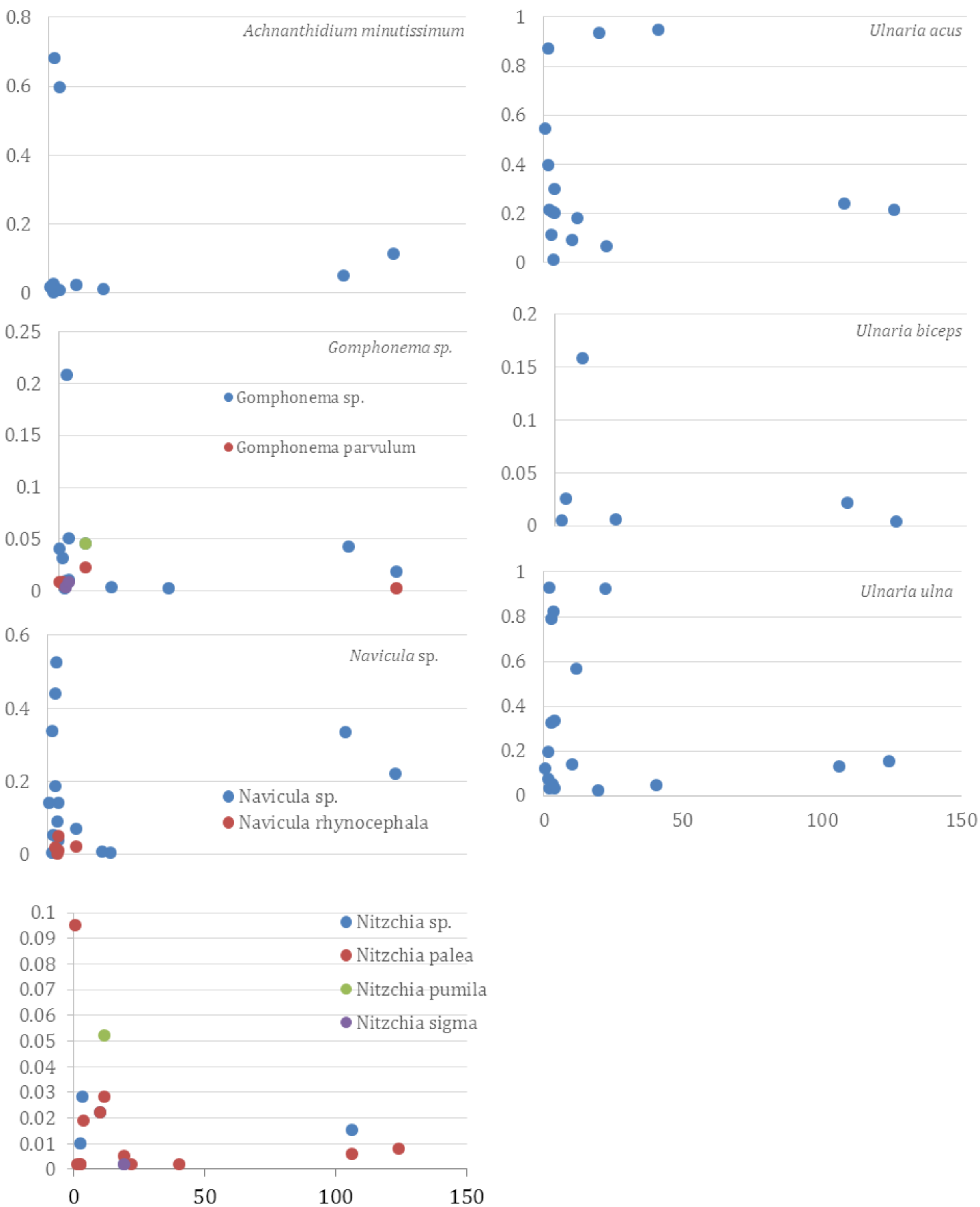



Appendix Q. Correlation between diatom abundance in each sampling event and Dissolved Oxygen.

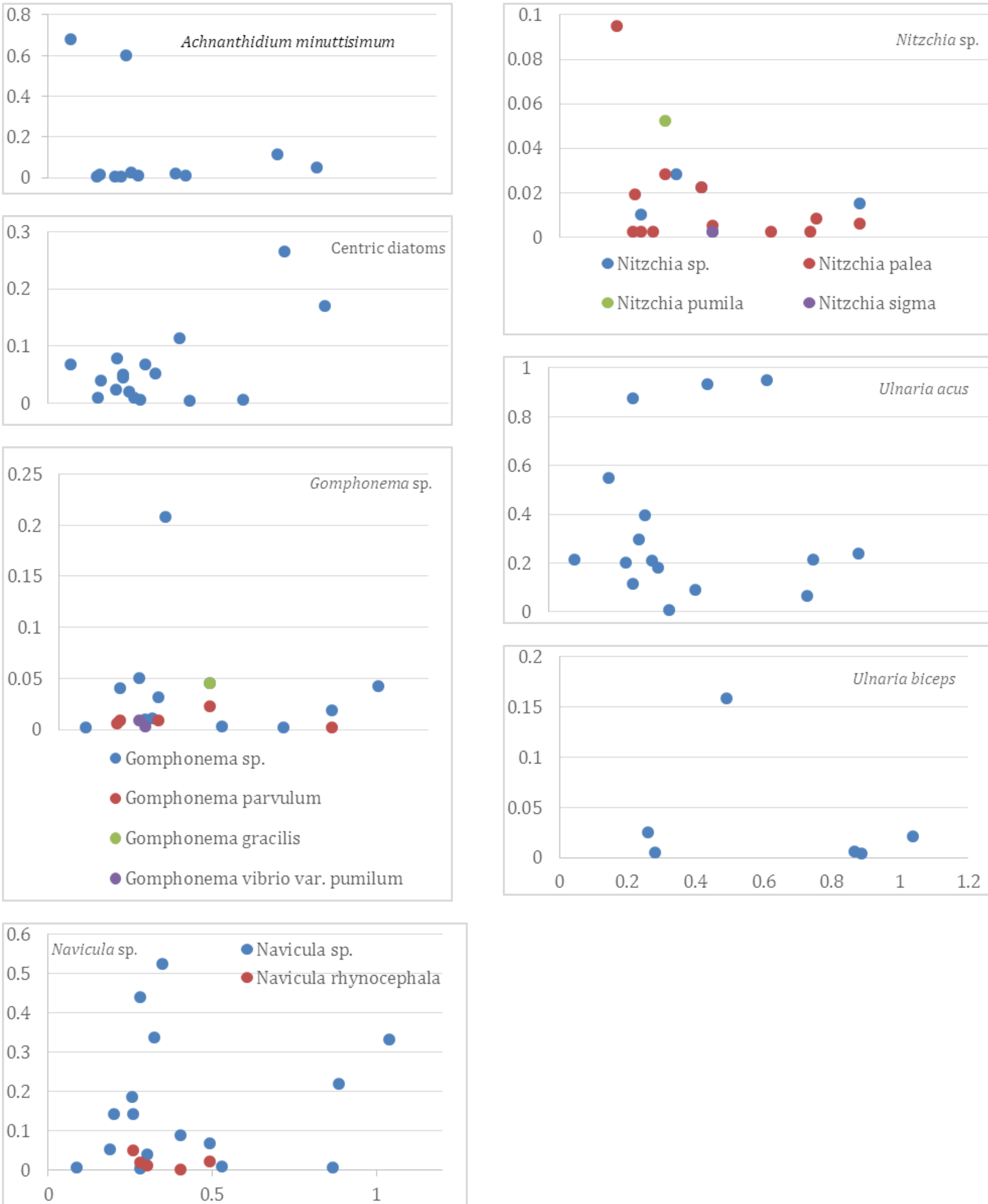


**Preliminary data from the first year of sampling**

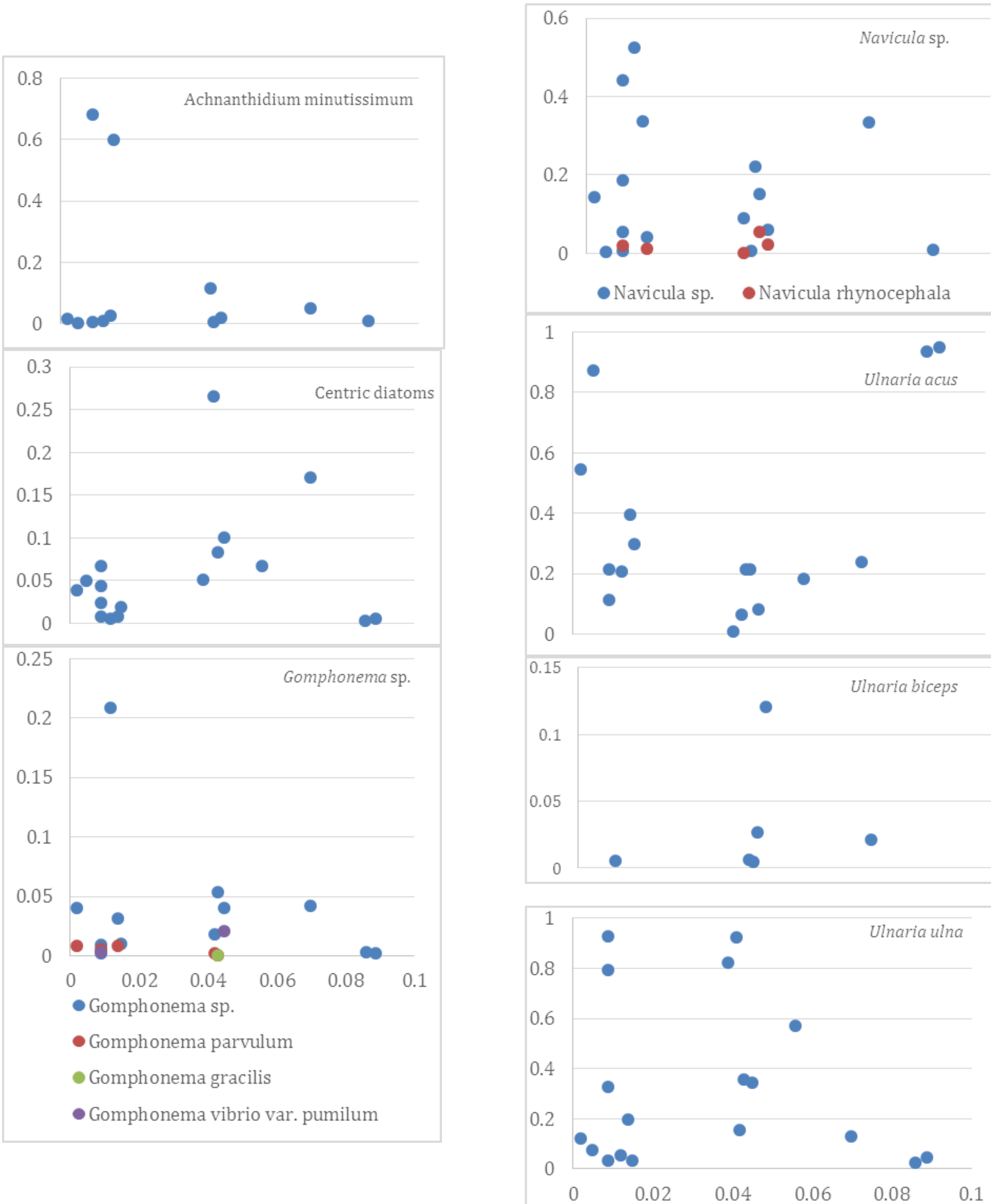
Appendix R. Relationship between relative abundance of common species and Chlorophyll A µg/L for the first year of sampling



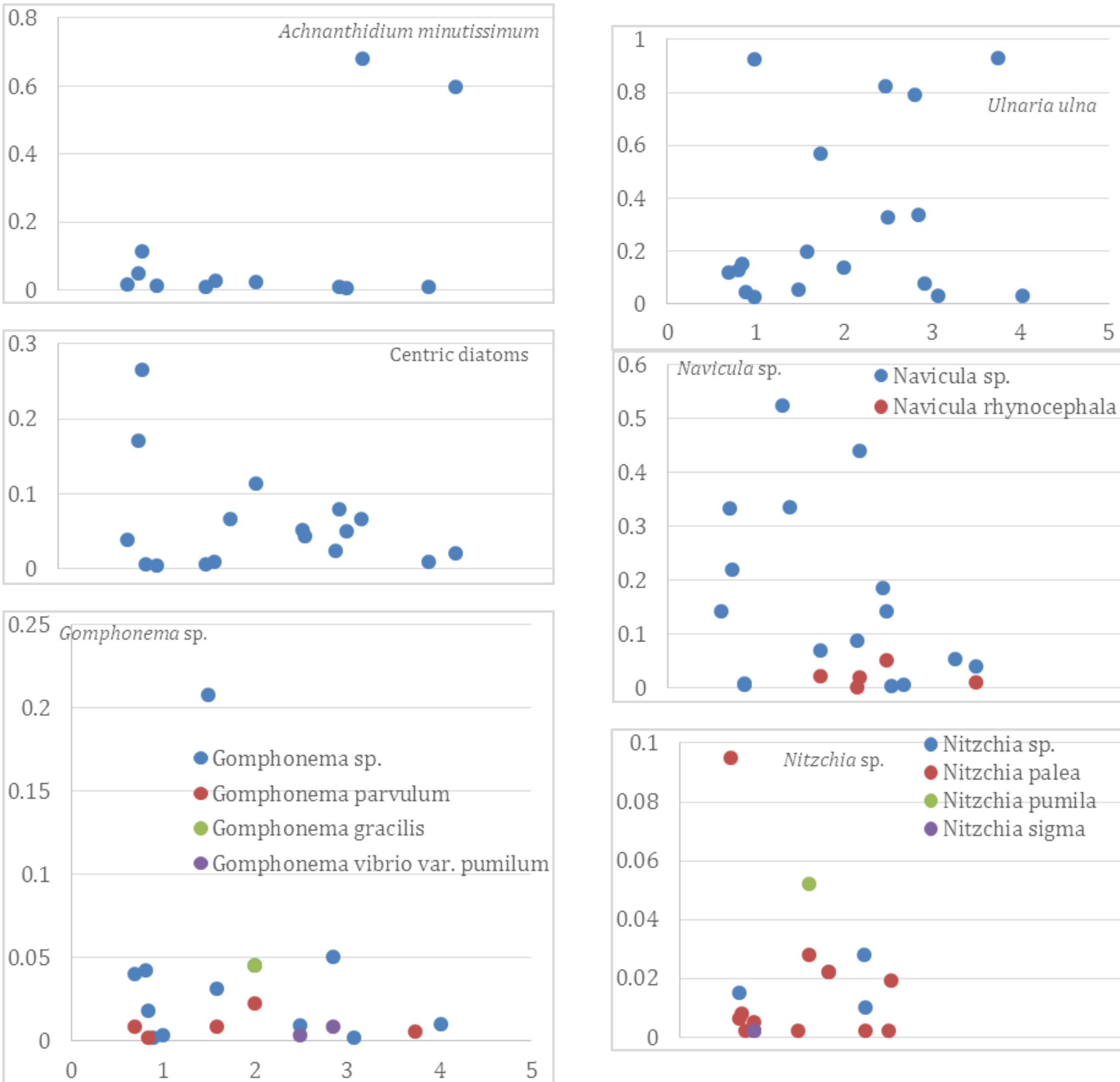
Appendix S. Relationship between relative abundance of common species and Total Nitrogen mg/L for the first year of sampling.



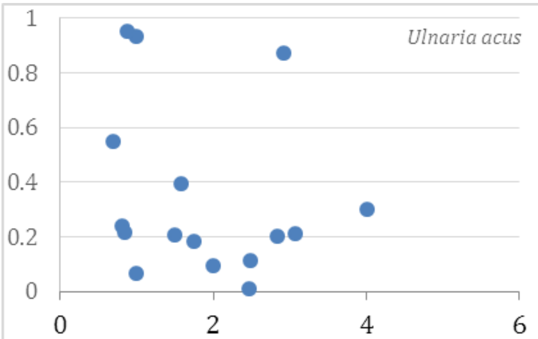
Appendix T. Relationship between relative abundance of common species and Total Phosphorous mg/L for the first year of sampling.

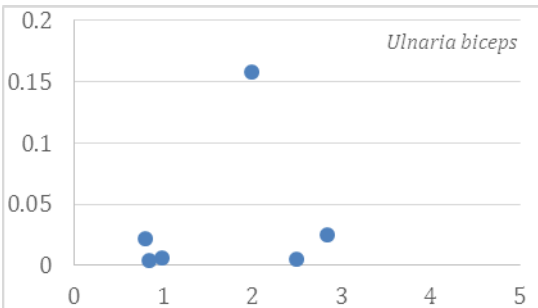


Appendix U. Relationship between relative abundance of common species and Secchi Disc Transparency for the first year of sampling



Appendix R. Relationship between relative abundance of common species and Chlorophyll A µg/L for the first year of sampling



Appendix V. Diatom taxa relative abundance for the first year of sampling.