University of Puerto Rico
College of Agricultural Sciences
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NUTRIENT LEVELS ASSOCIATED WITH ECOLOGICAL THRESHOLDS OF IMPAIRMENT IN RESERVOIRS OF PUERTO RICO
PREQB CONTRACT NO: 2012-000022

FINAL PROJECT REPORT
ENCOMPASSING PERIOD FROM
DECEMBER 15TH, 2011 TO SEPTEMBER 30TH, 2014

Project Leader

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1.0 Executive Summary

Twenty five samplings events were performed (twenty four at Toa Vaca) on five reservoirs of Puerto Rico. Median TN, TP and chlorophyll *a* (Chl-*a*) concentrations for surface samples (1m depth) for all reservoirs combined are: 0.33 mg/L, 17.0 µg /L, and 9.76µg/L, respectively. Cidra, and La Plata reservoirs exhibited significantly higher concentrations of nutrients and Chl-*a* than the other reservoirs. On the other hand, Cerrillos and Patillas exemplify reservoirs closer to reference conditions, and statistically placed at the bottom of the reservoir list in both nutrients and Chl-a concentrations. Toa Vaca and Guajataca embody reservoirs at a midpoint of the productivity spectrum. As expected, reservoirs with higher nutrient and productivity status (i.e., Cidra, La Plata) have significantly lower depth of light penetration (i.e., Secchi depth) than reservoirs at the lower end of the nutrient and Chl-*a* spectrum (i.e., Patillas, Cerrillos). Pearson correlation coefficients for the complete data set showed strong positive correlations between TN and Chl-*a* (r = 0.65), and TP and Chl-*a* (r = 0.73). Nutrient numeric criteria were established by evaluating the relationship between nutrients (i.e., TN and TP) and different ecological thresholds of impairement. A Chl-*a* concentration of 24 µg/L was selected as our impairment threshold based on evidence demonstrating that the extent of light penetration was significantly reduced at said Chl-*a* level (due to excessive phytoplankton biomass productivity), hindering in turn the reservoirs' capacity to comply with the aquatic life criteria. A least squares method of change-point analysis was used to determine total nitrogen and total phosphorous concentrations associated with our impairment threshold (i.e., 24 µg/L Chl-*a*). The resulting values, 0.035 mg/L for TP and 0.43 mg/L for TN, can be considered as nutrient thresholds associated with impairment. To protect against impairment we incorporated a margin of safety based on the concentrations defining the lower 5% confidence interval of the bootstrap distribution of values associated with our Chl-*a* impairment threshold (i.e., P(05)-one sided tail). **The results: 0.026 mg/L for TP and 0.41mg/L for TN, are proposed as numeric criteria for establishing the nutrient standards for reservoirs of Puerto Rico**.

A basic chemical composition analysis was performed on reservoir samples to evaluate the distribution of several elements that are relevant to primary productivity and/or potable water purposes. Differences in concentrations of major cations (i.e., Ca, Fe, Al, Mn) between reservoirs could in most cases be attributed to the lithology of their respective basins. In general, total aluminum, iron and manganese concentrations increased with depth and were negatively correlated with pH, dissolved oxygen and oxidation reduction potential values. Said result emphasizes the role of reductive dissolution and mineralization reactions on the fate of different elements in nature.

The net-sized phytoplankton species of the reservoirs were quantified and characterized. These were distributed within six phyla: Cyanobacteria, Charophyta, Chlorophyta, Euglenozoa, Myzozoa, and Ochrophyta. Diatoms were included as part of the Ochrophyta in the phytoplankton quantitative analyses (as live pennate, empty pennate,

live centric and empty centric). The dominant organisms across all the samplings were the dinoflagellate *Peridiniopsis* sp., found in all rserevoirs, followed by the chlorophyceans *Tetraedron minimum* and *Pediastrum simplex*. Patterns of phytoplankton diversity were site specific. A triplot analysis of the phytoplankton functional groups (G1-G7), reservoirs and water quality parameters was conducted for year one (2012) data-set. The analysis nearly explained 60% of the variation (sum of both axes), and separated the six reservoirs into two groups, with three members each. The first group of reservoirs was typified as dominated by unicellular flagellates (functional group 5). Meanwhile group 2 (G2), mainly composed of chrysophytes, related positively to nitrate, and showed strong negative relationships with TN, TP, oxygen and chl-a, as predicted by a previous model.

A detailed diatom survey was also conducted for years 2012 and 2013. Dominant taxa present in oligotrophic (based on Carlson's Index) reservoirs were *Achnanthidium minutissimum*, centric diatoms, *Navicula* sp., and *Ulnaria acus, Ulnaria ulna.* For mesotrophic reservoirs, the dominant taxa were *Navicula* sp., *Synedra* sp., *Ulnaria acus* and *Ulnaria ulna.* In eutrophic reservoirs, centric diatoms, *Synedra* sp., and *Ulnaria ulna* were the dominant species. *Ulnaria ulna* was the most common and abundant species, present in all six reservoirs, always above 25% of relative abundance regardless of the trophic state.

Phtoplankton biovolumes were calculated and algal biomasses were estimated for the 3 years of sampling. Dinoflagellates comprised the largest fraction of the biovolumes in all reservoirs, except La Plata, where green algae dominated. Euglenophyte followed in biovolume magnitude in all reservoirs. Cyanobacterial biovolume was relatively important in Guajataca, mainly due to the sporadic blooms of *Microcystis*. However, cyanobacteria were negligible in Patillas. Therefore, the relation between cyanobaterial biovolume and trophic states was not clearly defined. Diatoms were numerically dominant in some reservoirs, like Cerrillos, but when their numbers are converted to biovolumes they felt short to dianoflagellates and green algae. A triplot analyses was performed for the following factors: cumulative relative algal biovolumes by major group, localities (reservoirs) and limnological parameters. Green algae biovolumes were more responsive to increased nutrient load (TN and TP). All other algal decreased their relative biovolumes at the expense of increased green algae.

The zooplankton community of the six reservoirs was characterized by a mix of species already known under similar environmental conditions in the Neotropical region, and a group of species with a totally opposed behavior. Decreased water transparency and higher concentrations of nutrients (TP and TN) were related to zooplankton density, particularly to rotifers. Sixty-five zooplanktonic taxa were identified. Rotifera was the richest group with 37 taxa. Cladocerans were represented by 20 taxa, while Copepoda by 8 taxa. This pattern is common in tropical freshwaters according to Neves et al. (2003).

The zooplankton community showed a typical structure, and clear rarity of large zooplankters like *Daphnia* spp. Copepoda were mainly represented by immature forms of nauplii and copepodites, but nauplii were not considered as these were below the sampling limit allowed by the 63 µm mesh and they are difficult to assign to particular taxa. However, adult cyclopoids showed a well-documented distribution pattern, with higher numerical densities at poor environmental quality, decreasing to low values in lakes of a better water quality.

Rotifers predominated in terms of densities in all reservoirs. Absolute abundances (instead of relative abundances) of rotifers show an increase with increased eutrophication. A sound relationship was observed between *Brachionus* rotifers (ind/L) and phosphorus (lnTP). A segmented curved approach had changepoint value for total phosphorus (parameter g), estimated as Ln of 3.28 (= 26.58 ppb). Also, an interesting relationship was detected between *Brachionus* rotifers (ind/L) and Secchi depth (m), where the changepoint value for Secchi depth (parameter g) was estimated as 1.16 meter. This Secchi depth value is a clear sign of impairment for local reservoirs.

2.0 General Introduction

There are three major indicators of ecological integrity in lakes/reservoirs, namely: trophic status (i.e., nutrient status and productivity), phytoplankton community structure, and dissolved oxygen dynamics. An analysis of these indicators in reservoirs of Puerto Rico raises major concerns regarding their long-term sustainability. All reservoirs are currently listed as impaired for noncompliance with the USEPA aquatic life (dissolved oxygen) criteria (PREQB, 2008). In addition, most of our reservoirs possess near favorable or favorable conditions, from a nutritional standpoint, to sustain the predominant growth of nuisance algae (Martínez et al., 2005).

Nutrient over enrichment of surface waters leads to profuse growth of aquatic macrophytic vegetation. It also leads to undesirable abundance of phytoplankton, which can result in the production of toxins that are hazardous to human health and make water treatment for drinking purposes more difficult and expensive. The eventual decay of macrophytic, planktonic and benthic biomass can lead to short- and long-term cycles of anoxia, which can result in fish kills (USEPA 1999; Campbell and Edwards, 2001). All these factors are in detriment to the designated uses of water bodies (drinking water supply, aquatic life use, navigation, fishing, or swimming) (USEPA, 2000).

The United States Environmental Protection Agency (USEPA) conducted a national assessment of lakes/reservoirs in contiguous states (USEPA, 2009). Elevated nutrient concentrations constituted the second most important cause of impairment affecting close to 20% of the total lake/reservoir population. Lakes with excess nutrients were two-and-a half-times more likely to exhibit poor biological health relative to those with adequate

nutrient levels. Existing evidence indicates that problems associated with excessive nutrient levels are more prevalent in Puerto Rico than in the continental U.S.A. Results from a recent study conducted by our research group revealed that sixty three percent of the reservoir population (12) had trophic state index (TSI (TP)) values corresponding to the eutrophic category. Thirty-two percent ranked as mesotrophic (6) while the remaining reservoir (Carraízo) was classified as hypereutrophic (Martínez et al., 2005). Reservoirs classified as eutrophic or hypereutrophic account for sixty-two percent of the total reservoir volume of the island.

Said study served as basis for the development of numeric reference criteria, the first step into the adoption of quantitative water quality standards aimed at preserving/restoring the integrity of reservoirs in Puerto Rico. Reference criteria constitute quantitative thresholds that distinguish reference or best attainable conditions (from a nutritional standpoint) from "nutrient enriched" reservoirs. Numeric criteria on the other hand define the transition between nutrient enrichment and impairment on the trophic status spectrum.

The primary goal of numeric nutrient standards is to prevent significant degradation of the ecological integrity of waters (rivers/streams, lakes/reservoirs, coastal waters) while at the same time preserving their designated uses. In Puerto Rico all reservoirs are evaluated based on the following designated uses: primary and secondary contact, aquatic life and sources of potable water. In the case of reservoirs probably the most protective use for establishing a numeric nutrient standard is aquatic life. From a human health standpoint nutrient (i.e., nitrate) concern levels associated with direct potable water consumption are orders of magnitude greater than those shown to cause significant ecological impairment. Other elements of human health concerns such as protecting against excessive cyanotoxin concentrations in the raw source of potable water are effectively addressed by adopting an ecological threshold that prevents profuse growth of aquatic macrophytic vegetation and recurring algae bloom formation.

Based on this conceptual framework we proposed using chlorophyll-*a* to define a quantitative ecological threshold that prevents significant degradation of the ecological integrity of our reservoirs. The rationale follows that used by USEPA in the most recent national assessment of lakes. In that study, USEPA used fixed thresholds of chlorophyll-*a* to categorize the nation's lakes according to trophic levels (USEPA, 2009). USEPA selected this parameter recognizing that the selected Chl-*a* thresholds were "longstanding accepted values from the peer reviewed scientific literature, which are well established, and widely and consistently used" (USEPA, 2009). On that study a value of 30 µg/L for Chl-*a* was used to define the boundary between eutrophic – hypereutrophic conditions. A similar Chl-*a* concentration (i.e., 30 µg/L) has been proposed as a transition point to cyanobacterial dominance in lentic waters of the U.S. (Dr. Alan Wilson, personal communication). Clearly hypereutrophic conditions are detrimental to the sustainability of aquatic systems and by definition represent a state

where the ecological integrity of the system has been compromised. We proposed using 30 µg/L Chl-*a* as a first approximation to establishing an ecological limit condition associated with impairment in reservoirs of Puerto Rico. An adjustment to that initial benchmark was later established based on stressor-response relationships from data gathered during a research project conducted between 2011 and 2014. This scheme assumes that nutrients are the primary factor controlling phytoplankton productivity in our reservoirs (i.e., the system is assumed to remain at a steady state despite the influence of other factors such as zooplankton activity), and that differences in Chl-*a* concentration among different algae genera do not influence significantly the relationship between Chl-*a* and nutrients. These assumptions seem reasonable in light of USEPAs recognition of Chl-a as the judgment criterion of trophic status across different ecoregions in the U.S.A. (USEPA, 2009).

The experimental strategy envisioned the periodical monitoring of six (6) reservoirs of the island (encompassing a range of trophic status) to generate sufficient variation in the data to establish nutrient-Chl-a relationships robust enough to define the numeric thresholds with a degree of confidence. In addition, a detailed analysis of phytoplankton community composition was conducted at each lake to assess the feasibility of using different thresholds of community structure (i.e., taxonomic richness, diversity, composition) to strengthen the regulatory framework. Finally, vertical profiles measurements of key limnological parameters (i.e., temperature, pH, dissolved oxygen, turbidity) were obtained at each reservoir to determine the extent of stratification and its potential influence on oxygen and nutrient availability.

2.1 Overall Objective:

- Perform a quantitative assessment of the relationships between nutrients and ecological thresholds of impairment as means of developing a framework for the implementation of numeric nutrient criteria for reservoirs of Puerto Rico.

2.2 Experimental Approach

For a detailed description of the methodology the reader is referred to the Quality Assurance Project Plan (QAPP) of this project (see Title page on Appendix A). Six of our most important reservoirs were chosen for evaluation (see reservoir description section). The reservoirs were selected to encompass a wide range of morphological characteristics (e.g., superficial area, depth), and trophic status.

2.2.1 Nutrient Status and Ancillary Water Column Parameters:

Results from twenty five sampling events performed on each reservoir (twenty four for Toa Vaca) are included in this report. Samples were obtained from the mid (center) section of each reservoir between 9:00 – 11:00 A.M (Appendix B). Sampling criteria required a minimum of 5 days without a 2yr - 24 hour rain event or greater (NOAA. 2006-

Appendix C). 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 (ºC), 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.

2.2.2 Plankton Community Structure

The work plan established three phytoplankton sampling events per reservoir per year. At each sampling, length and depth-integrated samples of the entire mixed layer (epilimnion) are gathered by longitudinal and oblique tows within the first meter using a bongo net system fitted with two metered 64 µm mesh nets. The samples are transferred to 500-mL bottles and preserved with 4% formalin and freshwater solution.

Using a Stemple pipette, two individual aliquots are taken from the diluted sample and dispensed into an Utermöhl chamber. After 48 hrs of settling, algal (=phytoplankton) counts are made with magnifications of 10-40X using an inverted microscope. Identification of algal taxa is made to the lowest taxonomic level using published criteria (Prescott 1970), which have been validated for tropical surface waters by our research group (Martínez et al., 2005).

Taxonomic richness (S-value) for algae is calculated by determining the number of taxa observed in the Utermöhl chambers. Numbers of taxa and cells per volume of sample are based upon the volume dispensed in the Utermöhl chambers.

Upon completion of the third year of sampling, algal community structure (= diversity or H-value) will be based on the taxonomic richness and the relative abundance of taxa, as follows:

$$H = -\sum \frac{Ni}{N} \ln \frac{Ni}{N}$$

where:
H = Shannon-Weaver 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.

For the zooplankton analyses, number of taxa and individuals per volume of sample is based upon the volume dispensed in the Sedgewick-Rafter chambers. Number of taxa per reservoir corresponds to the cumulative richness after scanning these chambers. Species were identified using published keys and Santos-Flores (2001).

2.2.3 Statistical Analysis

The statistical analysis focused on the relationship between nutrient parameters (TP and TN) with chlorophyll-*a*. Simple and multiple regression models were fitted for log-transformed values. Ecological thresholds were established based on conditional probability analysis (change point identification) (Paul and McDonald, 2005, Qian et al., 2003). This method yields geographically specific thresholds in relation to biological indicators. Confidence intervals for the thresholds were calculated using bootstrap (for the non parametric methods) or model-based approximation (for the parametric methods). Limnological parameters and plankton community structure indicators were described and related to nutrient parameters using multiple regression models.

3.0 Description of Reservoirs

Cerrillos

Cerrillos is located in the municipality of Ponce, in the south region of Puerto Rico. 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 (Table 1). 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). The predominant geological formations (i.e., Anon, Monserrate, and Lago Garzas and Yauco formations) consist of volcaniclastic terranes from the Tertiary and Cretaceous (Figure 1) (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 .

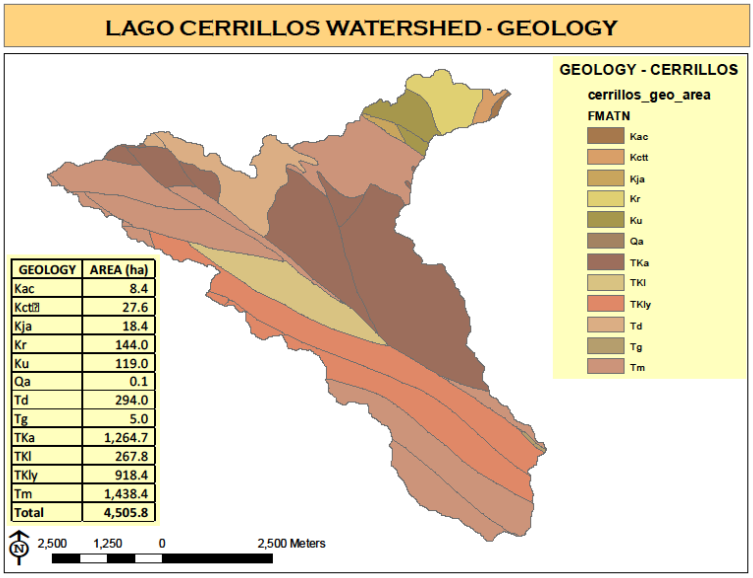
Accessed [04/23/2012]), (Figure 2). The Caguabo series consists of shallow, well drained, moderately permeable soils formed from material that weathered from basalt. These soils and present mainly on very steep slopes. The Mucara series consists of moderately deep, well drained, and moderately permeable 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 (Figure 3).

Table 1. Principal characteristics of the Cerrillos reservoir (from Ortiz et al., 2004 and Soler-López, 2008)

Year built	1991
Drainage area (km^2)	45.33
Superficial area (km^2)	2.12
Maximum depth (m)	65.0
Average depth (m)	27.8
Original storage capacity at max. conservation pool elevation (m^3x10^6)	38.03
Estimated storage capacity on last bathymetry (2008) (m^3x10^6)	37.26
Long-term sedimentation rate (m^3/yr)	45,794
Long-term annual water-storage capacity loss rate (%/yr)	0.12
Renewal frequency (times/yr)	0.9

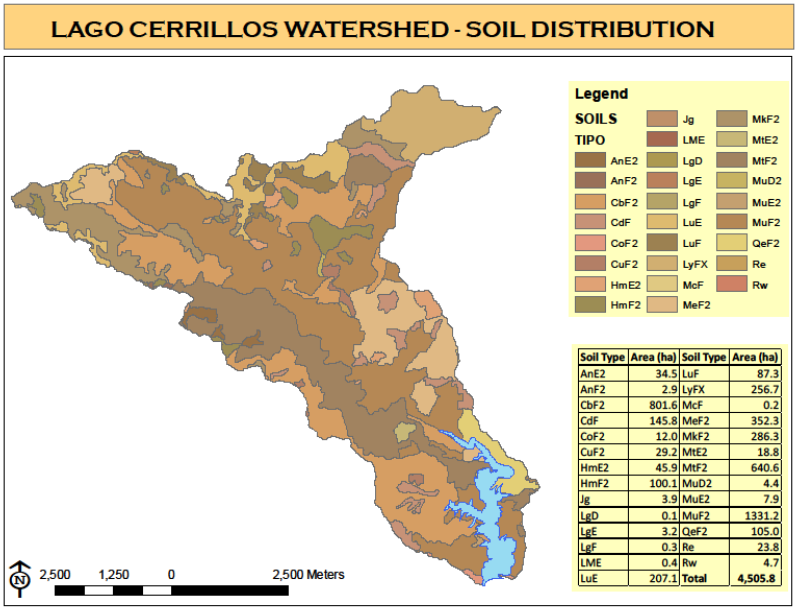
Nutrient data from previous studies from our group

Martínez et al., (2005) conducted a study to estimate reference nutrient conditions on reservoirs of Puerto Rico. Median concentrations (range values in parenthesis) from samples (8) collected at the center section of this reservoir at 1m depth were: 0.32 mg/L (0.16 – 0.52) for TKN-N; 0.023 mg/L (0.014-0.114) for NO_3-N; and 0.021 mg/L (0.012-0.045) for TP. The reservoir was classified as mesotrophic based on Carlson's TP trophic state index. Sotomayor et al., 2008, conducted monthly samplings from August 2006 to June 2007. Mean epilimnetic total N concentrations were 0.26 mg N/L and total P concentrations were 0.010 mg P/L. Median total N/total P ratios were 31 (+/- 11).



GEOLOGY	AREA (ha)
Kac	8.4
Kctt	27.6
Kja	18.4
Kr	144.0
Ku	119.0
Qa	0.1
Td	294.0
Tg	5.0
TKa	1,264.7
TKl	267.8
TKly	918.4
Tm	1,438.4
Total	**4,505.8**

Figure 1. Geology layer of the Cerrillos watershed.



Soil Type	Area (ha)	Soil Type	Area (ha)
AnE2	34.5	LuF	87.3
AnF2	2.9	LyFX	256.7
CbF2	801.6	McF	0.2
CdF	145.8	MeF2	352.3
CoF2	12.0	MkF2	286.3
CuF2	29.2	MtE2	18.8
HmE2	45.9	MtF2	640.6
HmF2	100.1	MuD2	4.4
Jg	3.9	MuE2	7.9
LgD	0.1	MuF2	1331.2
LgE	3.2	QeF2	105.0
LgF	0.3	Re	23.8
LME	0.4	Rw	4.7
LuE	207.1	**Total**	**4,505.8**

Figure 2. Soil series layer of the Cerrillos watershed.

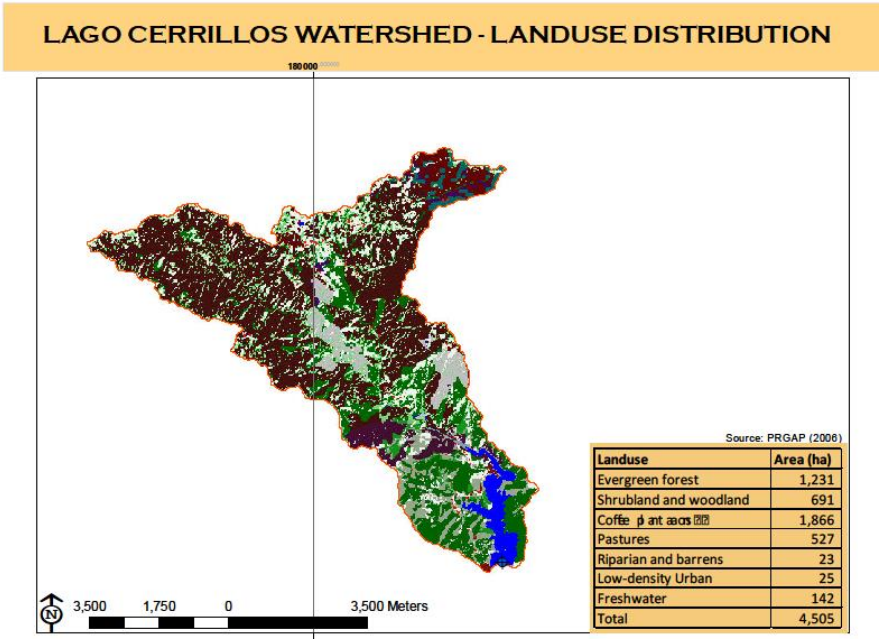


Figure 3. Land-use distribution of the Cerrillos watershed (legend in Appendix D).

Cidra

The Cidra reservoir is located in the municipality of Cidra, in the east-central region of Puerto Rico. The spillway crest is at 403 m above sea level (Ortiz et al., 2004, Table 2). 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 (Soler-López, 2007). The predominant geological formation at this watershed (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, (Figure 4) (USGS, 1998). 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]), (Figure 5). 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

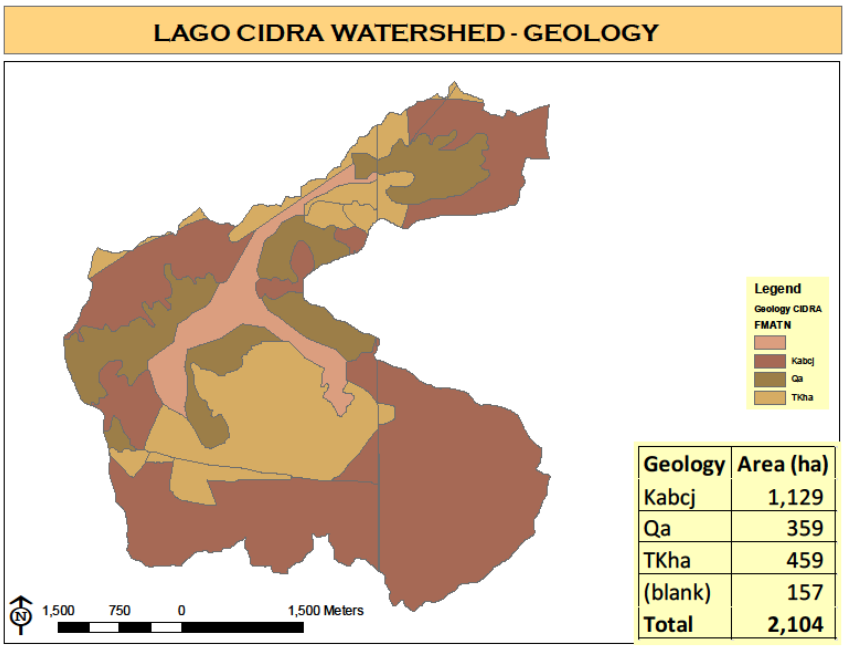
(50.1%). This watershed has the highest percent of land cover dedicated to human establishments, covering 8.5% of the area (Figure 6).

Table 2. Principal characteristics of the Cidra reservoir (from Ortiz et al., 2004 and Soler-López, 2007)

Year built	1946
Drainage area (km^2)	21.50
Superficial area (km^2)	1.06
Maximum depth (m)	18.5
Average depth (m)	5.4
Original storage capacity at max. conservation pool elevation (m^3x10^6)	6.54
Estimated storage capacity on last bathymetry (2007) (m^3x10^6)	5.63
Long-term sedimentation rate (m^3/yr)	15,000
Long-term annual water-storage capacity loss rate (%/yr)	0.23
Renewal frequency (times/yr)	2.6

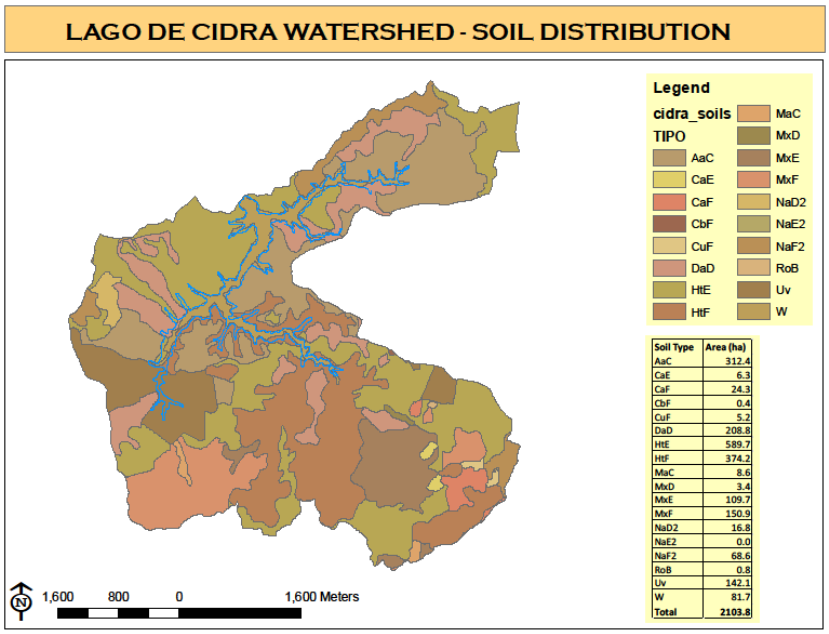
Nutrient data from previous studies from our group

Median concentrations (range values in parenthesis) from samples (8) collected at the center section (1m depth) of this reservoir during the "reference criteria study" were: 0.41 mg/L (0.34 – 0.78) for TKN-N; 0.053 mg/L (0.011-0.280) for NO_3-N; and 0.035 mg/L (0.010-0.088) for TP (Martínez et al., (2005)). The reservoir was classified as eutrophic based on Carlson's TP trophic state index (Martínez et al., (2005).



Geology	Area (ha)
Kabcj	1,129
Qa	359
TKha	459
(blank)	157
Total	**2,104**

Figure 4. Geology layer of the Cidra watershed.



Soil Type	Area (ha)
AaC	312.4
CaE	6.3
CaF	24.3
CbF	0.4
CuF	5.2
DaD	208.8
HtE	589.7
HtF	374.2
MaC	8.6
MxD	3.4
MxE	109.7
MxF	150.9
NaD2	16.8
NaE2	0.0
NaF2	68.6
RoB	0.8
Uv	142.1
W	81.7
Total	2103.8

Figure 5. Soil series distribution layer of the Cidra watershed.

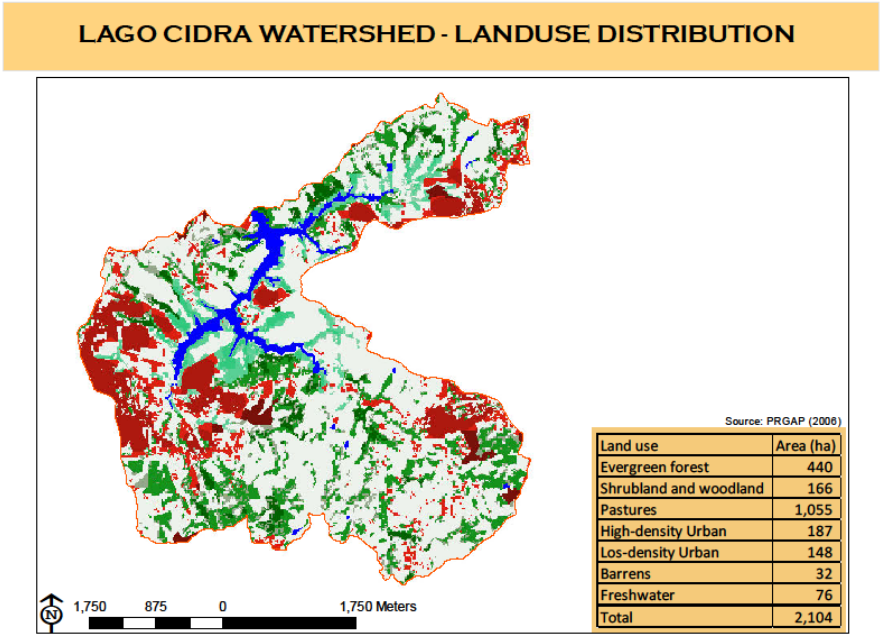


Figure 6. Land-use distribution layer of the Cidra watershed (legend in Appendix D).

Guajataca

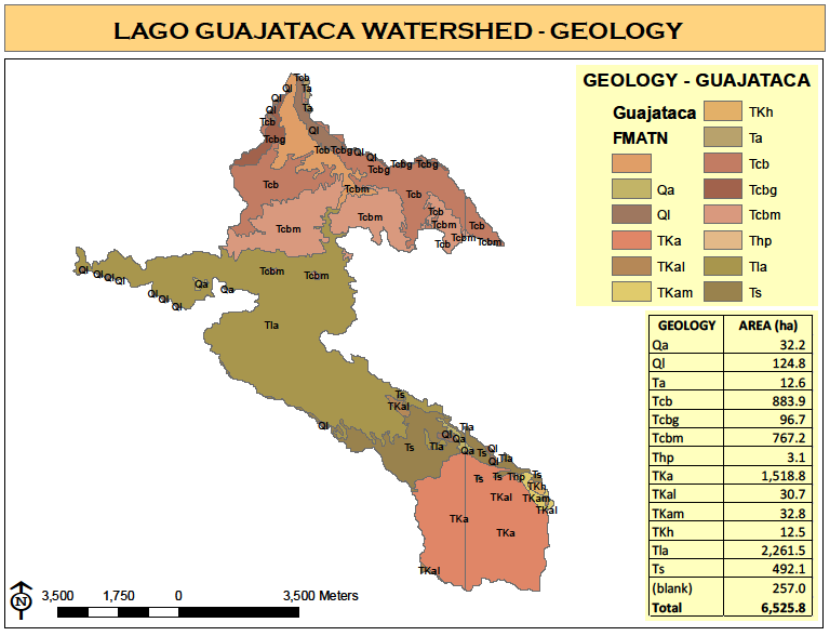
The Guajataca reservoir is located in the north-western region of Puerto Rico about 8 km south of the Quebradillas municipality and 10km northeast of the San Sebastián municipality. The reservoir was completed in 1928 (Table 3). It drains waters from the Río Guajataca watershed which covers an area of 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 (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 (Figure 7) (USGS, 1998). 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]), (Figure 8). 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 (Figure 9).

Table 3. Principal characteristics of the Guajataca reservoir (from Ortiz et al., 2004 and Soler-López, 2000).

Year built	1928
Drainage area (km^2)	79.77
Superficial area (km^2)	3.42
Maximum depth (m)	27.0
Average depth (m)	12.4
Original storage capacity at max. conservation pool elevation (m^3x10^6)	48.46
Estimated storage capacity on last bathymetry (1999) (m^3x10^6)	42.28
Long-term sedimentation rate (m^3/yr)	87,000
Long-term annual water-storage capacity loss rate (%/yr)	0.20
Renewal frequency (times/yr)	2.5

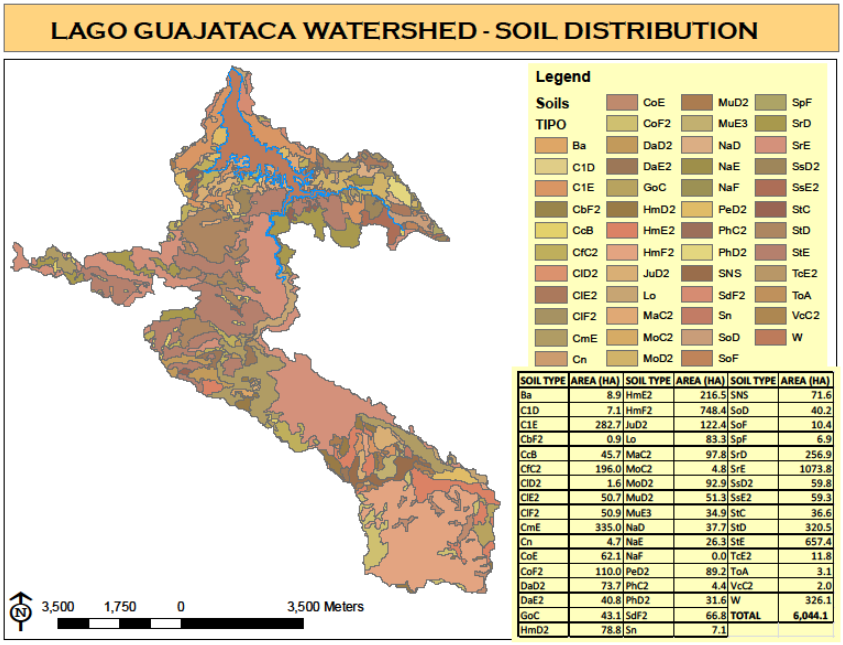
Nutrient data from previous studies from our group

Median concentrations (range values in parenthesis) from samples (8) collected at the dam section of this reservoir (1m depth) during the reference criteria study (2003-2005) were: 0.25 mg/L (0.02 – 0.42) for TKN-N; 0.114 mg/L (0.011-0.485) for NO_3-N; and 0.017 mg/L (0.010-0.032) for TP (Martínez et al., (2005)). The reservoir was classified as mesotrophic based on Carlson's TP trophic state index (Martínez et al., (2005). Pantoja-Agreda et al. (2009) conducted a study to characterize the spatial, temporal and vertical dynamics of the phytoplanktonic community at Guajataca as related to different environmental factors, including nutrients. Median concentrations (range values in parenthesis) from samples (19) collected at the dam section (1m depth) in said study were: 0.26 mg/L (0.10 – 0.57) for TKN-N; 0.024 mg/L (0.006-0.071) for NO_3-N; and 0.025 mg/L (0.010-0.043) for TP. More recently, Martínez et al. (2011), conducted a study to evaluate spatial variability in several water quality parameters at La Plata and Guajataca. Median concentrations (range values in parenthesis) from samples (12) collected at the center section (1m depth) in said study were: 0.52 mg/L (0.25 – 4.53) for TKN-N; 0 mg/L (nd-0.01) for NO_3-N; and 0.019 mg/L (0.004-0.029) for TP (Martínez et al. (2011).



GEOLOGY	AREA (ha)
Qa	32.2
Ql	124.8
Ta	12.6
Tcb	883.9
Tcbg	96.7
Tcbm	767.2
Thp	3.1
TKa	1,518.8
TKal	30.7
TKam	32.8
TKh	12.5
Tla	2,261.5
Ts	492.1
(blank)	257.0
Total	**6,525.8**

Figure 7. Geology layer of the Guajataca watershed.



SOIL TYPE	AREA (HA)	SOIL TYPE	AREA (HA)	SOIL TYPE	AREA (HA)
Ba	8.9	HmE2	216.5	SNS	71.6
C1D	7.1	HmF2	748.4	SoD	40.2
C1E	282.7	JuD2	122.4	SoF	10.4
CbF2	0.9	Lo	83.3	SpF	6.9
CcB	45.7	MaC2	97.8	SrD	256.9
CfC2	196.0	MoC2	4.8	SrE	1073.8
ClD2	1.6	MoD2	92.9	SsD2	59.8
ClE2	50.7	MuD2	51.3	SsE2	59.3
ClF2	50.9	MuE3	34.9	StC	36.6
CmE	335.0	NaD	37.7	StD	320.5
Cn	4.7	NaE	26.3	StE	657.4
CoE	62.1	NaF	0.0	TcE2	11.8
CoF2	110.0	PeD2	89.2	ToA	3.1
DaD2	73.7	PhC2	4.4	VcC2	2.0
DaE2	40.8	PhD2	31.6	W	326.1
GoC	43.1	SdF2	66.8	**TOTAL**	**6,044.1**
HmD2	78.8	Sn	7.1		

Figure 8. Soil series layer of the Guajataca watershed.

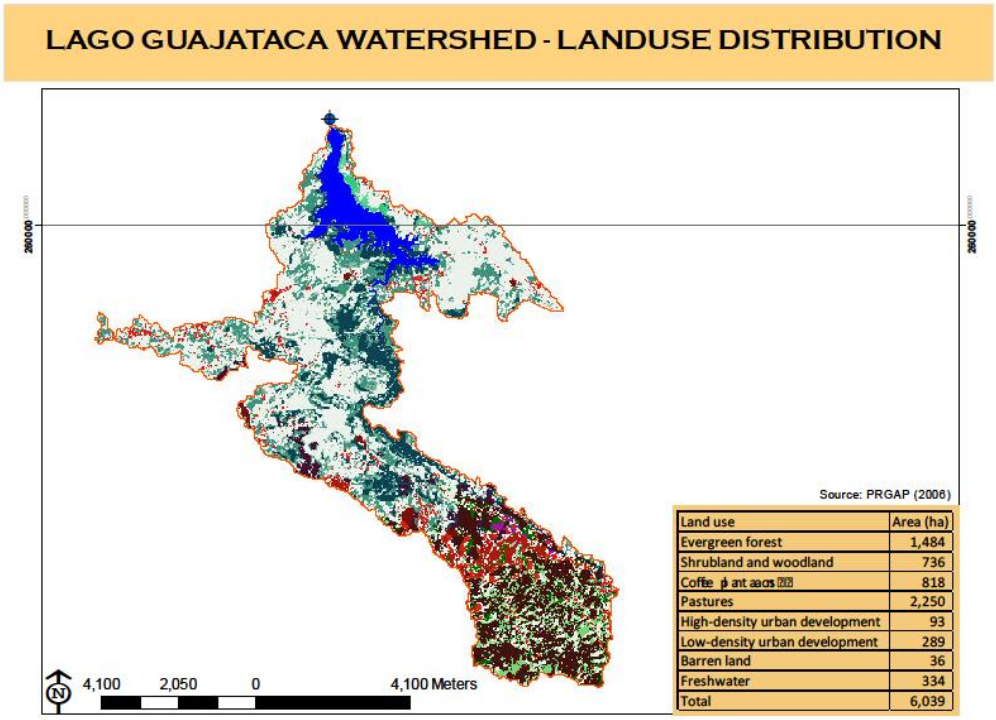


Figure 9. Land-use distribution layer of the Guajataca watershed (legend in Appendix D).

La Plata

La Plata reservoir is located between the municipalities of Toa Alta and Naranjito 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 (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) Figure 10) (USGS, 1998). 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]), (Figure 11). 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 (Figure 12).

Table 4. Principal characteristics of the La Plata reservoir (from Ortiz et al., 2004 and Soler-López, 2008)

Year built	1974
Drainage area (km^2)	468.8
Superficial area (km^2)	3.32
Maximum depth (m)	27.0
Average depth (m)	10.7
Original storage capacity at max. conservation pool elevation (m^3x10^6)	40.21
Estimated storage capacity on last bathymetry (2006) (m^3x10^6)	31.27
Long-term sedimentation rate (m^3/yr)	280,000
Long-term annual water-storage capacity loss rate (%/yr)	0.69
Renewal frequency (times/yr)	8.2

Nutrient data from previous studies from our group

Median concentrations (range values in parenthesis) from samples (8) collected at the dam section of this reservoir (1m depth) during the reference criteria study (2003-2005) were: 0.32 mg/L (0.21 – 0.58) for TKN-N; 0.081 mg/L (0.040-0.463) for NO_3-N; and 0.031 mg/L (0.013-0.065) for TP (Martínez et al., (2005)). The reservoir was classified as eutrophic based on Carlson's TP trophic state index (Martínez et al., (2005). More recently, Martínez and Sotomayor (2011) conducted a study to evaluate dissolved oxygen dynamics at the reservoir. Median concentrations (range values in parenthesis) from samples (19) collected at the center section (1m depth) in said study were: 0.78 mg/L (0.41 – 3.40) for TKN-N; 0.013 mg/L (nd-0.79) for NO_3-N; and 0.043 mg/L (0.012-0.148) for TP.

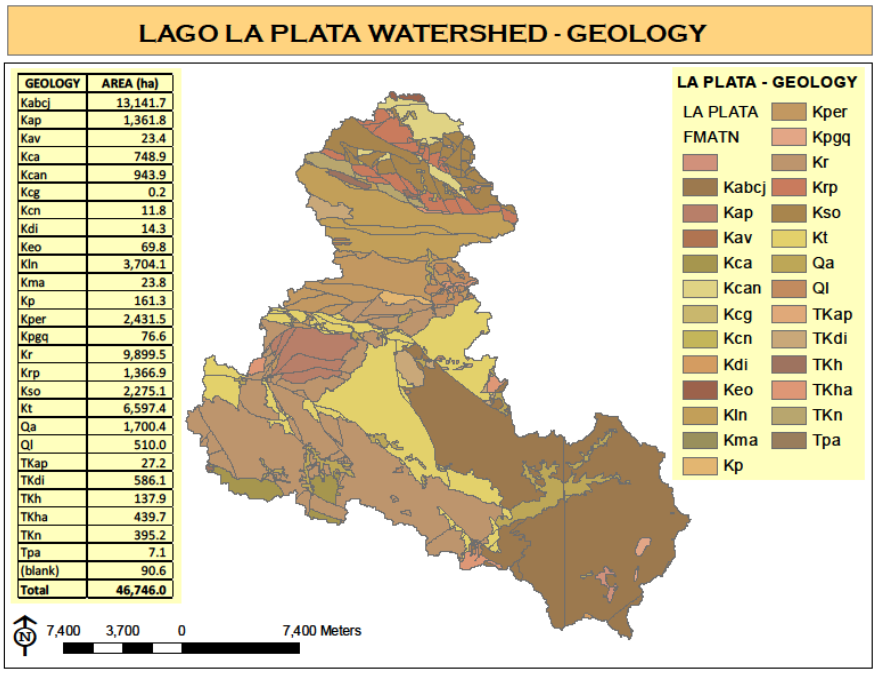


Figure 10. Geology layer of La Plata watershed.

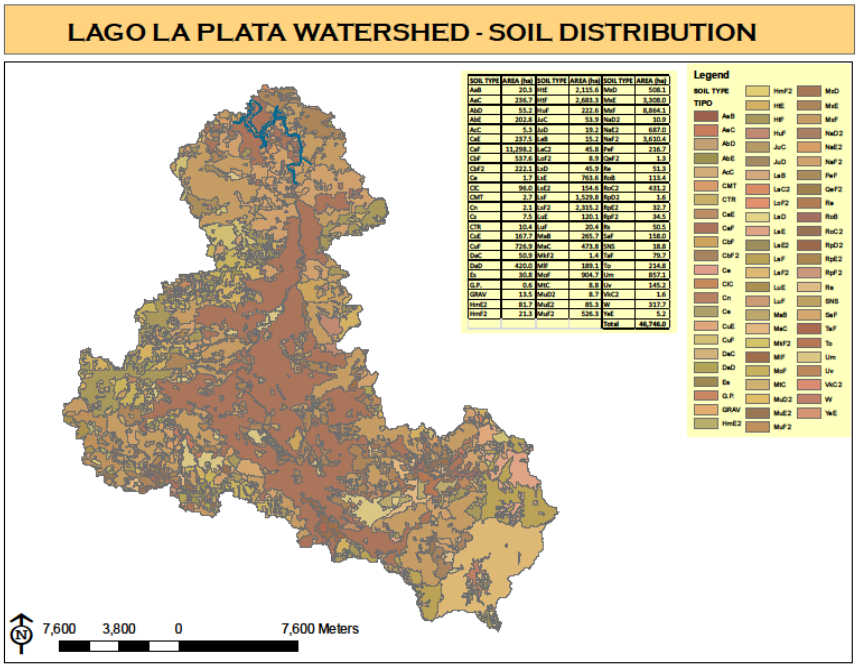


Figure 11. Soil series distribution layer of La Plata watershed.

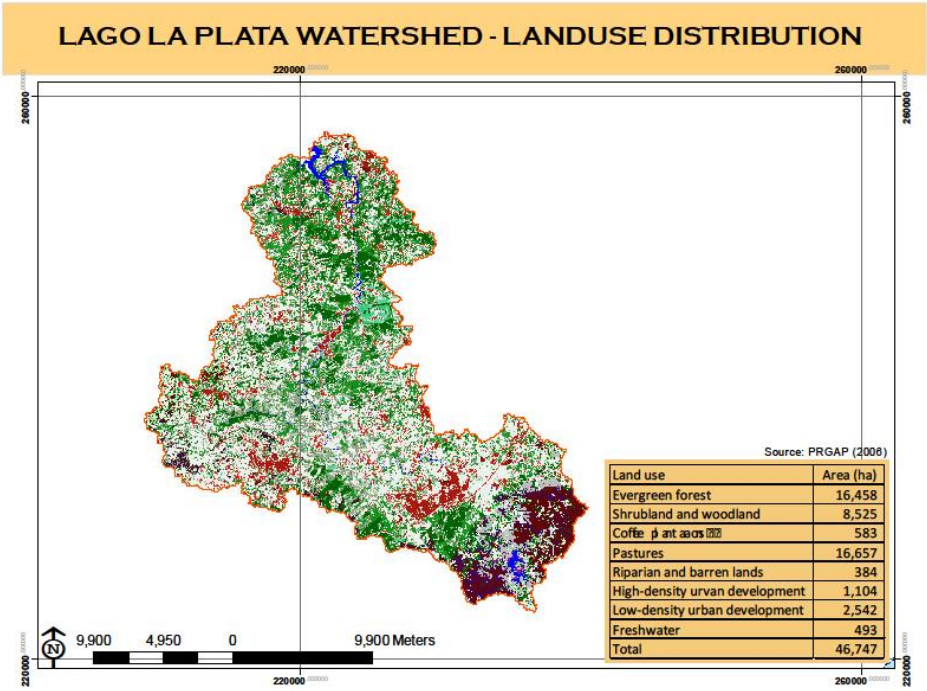


Figure 12. Land-use distribution layer of La Plata watershed (legend in Appendix D).

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. 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, Table 5). The reservoir impounds the waters of Río Patillas and Río Marín. A bathymetry study concluded in 2007 reported a water storage loss of 23% 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 (Figure 13) (USGS, 1998). 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]), (Figure 14). 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 (Figure 15). This is by far the highest percent of any

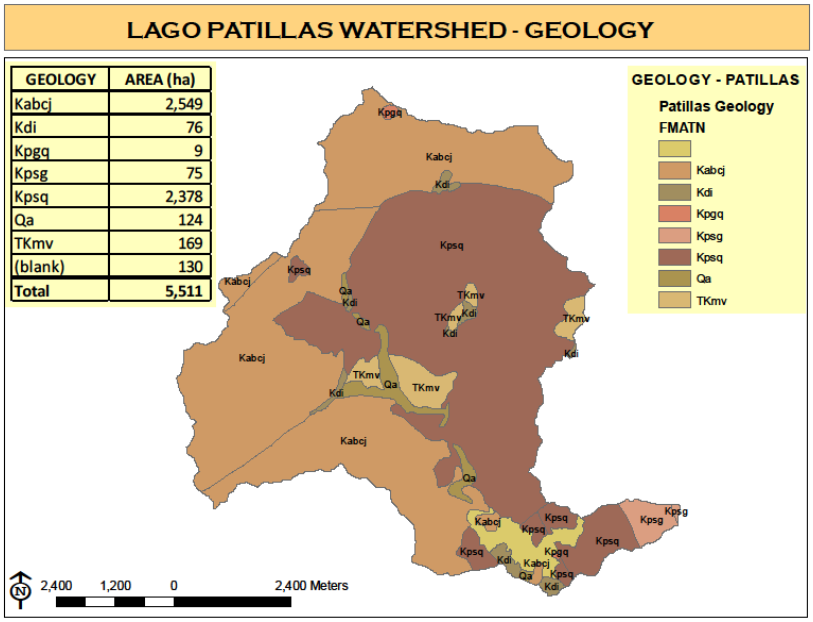
of the reservoirs being studied. Human establishments represent just 0.51% of the area, the lowest of the reservoirs under evaluation.

Table 5. Principal characteristics of the Patillas reservoir (from Ortiz et al., 2004 and Soler-López, 2010).

Year built	1914
Drainage area (km^2)	65.26
Superficial area (km^2)	1.50
Maximum depth (m)	24
Average depth (m)	9.3
Original storage capacity (1961) at max. conservation pool elevation (m^3x10^6)	17.64
Estimated storage capacity on last bathymetry (2007) (m^3x10^6)	13.57
Long-term sedimentation rate (m^3/yr)	27,000
Long-term annual water-storage capacity loss rate (%/yr)	0.5
Renewal frequency (times/yr)	4.5

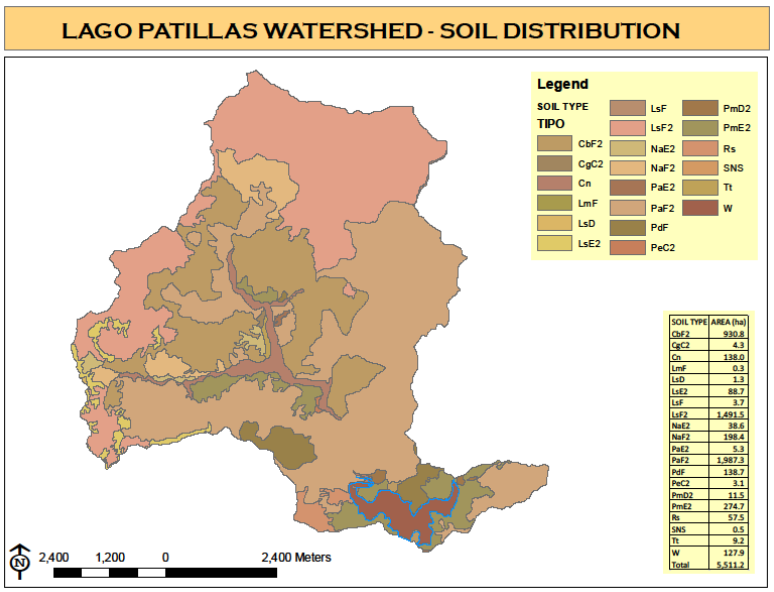
Nutrient data from previous studies from our group

Median concentrations (range values in parenthesis) from samples (8) collected at the center section of this reservoir (1m depth) during the reference criteria study (2003-2005) were: 0.25 mg/L (0.154 – 0.39) for TKN-N; 0.031 mg/L (0.007-0.15) for NO_3-N; and 0.018 mg/L (0.010-0.025) for TP (Martínez et al., (2005)). The reservoir was classified as mesotrophic based on Carlson's TP trophic state index (Martínez et al., (2005).



GEOLOGY	AREA (ha)
Kabcj	2,549
Kdi	76
Kpgq	9
Kpsg	75
Kpsq	2,378
Qa	124
TKmv	169
(blank)	130
Total	**5,511**

Figure 13. Geology layer of Patillas watershed.



SOIL TYPE	AREA (ha)
CbF2	930.8
CgC2	4.3
Cn	138.0
LmF	0.3
LsD	1.3
LsE2	88.7
LsF	3.7
LsF2	1,491.5
NaE2	38.6
NaF2	198.4
PaE2	5.3
PaF2	1,987.3
PdF	138.7
PeC2	3.1
PmD2	11.5
PmE2	274.7
Rs	57.5
SNS	0.5
Tt	9.2
W	127.9
Total	5,511.2

Figure 14. Soil series distribution layer of Patillas watershed.

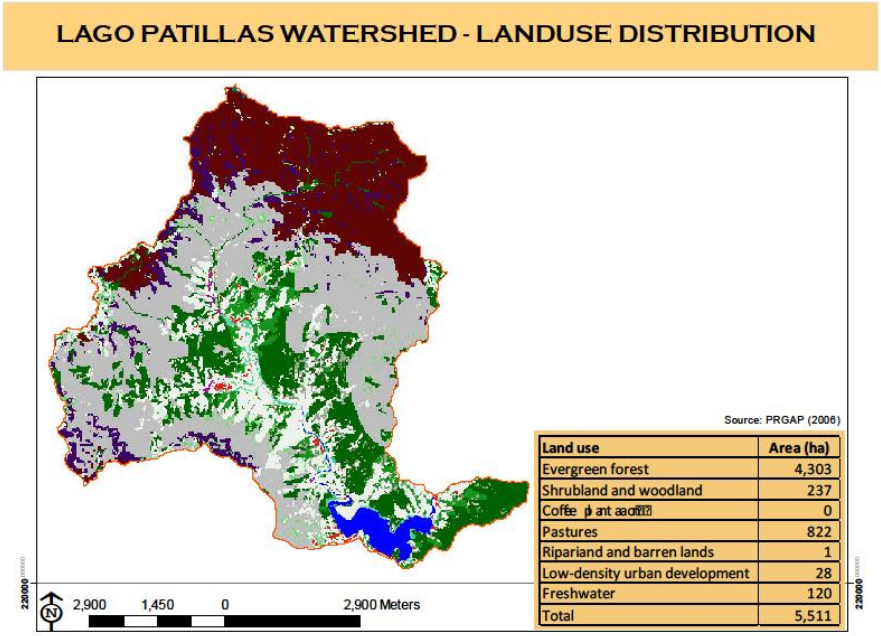


Figure 15. Land use distribution layer of Patillas watershed (legend in Appendix D).

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. The normal pool elevation is 164.90 m above sea level (Ortiz et al., 2004, Table 6). 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 (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 (Figure 16), (USGS, 1998). 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]), (Figure 17). 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) cover 1.43% of the area (Figure 18).

Table 6. Principal characteristics of the Toa Vaca reservoir (from Ortiz et al., 2004 and Soler-López, 2008).

Year built	1972
Drainage area (km^2)	56.72
Superficial area (km^2)	3.21
Maximum depth (m)	53.9
Average depth (m)	19.9
Original storage capacity at max. conservation pool elevation (m^3x10^6)	68.94
Estimated storage capacity on last bathymetry (2002) (m^3x10^6)	64.08
Long-term sedimentation rate (m^3/yr)	162,000
Long-term annual water-storage capacity loss rate (%/yr)	0.23
Renewal frequency (times/yr)	0.3

Nutrient data from previous studies from our group

Median concentrations (range values in parenthesis) from samples (8) collected at the center section of this reservoir (1m depth) during the reference criteria study (2003-2005) were: 0.35 mg/L (0.10 – 0.53) for TKN-N; 0.059 mg/L (0.002-0.10) for NO_3-N; and 0.028 mg/L (0.010-0.041) for TP (Martínez et al., 2005). The reservoir was classified as eutrophic based on Carlson's TP trophic state index (Martínez et al., 2005).

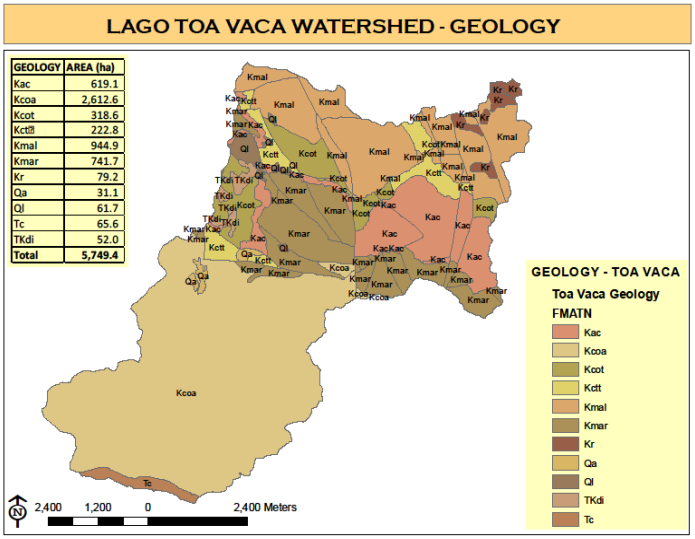
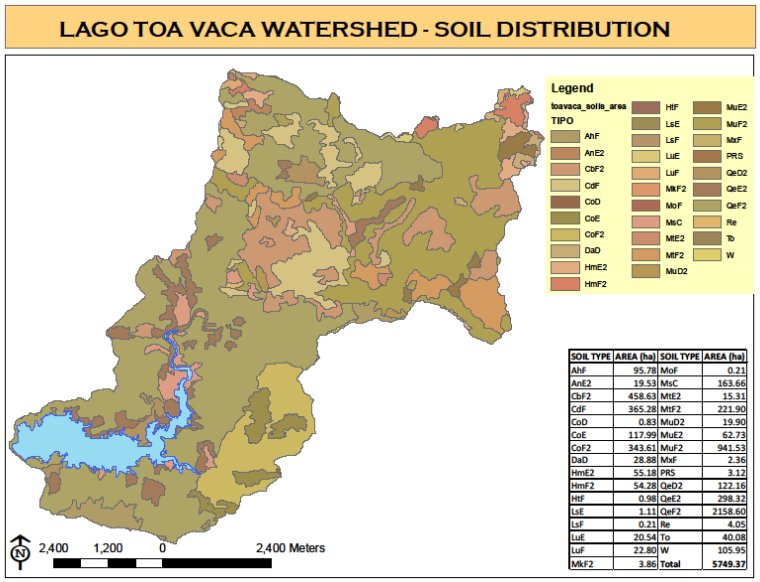
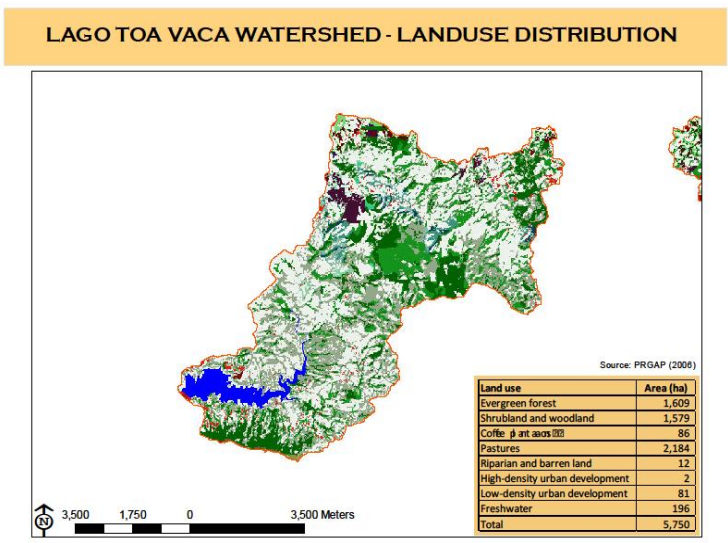


Figure 16. Geology layer of Toa Vaca watershed.



SOIL TYPE	AREA (ha)	SOIL TYPE	AREA (ha)
AhF	95.78	MoF	0.21
AnE2	19.53	MsC	163.66
CbF2	458.63	MtE2	15.31
CdF	365.28	MtF2	221.90
CoD	0.83	MuD2	19.90
CoE	117.99	MuE2	62.73
CoF2	343.61	MuF2	941.53
DaD	28.88	MxF	2.36
HmE2	55.18	PRS	3.12
HmF2	54.28	QeD2	122.16
HtF	0.98	QeE2	298.32
LsE	1.11	QeF2	2158.60
LsF	0.21	Re	4.05
LuE	20.54	To	40.08
LuF	22.80	W	105.95
MkF2	3.86	Total	5749.37

Figure 17. Soil series distribution layer of Toa Vaca watershed.



Land use	Area (ha)
Evergreen forest	1,609
Shrubland and woodland	1,579
Coffe [illegible]	86
Pastures	2,184
Riparian and barren land	12
High-density urban development	2
Low-density urban development	81
Freshwater	196
Total	5,750

Figure 18. Land-use distribution layer of Toa Vaca watershed (legend in Appendix D).

4.0 Limnological Conditions at the Surface (1m)

4.1. Sampling Dates

Twenty five samplings events were performed at each reservoir (twenty four for Toa Vaca), (Table 7). This represents 104% of the total sampling events scheduled (100% for

Toa Vaca). Sampling at Toa Vaca was temporarily hindered by the initial effects of an aireation project that began operations in late September, 2012. Due to the lack of dissolved oxygen in the water column during the initial months after the project's start up it was decided to postpone the sampling events until the system had become more stable. Although said situation was beyond our control we completed the sampling scheduled within the project's timeframe.

Table 7. Sampling events perfomed

Reservoir	Sampling Dates (mm/dd) for Water Quality and General Limnology	Sampling Dates (mm/dd) for Phytoplankton Taxonomy
Cerrillos	***Year 2012***: 02/22; 03/19; 04/02; 04/24; 05/15; 06/26; 07/02; 08/30; 10/11; 11/01; 11/27 ***Year 2013:*** 01/17; 2/05; 02/26; 03/19; 05/15; 6/13; 06/28; 08/29; 09/26; 10/16 ***Year 2014:*** 02/04; 02/25; 03/27, 4/22; 6/19	***Year 2012***: 03/19; 04/24; 07/02 ***Year 2013:*** 01/17; 05/15; 09/26 ***Year 2014:*** 02/04; 4/22; 6/19
Cidra	***Year 2012***: 02/28; 03/21; 04/11; 05/01; 05/24; 06/14; 07/12; 08/21; 09/04; 10/15; 11/15 ***Year 2013***: 01/15; 02/07; 02/28; 03/13; 06/11; 07/02; 08/13; 09/03; 10/03 ***Year 2014***: 02/05; 02/27; 03/26; 4/15; 5/6; 6/3	***Year 2012***: 03/21; 05/01; 07/12 ***Year 2013:*** 01/15; 06/11; 09/03 ***Year 2014:*** 02/05; 4/15; 6/3

Table 7 (cont).

Reservoir	Sampling Dates (mm/dd) for Water Quality and General Limnology	Sampling Dates (mm/dd) for Phytoplankton Taxonomy
Guajataca	***Year 2012:*** 03/06; 03/27; 04/19; 05/08; 05/29; 06/12; 07/10; 08/15; 10/02; 10/30; 11/20; ***Year 2013:*** *02/01;* 02/21; 03/05; 04/17; 05/22; 06/20; 07/03; 08/22; 09/17 ***Year 2014:*** 01/28; 02/18; 03/18; 04/08; 5/29; 7/1	***Year 2012:*** 03/06; 04/19 ; 07/10 ***Year 2013:*** 02/01; 05/22; 09/17 ***Year 2014:*** 01/28; 04/08; 7/1
La Plata	***Year 2012:*** 03/01; 03/15; 04/10; 04/26; 05/22; 06/06; 07/11; 08/16; 10/17; 11/07; 12/03; ***Year 2013:*** 01/24; 02/14; 03/07; 04/12; 06/06; 08/07; 09/10; 10/08; 10/23 ***Year 2014:*** 01/23; 02/14; 03/20; 04/10; 5/27; 6/12	***Year 2012:*** 03/15 ; 04/26; 07/11 ***Year 2013:*** 01/24; 06/06; 09/10 ***Year 2014:*** 01/23; 04/10; 6/12
Patillas	***Year 2012:*** 02/14; 03/13; 04/03; 04/17; 05/10; 05/30; 06/27; 09/12; 10/03; 11/13; 11/29; ***Year 2013:*** 01/22; 02/13; 03/12; 04/02; 06/18; 07/09; 08/08; 09/19; 10/10 ***Year 2014:*** 01/30; 03/04; 04/01; 4/29; 6/5; 6/24	***Year 2012:*** 03/13 ; 04/17; 06/27 ***Year 2013:*** 01/22; 06/18; 09/19 ***Year 2014:*** 01/30; 4/29; 6/24

Table 7:cont.

Reservoir	Sampling Dates (mm/dd) for Water Quality and General Limnology	Sampling Dates (mm/dd) for Phytoplankton Taxonomy
Toa Vaca	***Year 2012:*** 02/08; 03/08; 03/29; 05/02; 05/23; 06/13; 07/03; 09/06; 11/08; 12/04; ***Year 2013***: 04/24; 05/08; 06/04; 06/25; 08/15; 09/12; 10/02; 10/24; ***Year 2014:*** 02/13; 03/06; 04/03; 4/24; 5/22; 6/26	***Year 2012:*** 03/8; 5/02; 07/03 ***Year 2013***: 05/08; 09/12 ***Year 2014:*** 02/13; 4/24; 5/22; 6/26

4.2. Descriptive Statistics

Descriptive statistics of water-quality data for all lakes combined (1m depth) are presented in Table 8, and for individual lakes in Table 9. Median values (range in parenthesis) for all reservoirs combined are 0.33 mg/L (0.048 – 3.18), 17.0 µg /L (3.0 – 220.0), and 9.76µg/L (0.56 – 496.36) for TN, TP and Chl-a. On the other hand, median values for Secchi depth and dissolved oxygen are 1.70m (0.5 - 4.3) and 7.99mg/L (1.61 – 12.54), respectively. Finally, median values for pH and temperature are 8.21 (6.56 – 9.30) and 27.53 (23.42 – 30.29), respectively.

4.2.1. Comparison Between Reservoirs

As intended, the reservoirs chosen exemplify the nutrient status/productivity spectrum of reservoirs in the island (Table 10). Cidra, and La Plata represent reservoirs at the higher end of the productivity/nutrient enrichment spectrum, and statistically, they placed among the top two in nutrient concentrations (i.e., TKN, TN, and TP), and Chl-a (Table 10, Figures 19-22). On the other hand, Cerrillos and Patillas typify those closer to reference conditions, and statistically they placed at the bottom both in nutrients and Chl-a concentrations. Finally, Toa Vaca and Guajataca represent a transition phase in the productivity spectrum and statistically placed at a mid-point in terms of nutrients and chlorophyll *a* concentrations. The validation of the range of nutrient and productivity status covered by the selected reservoirs is an essential component of the overall experimental strategy of this project. As expected, reservoirs with higher nutrient and productivity status (i.e., Cidra, La Plata) have lower depth of light penetration (i.e., Secchi depth) than reservoirs with relatively lower nutrient and Chl-a values (i.e., Patillas, Cerrillos) (Table 11; Figure 23). In general, the obtained values are within previous

reported levels, with possibly the exception of Patillas, Cerrillos and Guajataca, which currently appear to have lower TP concentrations than those of 2005, and Cidra, which at present exhibits higher TN concentrations than those reported by Martínez et al. (2005).

Table 8. Descriptive Statistics (all reservoirs combined).

Variable	Minimum	Lower Quartile	Median	Mean	Std Dev	Upper Quartile	Maximum
TKN_N	0.0467	0.2127	0.3154	0.4034	0.3220	0.4974	3.1749
NO3_N	0.0010	0.0020	0.0059	0.0168	0.0312	0.0165	0.2883
TN	0.0477	0.2290	0.3283	0.4203	0.3253	0.5238	3.1823
TN_TP	3.8481	13.1973	18.6706	24.0092	17.5460	29.1201	140.0780
TP	0.0030	0.0090	0.0170	0.0253	0.0268	0.0315	0.2200
DP	0.0000	0.0000	0.0000	0.0003	0.0015	0.0000	0.0160
Chla	0.5600	4.8540	9.7575	19.5450	38.4561	17.2765	496.3600
lntkn_n	-3.0647	-1.5478	-1.1540	-1.1505	0.7161	-0.6984	1.1553
lnno3_n	-6.8918	-6.1986	-5.1378	-5.0381	1.3496	-4.1054	-1.2436
lntn	-3.0432	-1.4740	-1.1137	-1.0933	0.6809	-0.6466	1.1576
lntp	-5.8091	-4.7105	-4.0745	-4.0737	0.8830	-3.4579	-1.5141
lntn_tp	1.3476	2.5800	2.9269	2.9804	0.6119	3.3714	4.9422
lnchla	-0.5798	1.5798	2.2780	2.3031	1.0663	2.8493	6.2073
temp	23.4200	25.9750	27.5250	27.2985	1.5867	28.5350	30.2900
CE	0.1200	0.1750	0.2360	0.2355	0.0645	0.2755	0.4040
pH	6.5600	7.8050	8.2050	8.1439	0.4529	8.4700	9.3000
ORP	-26.0000	154.8000	217.3500	207.2810	81.8736	270.3000	386.9000
Turb	0.0000	0.0000	1.0000	3.0518	4.9964	4.2000	40.8000
DO	1.6100	7.2200	7.9900	7.9639	1.4846	8.5700	12.5400
Sechi	0.5000	1.2000	1.7000	1.8744	0.8130	2.5000	4.3000

Table 9. Descriptive statistics by reservoirs

lakename	N Obs	Variable	Minimum	Lower Quartile	Median	Mean	Std Dev	Upper Quartile	Maximum
Cerrillo	100	TKN_N	0.0467	0.1575	0.2116	0.2154	0.1001	0.2714	0.4558
		NO3_N	0.0010	0.0010	0.0053	0.0149	0.0253	0.0106	0.0942
		TN	0.0523	0.1701	0.2238	0.2303	0.1038	0.2923	0.4568
		TN_TP	3.8481	15.6500	22.9700	28.7298	22.8556	35.9959	140.0780
		TP	0.0030	0.0060	0.0100	0.0100	0.0043	0.0120	0.0220
		DP	0.0000	0.0000	0.0000	0.0001	0.0005	0.0000	0.0030
		Chla	1.1100	3.5035	4.7880	5.8533	3.8854	7.5485	20.4720
		lntkn_n	-3.0647	-1.8484	-1.5533	-1.6908	0.6337	-1.3040	-0.7858
		lnno3_n	-6.8918	-6.8918	-5.2391	-5.3071	1.4438	-4.5539	-2.3628
		lntn	-2.9505	-1.7717	-1.4968	-1.6018	0.5725	-1.2306	-0.7835
		lntp	-5.8091	-5.1160	-4.6052	-4.7085	0.4810	-4.4228	-3.8167
		lntn_tp	1.3476	2.7504	3.1342	3.1067	0.7243	3.5831	4.9422
		lnchla	0.1044	1.2529	1.5661	1.5611	0.6639	2.0212	3.0191
		temp	24.2200	24.7550	27.0100	26.5378	1.5341	27.8250	29.1000
		CE	0.2120	0.2270	0.2300	0.2321	0.0101	0.2350	0.2530
		pH	6.5600	8.1150	8.3850	8.2953	0.4252	8.6100	8.8900
		ORP	31.5000	177.3000	228.5500	215.3150	83.7520	266.5000	386.9000
		Turb	0.0000	0.0000	0.0000	1.2230	3.0982	0.5000	22.3000
		DO	4.5600	7.8150	8.1200	8.0811	1.2938	8.8100	10.6200
		Sechi	1.2000	2.3000	2.7000	2.6945	0.5715	3.1000	4.0000
Cidra	100	TKN_N	0.2551	0.5199	0.6899	0.8129	0.4882	0.9504	3.1749
		NO3_N	0.0010	0.0045	0.0123	0.0246	0.0268	0.0440	0.1034
		TN	0.3013	0.5665	0.7393	0.8375	0.4781	0.9611	3.1823
		TN_TP	9.4163	14.6681	20.2146	21.4213	8.1113	25.9864	55.3251
		TP	0.0160	0.0250	0.0330	0.0463	0.0397	0.0540	0.2200
		DP	0.0000	0.0000	0.0000	0.0005	0.0013	0.0000	0.0100
		Chla	2.9770	16.0305	28.3800	60.6478	78.6662	84.5685	496.3600
		lntkn_n	-1.3661	-0.6540	-0.3712	-0.3390	0.4969	-0.0510	1.1553
		lnno3_n	-6.8918	-5.4001	-4.3977	-4.4230	1.3223	-3.1229	-2.2690
		lntn	-1.1996	-0.5683	-0.3021	-0.2945	0.4655	-0.0400	1.1576
		lntp	-4.1352	-3.6889	-3.4112	-3.2935	0.6103	-2.9188	-1.5141
		lntn_tp	2.2424	2.6857	3.0064	2.9990	0.3602	3.2576	4.0132
		lnchla	1.0909	2.7745	3.3455	3.5802	0.9970	4.4375	6.2073
		temp	23.4200	25.1700	26.4550	26.5078	1.6406	28.2700	28.8300
		CE	0.1360	0.1635	0.1750	0.1812	0.0253	0.1940	0.2320
		pH	6.5800	7.5000	7.7650	7.7580	0.3870	8.0600	8.4600
		ORP	-26.0000	161.6000	244.6000	217.3610	86.7823	279.3500	343.8000
		Turb	0.0000	4.7500	6.7000	8.2580	8.1260	8.6000	40.8000
		DO	3.7100	6.6150	7.5000	7.3832	1.1871	8.2250	9.3100
		Sechi	0.6000	0.9000	1.0000	0.9963	0.1705	1.1000	1.5000

Table 9 (cont.)

lakename	N Obs	Variable	Minimum	Lower Quartile	Median	Mean	Std Dev	Upper Quartile	Maximum
Guajatac	100	TKN_N	0.0933	0.2065	0.2808	0.2742	0.0914	0.3263	0.5989
		NO3_N	0.0010	0.0010	0.0046	0.0052	0.0045	0.0069	0.0219
		TN	0.0943	0.2135	0.2845	0.2793	0.0915	0.3328	0.6043
		TN_TP	8.0248	16.9875	27.7405	32.6391	20.0622	39.5623	97.8401
		TP	0.0030	0.0060	0.0100	0.0109	0.0054	0.0120	0.0240
		DP	0.0000	0.0000	0.0000	0.0002	0.0006	0.0000	0.0030
		Chla	1.4000	4.3665	7.1545	7.4805	3.8405	9.7575	17.9530
		lntkn_n	-2.3716	-1.5775	-1.2702	-1.3531	0.3583	-1.1200	-0.5127
		lnno3_n	-6.8918	-6.8918	-5.3757	-5.6497	0.9183	-4.9783	-3.8211
		lntn	-2.3608	-1.5441	-1.2570	-1.3328	0.3540	-1.1001	-0.5037
		lntp	-5.8091	-5.1160	-4.6052	-4.6502	0.5346	-4.4228	-3.7297
		lntn_tp	2.0825	2.8324	3.3229	3.3174	0.5813	3.6776	4.5833
		lnchla	0.3365	1.4739	1.9677	1.8615	0.5864	2.2780	2.8878
		temp	25.4700	26.3650	27.8550	27.6466	1.3263	28.6950	30.1000
		CE	0.1970	0.2465	0.2640	0.2643	0.0282	0.2855	0.3070
		pH	7.4200	8.2150	8.3000	8.3091	0.2285	8.4300	8.9300
		ORP	76.9000	152.1500	203.4000	198.4660	67.4985	250.8500	338.9000
		Turb	0.0000	0.0000	0.8000	1.8070	2.7810	2.4000	10.0000
		DO	7.2700	8.0350	8.4000	8.4758	0.8035	8.5550	11.5300
		Sechi	1.0000	1.5000	1.7500	1.9486	0.7059	2.2250	4.3000
La Plata	100	TKN_N	0.2100	0.4006	0.5351	0.5658	0.2160	0.6887	1.2740
		NO3_N	0.0010	0.0036	0.0056	0.0264	0.0577	0.0170	0.2883
		TN	0.2159	0.4092	0.5488	0.5922	0.2351	0.7656	1.2787
		TN_TP	3.9976	9.4688	12.1288	12.4679	4.3741	14.5530	30.6121
		TP	0.0160	0.0355	0.0490	0.0519	0.0255	0.0620	0.1550
		DP	0.0000	0.0000	0.0000	0.0004	0.0009	0.0000	0.0040
		Chla	7.2400	14.0000	22.0050	26.3109	16.4755	34.7970	87.2950
		lntkn_n	-1.5607	-0.9149	-0.6253	-0.6387	0.3757	-0.3729	0.2422
		lnno3_n	-6.8918	-5.6233	-5.1770	-4.8534	1.4540	-4.0718	-1.2436
		lntn	-1.5331	-0.8937	-0.6000	-0.6003	0.3955	-0.2671	0.2459
		lntp	-4.1352	-3.3383	-3.0159	-3.0665	0.4727	-2.7806	-1.8643
		lntn_tp	1.3857	2.2480	2.4956	2.4662	0.3418	2.6778	3.4214
		lnchla	1.9796	2.6390	3.0913	3.0989	0.5819	3.5495	4.4693
		temp	25.6200	26.6600	27.9100	27.8373	1.3668	28.8500	30.2600
		CE	0.2350	0.2660	0.3180	0.3128	0.0453	0.3460	0.4040
		pH	8.1100	8.3900	8.6500	8.6268	0.2875	8.8550	9.3000
		ORP	24.3000	162.0000	200.4000	195.6290	70.5696	244.0000	314.3000
		Turb	0.0000	1.2000	2.5000	3.4760	4.1241	4.5000	19.4000
		DO	6.2300	8.1650	9.4350	9.3657	1.5608	10.4200	12.5400
		Sechi	0.5000	1.0000	1.4000	1.3457	0.4226	1.7000	2.3000

Table 9 (cont.)

lakename	N Obs	Variable	Minimum	Lower Quartile	Median	Mean	Std Dev	Upper Quartile	Maximum
Patillas	100	TKN_N	0.0467	0.1594	0.2030	0.2017	0.0936	0.2660	0.5429
		NO3_N	0.0010	0.0041	0.0084	0.0183	0.0237	0.0230	0.1176
		TN	0.0477	0.1635	0.2263	0.2201	0.0955	0.2820	0.5503
		TN_TP	4.8698	20.3883	27.1320	32.9322	20.0352	41.5145	110.1004
		TP	0.0030	0.0060	0.0060	0.0076	0.0032	0.0100	0.0200
		DP	0.0000	0.0000	0.0000	0.0001	0.0007	0.0000	0.0050
		Chla	0.5600	3.4520	5.0480	5.6618	3.2787	7.6460	15.7780
		lntkn_n	-3.0647	-1.8362	-1.5946	-1.7514	0.6191	-1.3243	-0.6109
		lnno3_n	-6.8918	-5.5055	-4.7849	-4.7559	1.3079	-3.7709	-2.1401
		lntn	-3.0432	-1.8108	-1.4861	-1.6406	0.5601	-1.2659	-0.5972
		lntp	-5.8091	-5.1160	-5.1160	-4.9599	0.4117	-4.6052	-3.9120
		lntn_tp	1.5831	3.0149	3.3007	3.3193	0.6171	3.7259	4.7014
		lnchla	-0.5798	1.2389	1.6190	1.5224	0.7354	2.0342	2.7586
		temp	25.3800	26.3750	28.0700	27.8556	1.5648	29.0400	30.2900
		CE	0.1200	0.1350	0.1440	0.1434	0.0119	0.1520	0.1650
		pH	7.1000	7.6400	7.7400	7.8504	0.3627	7.9650	8.8400
		ORP	22.6000	141.8500	242.2000	218.5520	92.7383	293.9500	358.5000
		Turb	0.0000	0.0000	0.1500	1.3150	2.7778	0.5500	14.8000
		DO	6.3000	7.2300	7.7700	7.6700	0.6399	8.1000	9.2400
		Sechi	1.2000	1.8250	2.5000	2.5294	0.7189	3.2000	3.8000
Toa Vaca	96	TKN_N	0.0933	0.2384	0.3243	0.3484	0.1388	0.4048	0.8727
		NO3_N	0.0010	0.0020	0.0051	0.0114	0.0160	0.0090	0.0612
		TN	0.0981	0.2691	0.3445	0.3598	0.1352	0.4144	0.8737
		TN_TP	5.1618	11.2008	14.5130	15.5259	7.2045	17.4240	51.3926
		TP	0.0120	0.0180	0.0220	0.0253	0.0111	0.0275	0.0660
		DP	0.0000	0.0000	0.0000	0.0008	0.0031	0.0000	0.0160
		Chla	1.5100	5.9450	12.1975	10.9731	5.8382	14.1690	28.6380
		lntkn_n	-2.3716	-1.4340	-1.1260	-1.1291	0.3944	-0.9043	-0.1362
		lnno3_n	-6.8918	-6.1986	-5.2826	-5.2482	1.2315	-4.7070	-2.7937
		lntn	-2.3220	-1.3127	-1.0656	-1.0895	0.3753	-0.8809	-0.1350
		lntp	-4.4228	-4.0174	-3.8167	-3.7507	0.3635	-3.5937	-2.7181
		lntn_tp	1.6413	2.4160	2.6750	2.6612	0.3917	2.8578	3.9395
		lnchla	0.4121	1.7823	2.5012	2.1903	0.7272	2.6510	3.3547
		temp	24.9200	26.2500	27.5000	27.4101	1.4630	28.5900	29.7500
		CE	0.2270	0.2615	0.2710	0.2808	0.0295	0.3035	0.3440
		pH	7.4000	7.8300	7.9650	8.0186	0.3179	8.2750	8.6000
		ORP	32.9000	137.3500	199.5500	197.9917	85.4693	270.6500	335.4000
		Turb	0.0000	0.4000	1.3500	2.1979	2.4352	3.4500	10.3000
		DO	1.6100	6.1000	7.0050	6.7597	1.6382	7.7450	9.5200
		Sechi	1.1000	1.4000	1.6000	1.7260	0.4816	2.0000	3.0000

Table 10. LSD Grouping for Means in selected reservoirs (*in a given column, means with the same letter are not significantly different using a=0.05*)

Lake Name	TKN-N (mg/L)	TN (mg/L)	TP (mg/L)	Chl-a (µg/L)
Cidra	0.72 A	0.76 A	0.037 B	36.93 A
La Plata	0.53 B	0.55 B	0.045 A	21.83 B
Toa Vaca	0.32 C	0.33 C	0.023 C	9.03 C
Guajataca	0.26 D	0.27 D	0.010 D	6.43 D
Cerrillos	0.18 E	0.20 E	0.009 D	4.76 E
Patillas	0.17 E	0.19 E	0.007 E	4.56 E

Table 11. LSD Grouping for Means for Secchi depth in selected reservoirs (*means with the same letter are not significantly different using a=0.05*).

Lake Name	Secchi depth (m)
Cerrillos	2.69 A
Patillas	2.54 B
Guajataca	1.95 C
Toa Vaca	1.72 D
La Plata	1.35 E
Cidra	1.00 F

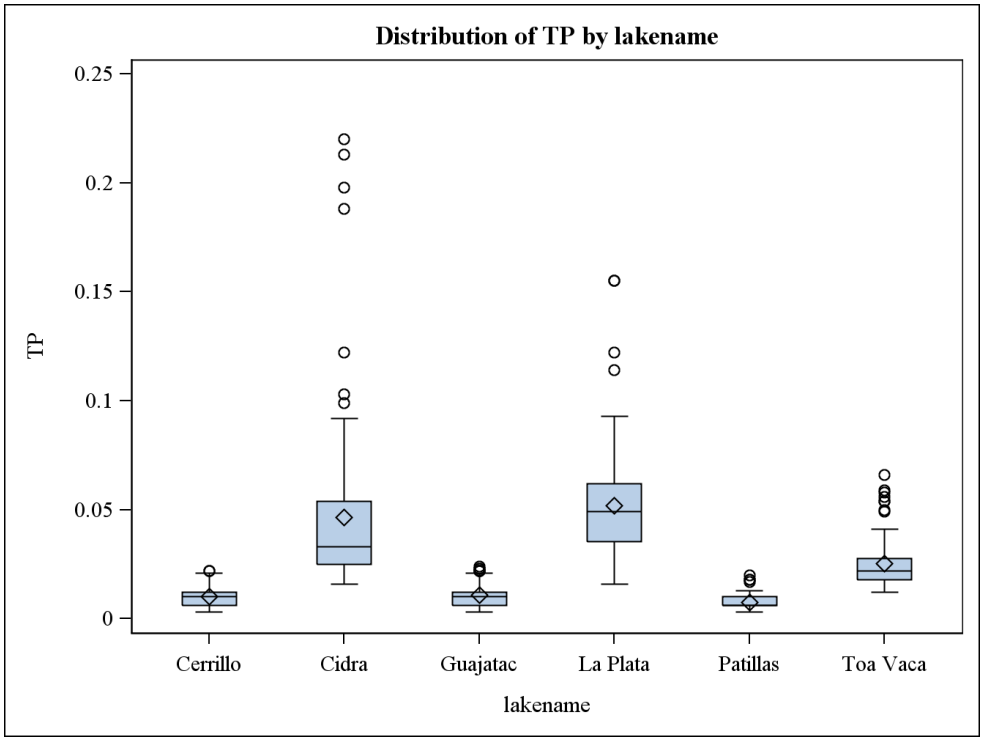


Figure 19: Distribution of total phosphorus concentrations (mg/L) in the selected reservoirs. The top and bottom box margins represent the 75th and 25th percentile of the distribution of values. The center line constitutes the median and the diamond symbol the average (mean).

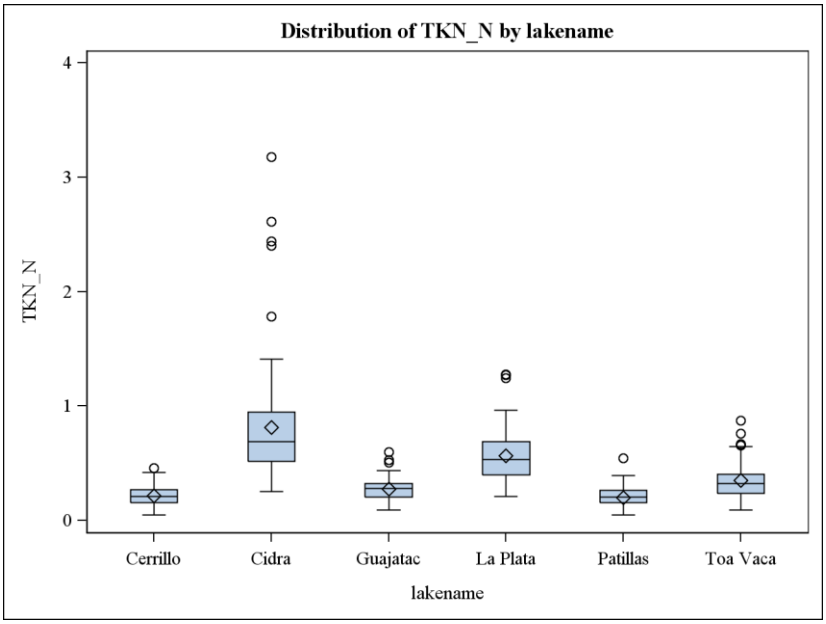


Figure 20: Total Kjeldahl nitrogen (mg/L) distribution in the selected reservoirs. The top and bottom box margins represent the 75th and 25th percentile of the distribution of values. The center line constitutes the median and the symbol diamond symbol the average (mean).

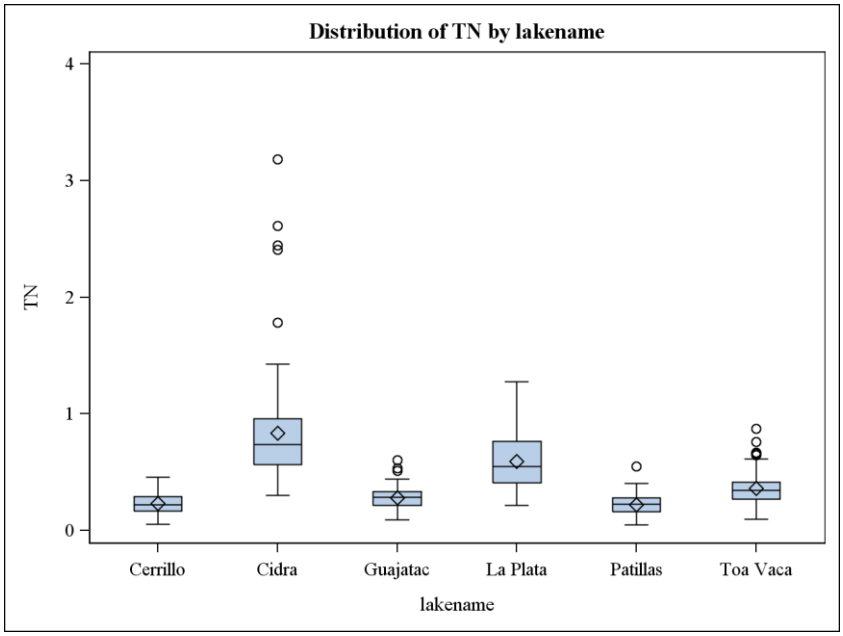


Figure 21: Distribution of total nitrogen concentrations (mg/L) in the selected reservoirs. The top and bottom box margins represent the 75th and 25th percentile of the distribution of values. The center line constitutes the median and the diamond symbol the average (mean).

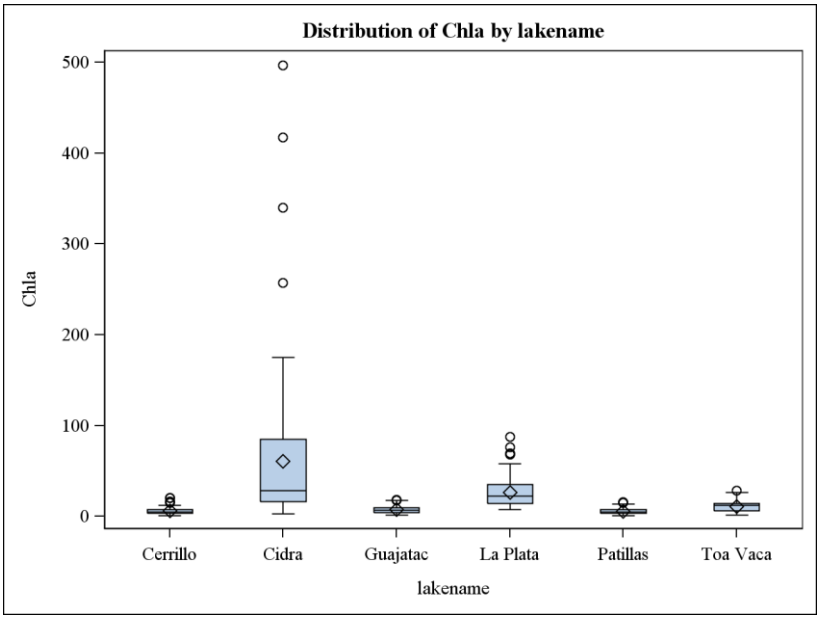


Figure 22: Distribution of chlorophyll *a* concentrations (µg/L) in the selected reservoirs. The top and bottom box margins represent the 75th and 25th percentile of the distribution of values. The center line constitutes the median and the diamond symbol the average (mean).

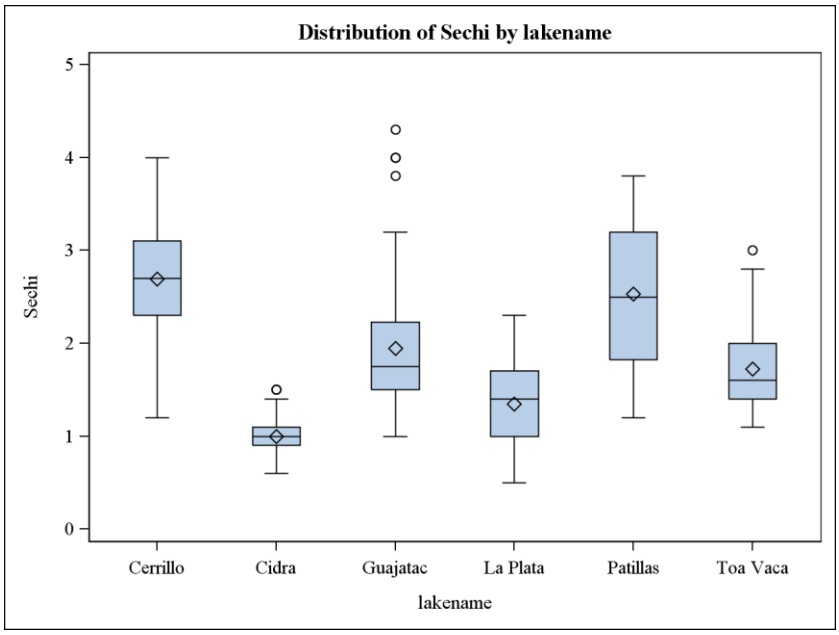


Figure 23: Distribution of Secchi depth values (m) at the selected reservoirs. The top and bottom box margins represent the 75th and 25th percentile of the distribution of values. The center line constitutes the median and the diamond symbol the average (mean).

4.2.2. Relationships Among Water-Quality Parameters

Pearson correlation coefficients for the complete data set showed strong positive correlations between TKN and Chl-a (r = 0.64), TN and Chl-a (r = 0.65), and TP and Chl-a (r = 0.73) (Table 12) (Figures 24 - 26). Both TN and TP were positively correlated (r=0.72) so it is not surprising that both nutrients exhibited similar correlation values relative to Chl-a. The relationship between TP and Chl-a is similar to the one obtained by Martínez et al. (2011, unpublished data) during a study conducted at Guajataca and La Plata. This is encouraging as it suggests that the relationship between TP and productivity (as measured by Chl-a) is valid throughout reservoirs covering a wide range of the productivity spectrum in Puerto Rico. The relationship between TP and Chl-*a* is similar to that reported in numerous studies worldwide (Table 12). Differences in slopes have been attributed to the effect of grazing (presence of large grazers lower the slope), stratification (stratification reduces the slope), and algal composition community among other factors (Mazumder, 1993; Felip and Catalan, 2000; Fernandes-Cunha, 2013). Regression coefficients (r) seem to indicate that total phosphorus has less explanatory power of the chlorophyll *a* variability in tropical lakes/reservoirs than in temperate lakes (Table 12). Among the hypotheses suggested to explain this behavior are: a greater relevance of nitrogen as a potential limiting nutrient in tropical systems, greater presence on non-algal particulates in tropical systems, and differences in morphometry and hydrologic regimes (White, E. 1983, Huzar, V., et al., 2006). Despite this, phosphorus still represents a major controlling factor of primary productivity in tropical reservoirs.

Table 12: Selected regression equations for the relationship between total phosphorus and chlorophyll a.

Linear regression equation	**r^2**	**Description**	**Reference**
Ln Chla = 0.94Ln TP – 0.381	**0.54**	**Puerto Rico's reservoirs**	**This study**
Ln Chla = 0.94Ln TP – 0.806	0.71	North American and European Lakes	Mazumder, 1994
Ln Chla = 0.87Ln TP – 0.622	0.89	Eastern North American Lakes	Nürnberg, 1996
Ln Chla = 0.80Ln TP – 0.576	0.64	Worldwide Lakes	Nürnberg, 1996
Ln Chla = 0.87Ln TP – 0.898	0.69	North American and European Lakes	Praire, et al., 1989
Ln Chla = 0.70Ln TP – 0.35	0.42	Tropical and Subtropical Lakes	Huzar, et al., 2006

Table 12 (cont).

Linear regression equation	r^2	Description	Reference
Ln Chla = 1.10Ln TP – 1.94	0.53	Tropical and Subtropical Reservoirs	Fernandes-Cunha, et al., 2013
Ln Chla = 0.96Ln TP – 1.27	0.94	OECD study	White, 1989
Ln Chla = 1.44Ln TP – 2.44	0.72	Worldwide Lakes	Carlson, 1977

As expected, increases in algae biomass density (as measured by Chl-a) diminish the light penetration depth into the lake profile (i.e., Secchi Depth). This is evidenced by the negative correlation coefficient obtained between these parameters (r=-0.67). In fact, both Chl-a and nutrients (i.e., TP, TKN, TN) were negatively correlated with Secchi depth (Table 13, Figures 27, and 28). A positive correlation (r= 0.76) was observed between pH and dissolved oxygen, a reflection of the influence of photosynthesis reactions on the reactivity of the systems (Figure 29).

Table 13: Pearson correlation coefficient between selected variables

Pearson Correlation Coefficients, N = 596 Prob > |r| under H0: Rho=0									
	lntkn_n	**lnno3_n**	**lntn**	**lntp**	**lntn_tp**	**lnchla**	**temp**	**CE**	**pH**
lntkn_n	1.00000	0.03967 0.3337	0.99020 <.0001	0.71837 <.0001	0.06525 0.1116	0.64060 <.0001	-0.01873 0.6481	0.10261 0.0122	0.03460 0.3991
lnno3_n	0.03967 0.3337	1.00000	0.13884 0.0007	0.03054 0.4568	0.11043 0.0070	0.06030 0.1415	-0.25235 <.0001	-0.08478 0.0385	-0.28868 <.0001
lntn	0.99020 <.0001	0.13884 0.0007	1.00000	0.72262 <.0001	0.07002 0.0876	0.64534 <.0001	-0.06282 0.1255	0.08354 0.0415	-0.01150 0.7794
lntp	0.71837 <.0001	0.03054 0.4568	0.72262 <.0001	1.00000	-0.63895 <.0001	0.73226 <.0001	-0.01010 0.8057	0.25710 <.0001	0.12721 0.0019
lntn_tp	0.06525 0.1116	0.11043 0.0070	0.07002 0.0876	-0.63895 <.0001	1.00000	-0.33857 <.0001	-0.05534 0.1773	-0.27805 <.0001	-0.19638 <.0001
lnchla	0.64060 <.0001	0.06030 0.1415	0.64534 <.0001	0.73226 <.0001	-0.33857 <.0001	1.00000	-0.06143 0.1342	0.11166 0.0064	-0.02267 0.5807
temp	-0.01873 0.6481	-0.25235 <.0001	-0.06282 0.1255	-0.01010 0.8057	-0.05534 0.1773	-0.06143 0.1342	1.00000	-0.03394 0.4082	0.39487 <.0001
CE	0.10261 0.0122	-0.08478 0.0385	0.08354 0.0415	0.25710 <.0001	-0.27805 <.0001	0.11166 0.0064	-0.03394 0.4082	1.00000	0.39778 <.0001
pH	0.03460 0.3991	-0.28868 <.0001	-0.01150 0.7794	0.12721 0.0019	-0.19638 <.0001	-0.02267 0.5807	0.39487 <.0001	0.39778 <.0001	1.00000
ORP	0.03822 0.3517	0.12139 0.0030	0.06882 0.0932	-0.04079 0.3202	0.13545 0.0009	-0.05533 0.1773	0.00920 0.8227	-0.13756 0.0008	-0.16183 <.0001
Turb	0.29086 <.0001	0.19974 <.0001	0.30918 <.0001	0.34543 <.0001	-0.15443 0.0002	0.34364 <.0001	0.11412 0.0053	-0.16735 <.0001	0.00326 0.9367
DO	0.07803 0.0569	-0.10779 0.0084	0.05282 0.1978	0.14293 0.0005	-0.14748 0.0003	0.09451 0.0210	0.26555 <.0001	0.10342 0.0115	0.76127 <.0001
Sechi	-0.66376 <.0001	-0.11513 0.0049	-0.66416 <.0001	-0.66590 <.0001	0.22186 <.0001	-0.67386 <.0001	0.05975 0.1452	-0.16259 <.0001	-0.05474 0.1820

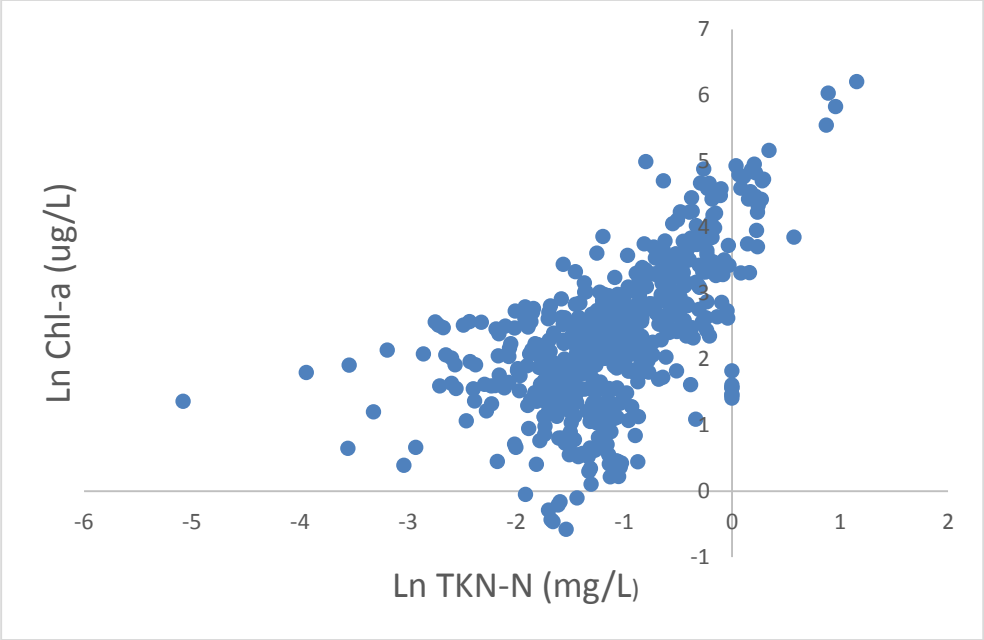


Figure 24: Relationship between total kjeldahl nitrogen (mg/L) and Chl-a (µg/L).

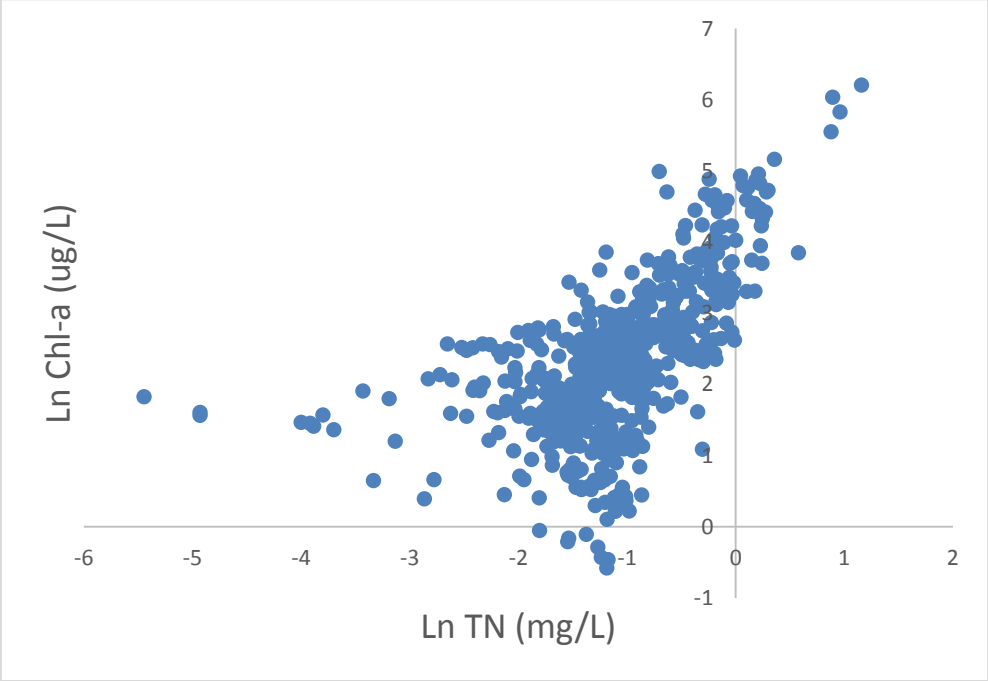


Figure 25: Relationship between total nitrogen (mg/L) and Chl-*a* (µg/L).

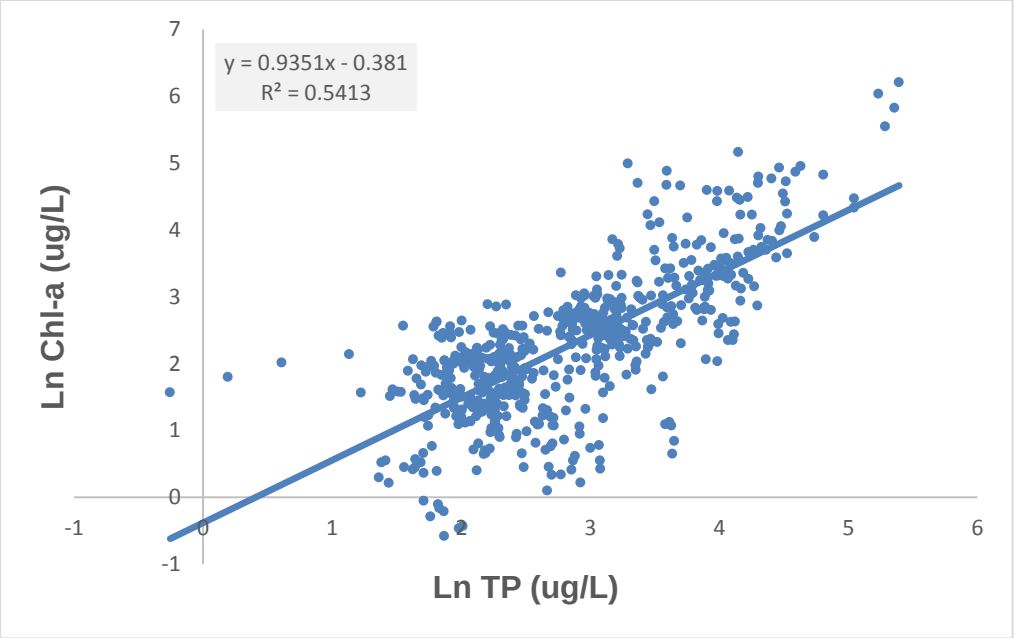


Figure 26: Relationship between total phosphorus (µg/L) and Chl-*a* (µg/L).

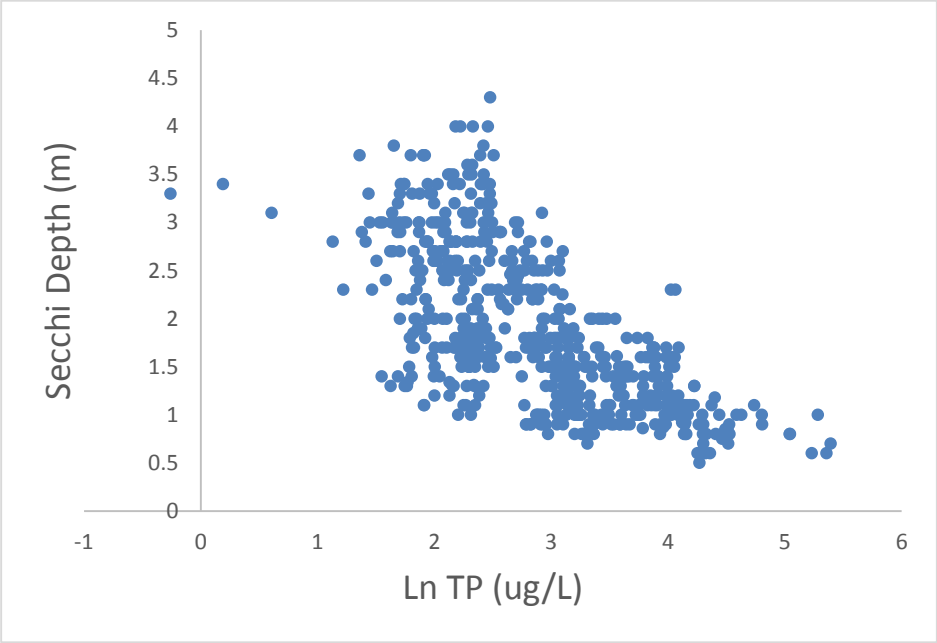


Figure 27: Relationship between total phosphorus and Secchi depth.

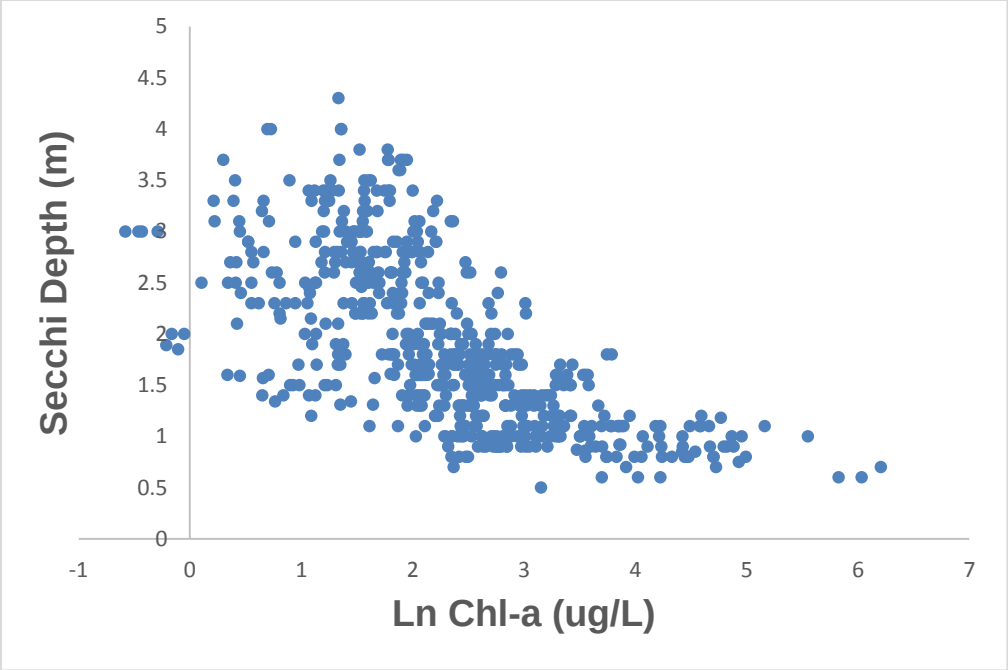


Figure 28: Relationship between chlorophyll *a* and Secchi depth.

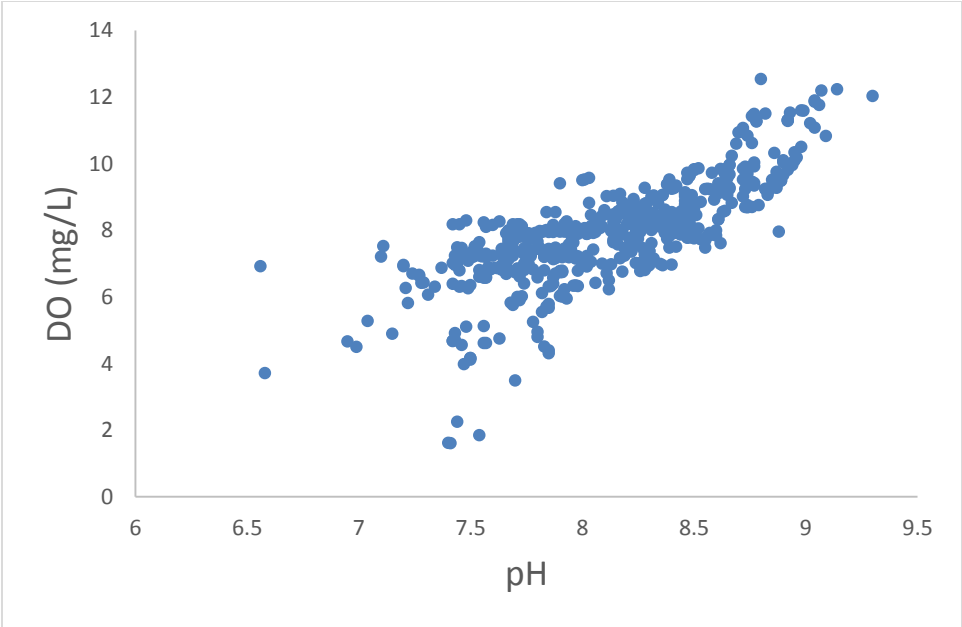


Figure 29: Relationship between dissolved oxygen and pH at the surface (1m).

4.2.3. Determination of numeric nutrient criteria

The relationships between chlorophyll *a*, Secchi Depth, and the aquatic life criteria were used to define an ecological threshlod of impairment for reservoirs of Puerto Rico. Secchi depth (SD) is a widely used tool in lake (reservoir) limnology. It offers a practical and reliable approach to estimating extent of light penetration in the water column. The extent of light penetration, particularly that corresponding to the photosynthetically active radiation [PAR (400-70nm)] fraction, greatly influences the spatial and temporal distribution of phytoplankton which in turn significantly impacts the dynamics of dissolved oxygen (DO), a critical parameter to aquatic life.

As stated, an inverse relationship exists between Chl-*a* (an index of algae biomass productivity) and the extent of light penetration as measured by SD. This relation has led to the worldwide adoption of SD as a trophic state defining criteria. Secchi depth values at or below 1m are considered indicative of impairment in lakes (Forsberg and Ryding, 1980; Nürnberg, 1996, Carlson and Simpson, 1996). We used our vertical profile data (DO, pH, temp., ORP) collected at Station B (i.e., station at the middle of the sampling transect) to explore in more detail the relationship between Chl-*a*, SD and DO in order to define a Chl-*a* threshold that could be used to establish the numeric criteria. The inverse relationship between SD and Chl-*a* (Figure 30) describes that there is a non-linear decrease in SD with Chl-*a*, followed by a well-defined threshold below which SD does not change significantly with further Chl-*a* increase (within the Chl-*a* range observed in this study). A segmented curve approach was used to identify the Chl-*a* value that best defines this transition (Table 14, Figure 30). The resulting value (23.6 µg/L Chl-*a*) represents a potential ecological threshold for establishing impairment conditions. At Chl-*a* values below 23.6 µg/L there is an exponential increase of light penetration with reductions in Chl-*a*, whereas at values greater than 23.6 µg/L Chl-*a*, SD values are less sensitive to change and have reached values that are considered indicative of impairment (i.e., 1m).

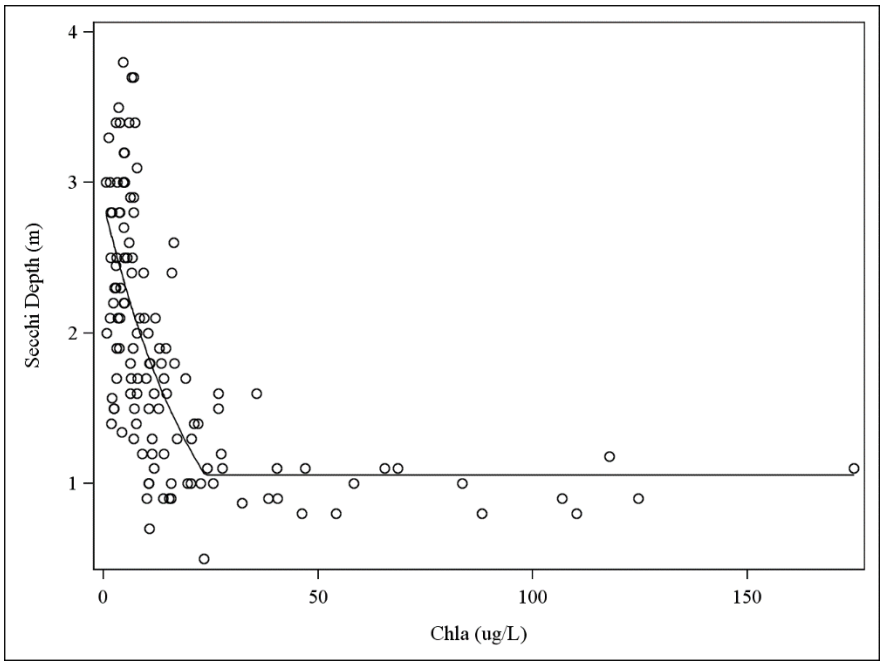


Figure 30: Relationship between chlorophyll *a* and Secchi depth at the water column profile station (Station B). Line represents the best fit of a segmented curved approach. The first segment was a negative exponential curve, and the second segment a horizontal line. The changepoint value for Chl-*a* (parameter g), was estimated as 23.6 µg/L.

Table 14: Results of a segmented curve fitting exercise on the Chl-a – SD data (station B).

Parameter Estimates					
Parameter	Estimate	Standard error	Pr > |t|	Lower interval	Upper interval
G	23.6	3.69	<.0001	16.316	30.95

The significance of the 23.6 µg/L Chl-*a* threshold as defining criteria is sustained by the relationship between Chl-*a* and DO obtained from the same data set. A plot of Chl-*a* values vs the maximum depth exhibiting DO values greater than 5mg/L results in a similar relationship as that of Chl-*a* and SD (Figure 31). We expressed DO concentrations in terms of the maximum depth in compliance with the USEPA aquatic life criteria (DO ≥ 5mg/L) due to the importance of this parameter for reservoirs of Puerto Rico. At present, all of our reservoirs are listed as impaired (PREQB 303(d) 2012) for violation to the USEPA DO criteria.

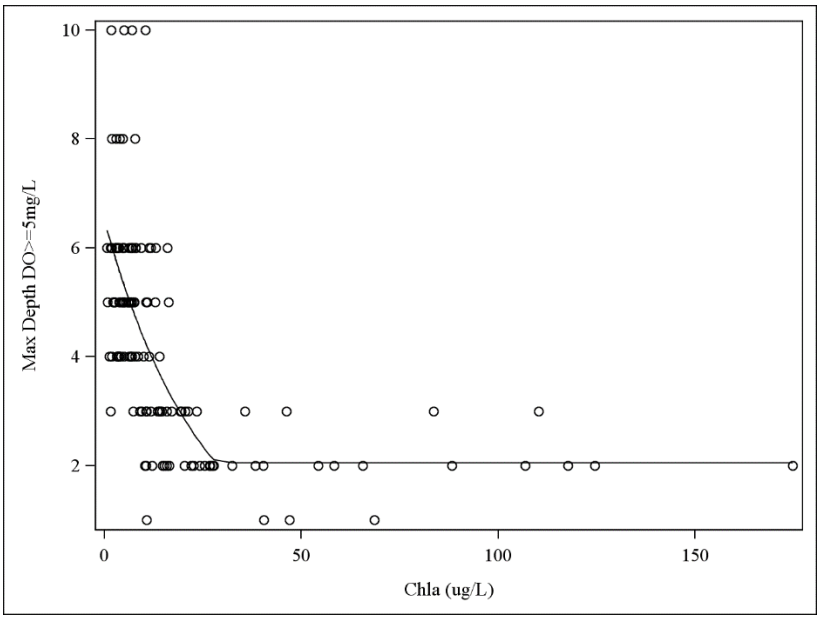


Figure 31: Relationship between chlorophyll *a* and the maximum depth with DO values ≥ 5mg/L. Data from at the water column profile station (Station B). Line represents the best fit of a segmented curved approach. The first segment was a negative exponential curve, and the second segment a horizontal line. The changepoint value for Chl-*a* (parameter g), was estimated as 28.3 µg/L.

The segmented curve approach for the relationship between Chl-*a* and DO yields a Chl-*a* concentration of 28.3 µg/L (Table 15). Significant increases in the maximum depth in compliance with the aquatic life criteria (i.e., DO ≥ 5mg/L) are observed at Chl-a concentrations below 28.3 µg/L. This type of data arrangement could be useful in developing a protocol for assessing aquatic life criteria compliance in reservoirs where hypolimnion anoxia may naturally occur. The value of 28.3 µg/L is similar to that obtained for the relationship between Chla- and SD. The latter (i.e., 23.6 µg/L) carries greater sensitivity due to the fact that SD values were recorded at 0.1m interval resolution whereas the DO profiles are based on discrete measurements obtained at 1m interval resolution. Altogether, the evidence suggests that at Chl-a values close to 24 µg/L the extent of light penetration in our reservoirs is significantly reduced (due to excessive phytoplankton biomass productivity) which hinders the capacity of our reservoirs to comply with the aquatic life criteria. On the other hand, an exponential increase in depth of light penetration is observed at Chl *a*- values < 24 µg/L. As stated this is reflected in an increase in the maximum depth with dissolved oxygen concentrations in compliance with the aquatic life criteria. We therefore suggest using 24 µg/L Chl-*a* as an impairment threshold to define the numeric criteria of reservoirs of Puerto Rico.

Table 15: Results of a segmented curve fitting exercise on the Chl-a – DO (max depth with DO values ≥ 5mg/L (station B).

Parameter Estimates					
Parameter	Estimate	Standard error	Pr > |t|	Lower interval	Upper interval
G	28.3	5.14	<.0001	18.1	38.5

TP and TN concentrations associated to our Chl-*a* impairment threshold (i.e., 24 µg/L) were established based on a change point analyses of the data. In order to determine a threshold (x_c) for a nutrient x, given that we know the corresponding threshold (y_c) for an environmental response y, consider $P(y>y_c \mid x>x_i)$ for all possible observed values of x_i. The assumption needed for interpreting these conditional probabilities is that they have a positive relationship with the nutrient (Paul and McDonald, 2005). The value x_c is determined as the changepoint value in the relationship $P(y>y_c \mid x>x_i)$ vs. x_i. In order to estimate this changepoint, several methods have been proposed (non-overlapping confidence intervals, non-linear model with a changepoint parameter, Bayesian changepoint model, and deviance reduction methods). Except for the deviance reduction methods and the nonlinear model, the other proposed methodologies assume normal distribution and constant variance. The deviance reduction method assumes a mixture model with two components, each with its own mean (and $\mu_2 \geq \mu_1$). Under the normal distribution assumption with equal variances, the changepoint is the value which minimizes the residual sum of squares. Not assuming normal distribution, the same method is equivalent to a least squares criterion, which is a commonly used method applied in many statistical settings. Here we apply the least squares method to determine Total Nitrogen and Total Phosphorus thresholds associated with our Chlorophyl-*a* impairment threshold. In each case, 1000 bootstrap samples were obtained, and the threshold was estimated as the average of the samples. 95% confidence intervals were estimated from the bootstrap samples using the percentile method.

Figure 32 show the relationship between the conditional probability and the nutrient level. The resulting values, 0.035 mg/L for TP and 0.43 mg/L for TN can be considered as nutrient thresholds associated with impairment (Table 16). To protect against impairment the Numeric Nutrient Criteria should incorporate a margin of safety. We have chosen to use the concentrations defining the lower end of the 90% confidence interval of the bootstrap distribution of values associated with our Chl-*a* impairment threshold (i.e., percentile 5) to prevent impairment. **These values are: 0.026 mg/L for TP and 0.41mg/L for TN**. Based on our data set reservoirs exhibiting said concentrations would also reach the Chl-*a* impairment threshold only 5% of the times. Thus we propose using 0.026 mg/L for TP and 0.41mg/L for TN as the nutrient criteria component of the eventual the numeric standards for reservoirs of Puerto Rico. The proposed values are in accord to nutrient standards adopted by other states of the U.S.A.

(http://cfpub.epa.gov/wqsits/nnc-development). It is recommended that the reservoirs condition be established based on median values of at least 5 consecutive samplings performed during the dry season.

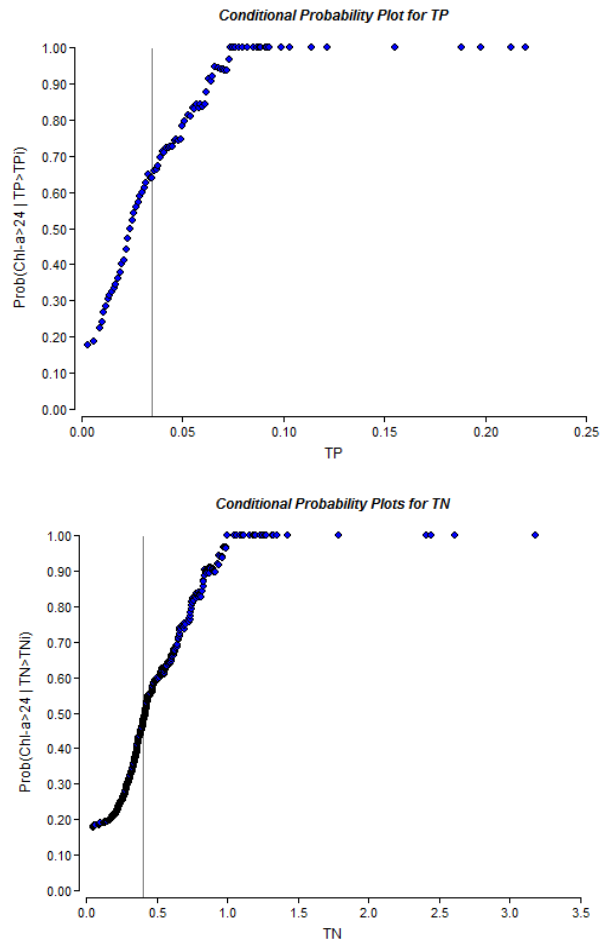


Figure 32: TP and TN conditional probability plots for our ecological Chl-a threshold.

Table 16: Changepoint estimates for TP and TN based on an impairment chlorophyll *a* threshold of 24 µg/L.

Stressor	Chl-*a* level (µg/L)	Changepoint	95% CI	
TP	24	0.0347	0.0260	0.0490
TN	24	0.4322	0.4035	0.4898

5.0 Water Column Profiles

In this section we present results of water column profiles for the following parameters: Temperature, pH, dissolved oxygen and oxidation-reduction potential (ORP). As stated previously, an aereation system was installed at Toa Vaca in late September, 2012. The operation of said system has altered the order of natural processes at the reservoir which is reflected in the parameters being measured. As such, results for Toa Vaca are divided in two phases: before and after the system's operation.

Water column profiles of turbidity, specific conductivity, total nitrogen and total phosphorus are presented in Appendix D.

5.1 Temperature

Tropical reservoirs commonly exhibit smaller temperature gradients within their water column (<10°C) relatively to their temperate counterparts. Despite this, tropical lakes/reservoirs usually experience long periods (≥ 8 months) of thermal stratification. This is a result of the considerable variations in the density of water at the temperature range that predominate in the tropics (i.e., 22°C to 31°C). At this range even small fluctuations in temperatures translate into a relatively stable stratified water column (Lewis Jr., 1996; Townsend, 1999; Sotomayor et al., 2007).

At Cerrillos we see a loss of thermal stratification with the arrival of our hemisphere winter months (i.e., November – February). This loss of stratification remains until mid-April, when stratification begins to set in. A more defined stratification persists until late October-November when the cycle repeats once again. The median surface temperature (1m) for Cerrillos during the monitoring period was 27.03°C, and that of bottom waters (≥10m) was 24.70°C (Figure 33). Similar to Cerrillos, temperatures at Cidra exhibit a loss of stratification during our hemisphere winter months. Said effect was more noticeable during the 2012-2013 "winter" (Nov. 2012- Feb. 2013) when relatively low temperatures were observed at the water surface, particularly during January-February, 2013. As with Cerrillos, thermal stratification sets in at mid-April and persists until late October, mid-November at Cidra. The median surface temperature (1m) for this reservoir was 26.49°C, and that of the bottom waters (≥10m) was 23.42°C (Figure 34). Contrary to what observed in Cerrillos and Cidra, both Guajataca and Patillas exhibit the beginning of a weak stratification earlier during the calendar year (Figures 35 and 36). The reservoirs remain stratified until November when surface water temperatures begin to drop significantly. As with Cidra, lower surface water temperatures persisted during the 2012-2013 "winter" (Nov. 2012- Feb. 2013) which slowed down the reinitiation of the thermal stratification process. Median surface temperatures for Guajataca and Patillas were: 27.91°C, and 28.27°C, while median temperatures of the bottom waters (≥10m) were: 25.04°C, and 25.40°C, respectively. La Plata has remained weakly to thermally stratified throughout the monitoring period (Figure 37). The median surface temperature (1m) for this reservoir was 28.14°C, and that of the bottom waters (≥10m) was 24.39°C. For Toa Vaca, the median

temperature at the surface (1m) and bottom waters (≥10m) are 27.51°C and 26.04°C respectively. As stated, the aireation system has significantly changed the thermal distribution pattern at this reservoir (Figure 38).

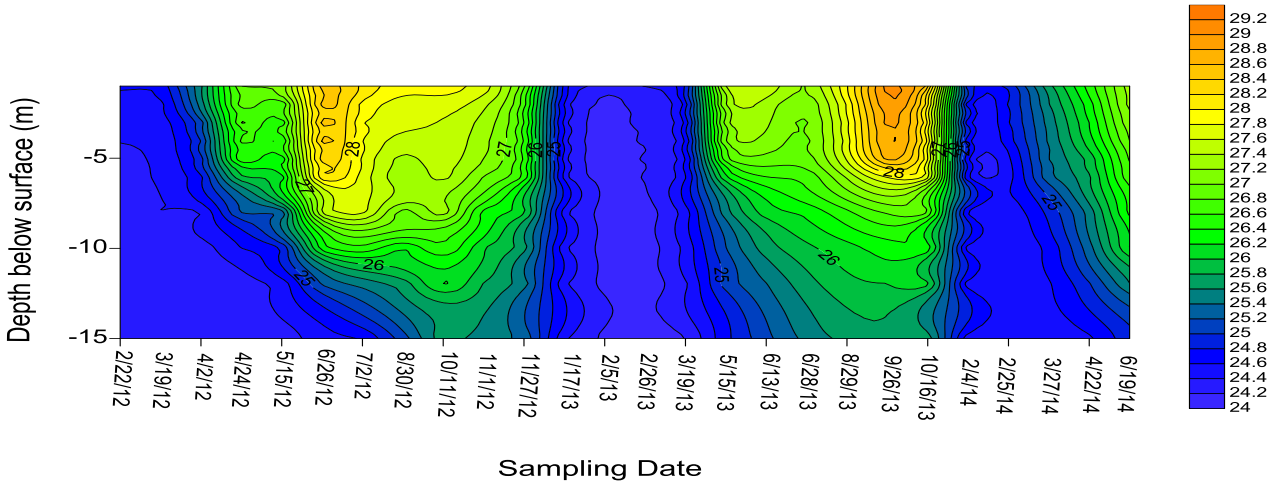


Figure 33: Temperature isopleth for Cerrillos.

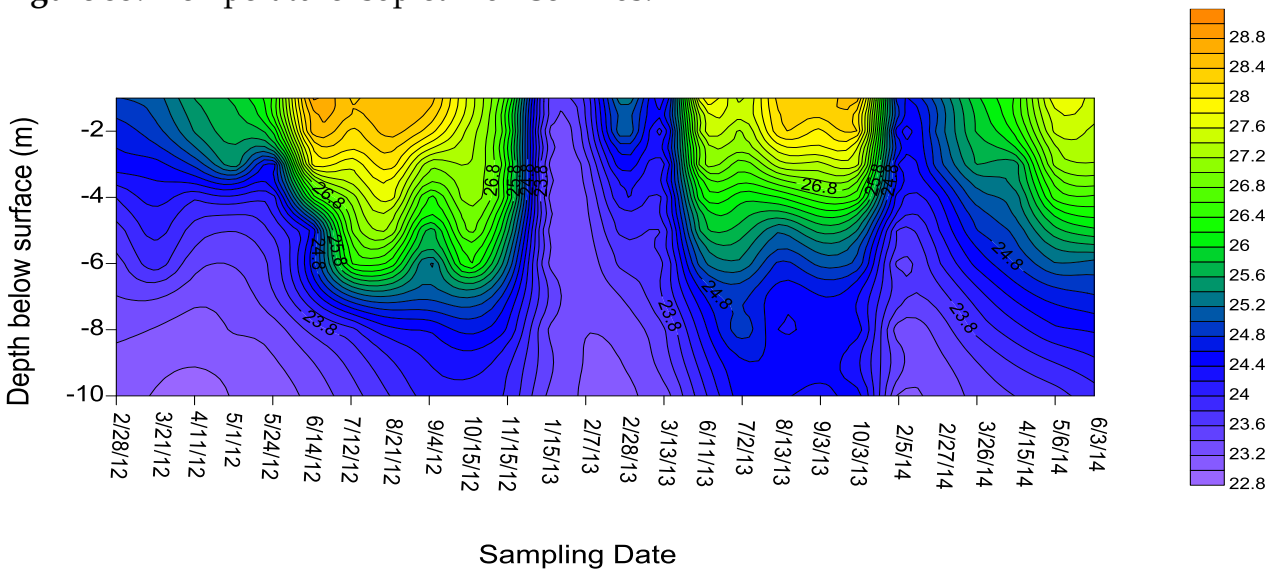


Figure 34: Temperature isopleth for Cidra.

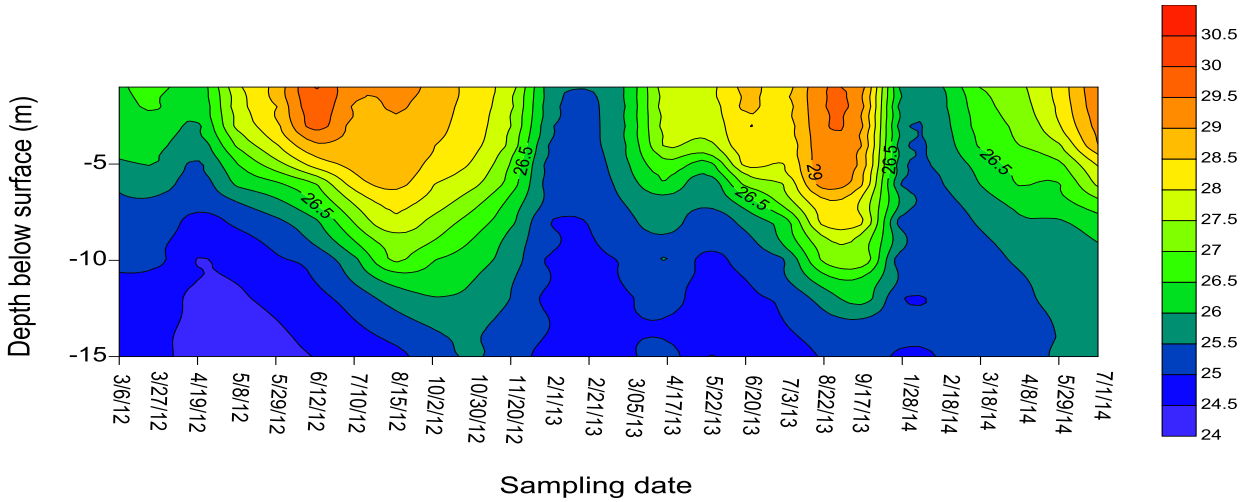


Figure 35: Temperature isopleth for Guajataca.

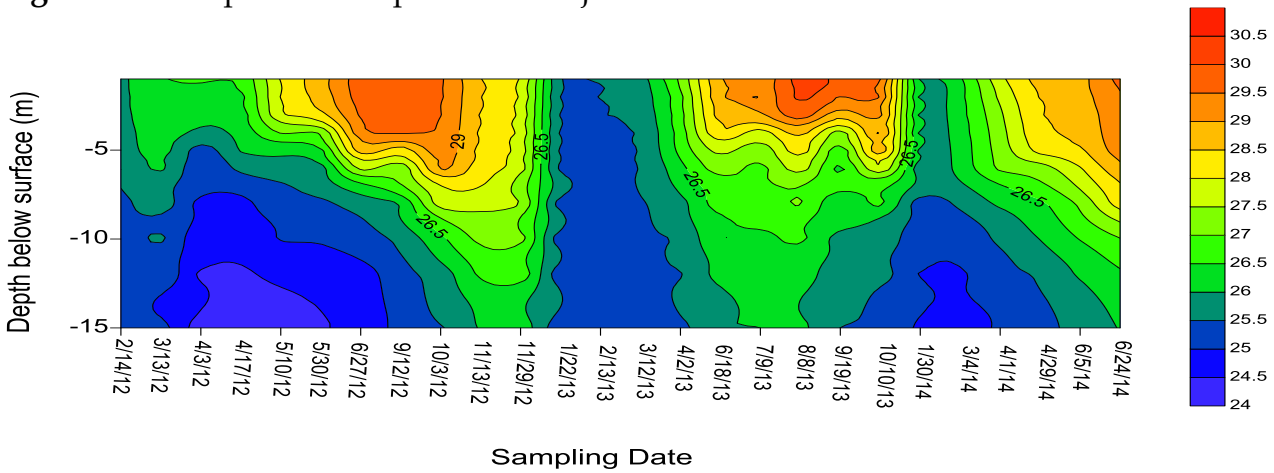


Figure 36: Temperature isopleth for Patillas.

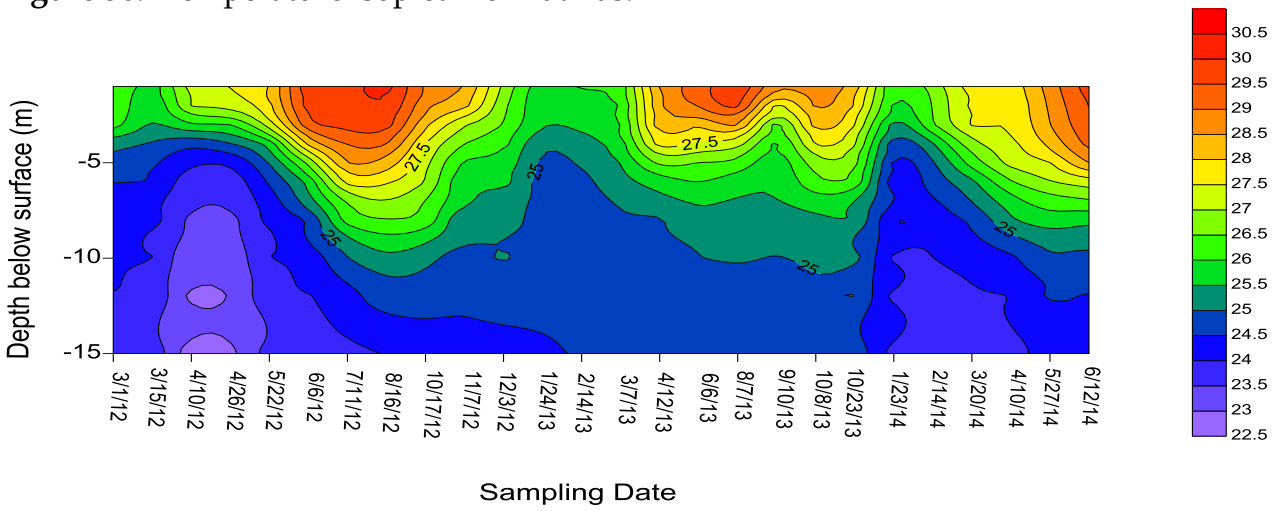


Figure 37: Temperature isopleth for La Plata.

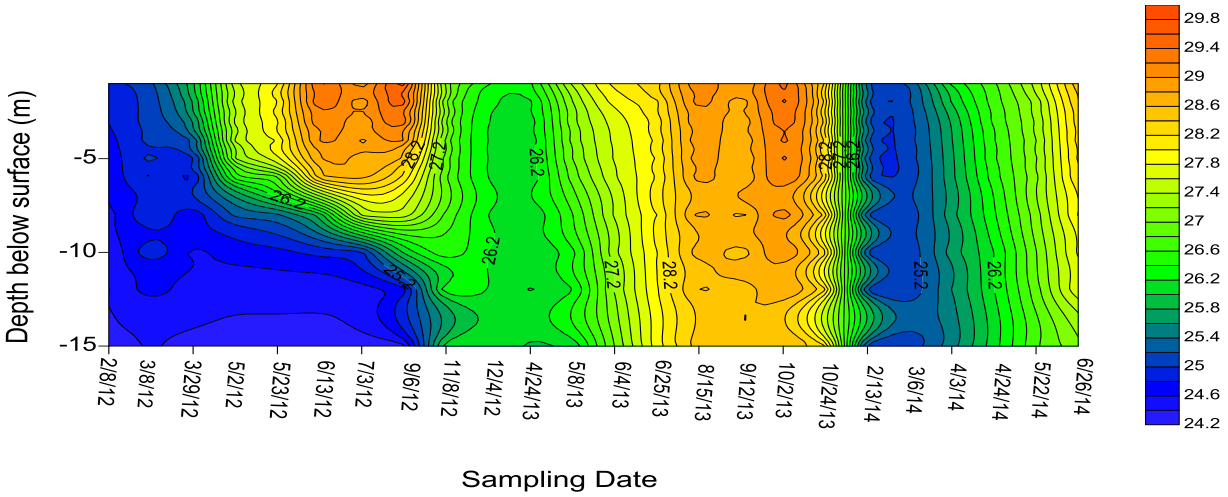


Figure 38: Temperature isopleth for Toa Vaca.

5.2 pH

The daytime pH of epilimnion waters (surface) is usually higher than at greater depths within the water column due to photosynthetic activities (i.e., dissolved carbon dioxide consumption) from the algal community. In contrast the pH of hypolimnion waters usually drops as a result of respiration (mineralization of organic matter) processes.

In Cerrillos, pH values reflected the process of thermal stratification, exhibiting uniform values throughout the water column during our "winter" months followed by notable increases in the pH of surface waters during stratification (Figure 39). A notable chemocline was observed at a depth of 7-8 meters during the period of stronger stratification. Median pH value at the surface (1m) was 8.41, and 7.42 at bottom waters (≥10m) at this reservoir. At Cidra, uniform pH values throughout the water column were noticeable during the 2012-2013 "winter" (Nov. 2012 – Feb. 2013) when low surface water temperatures predominated (Figure 40). At other times a chemocline around 3 meters was observed with greater pH values at the surface. Median pH value at the surface (1m) was 7.78, and 6.50 at bottom waters (≥10m). Guajataca and Patillas both showed a similar pattern, with a prevalent chemocline around 6-7 meters denoting a transition from higher pH values at the surface (Figures 41 and 42). This pattern was briefly disrupted during the 2012-2013 "winter" when the chemocline was eroded and relatively uniform pH values were observed throughout the water column as result of the loss in thermal stratification. Median pH values for Guajataca and Patillas at the surface were: 8.29, and 7.77, while values of the bottom waters (≥10m) were: 7.17, and 6.79, respectively. The effect of the 2012-2013 "winter" on the pH distribution of the water column was also observed at La Plata (Figure 43). At other times, a marked pH gradient is evident below a depth of 4 meters. Median pH value at the surface (1m) was 8.67, and 6.99 at bottom waters (>10m) for La Plata. For Toa Vaca, the median pH value at the surface (1m) was 7.99 and that of bottom waters was and 7.41 again reflecting the effect of the aireation system (Figure 44).

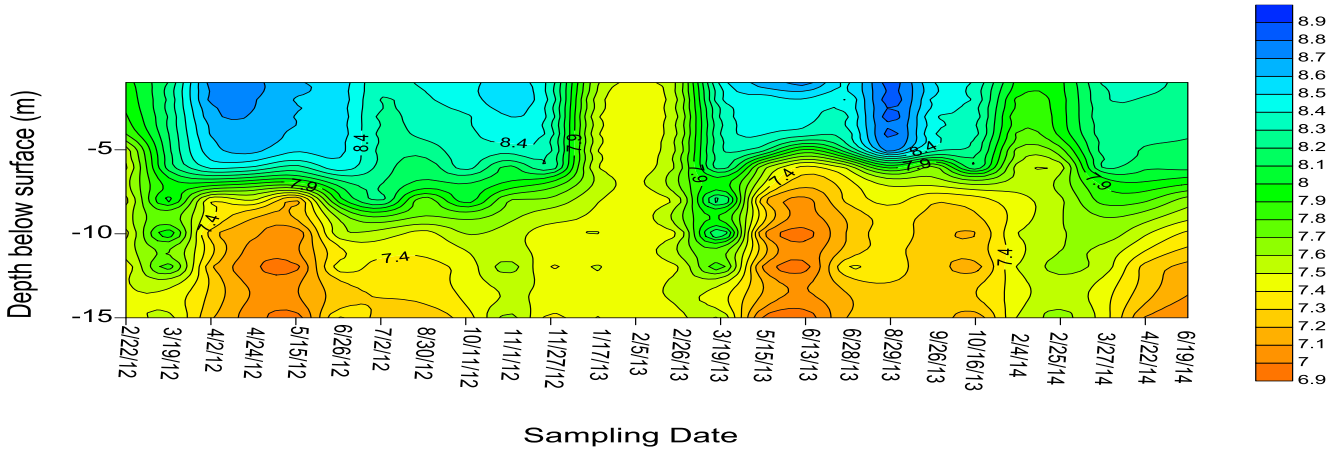


Figure 39: pH isopleth for Cerrillos.

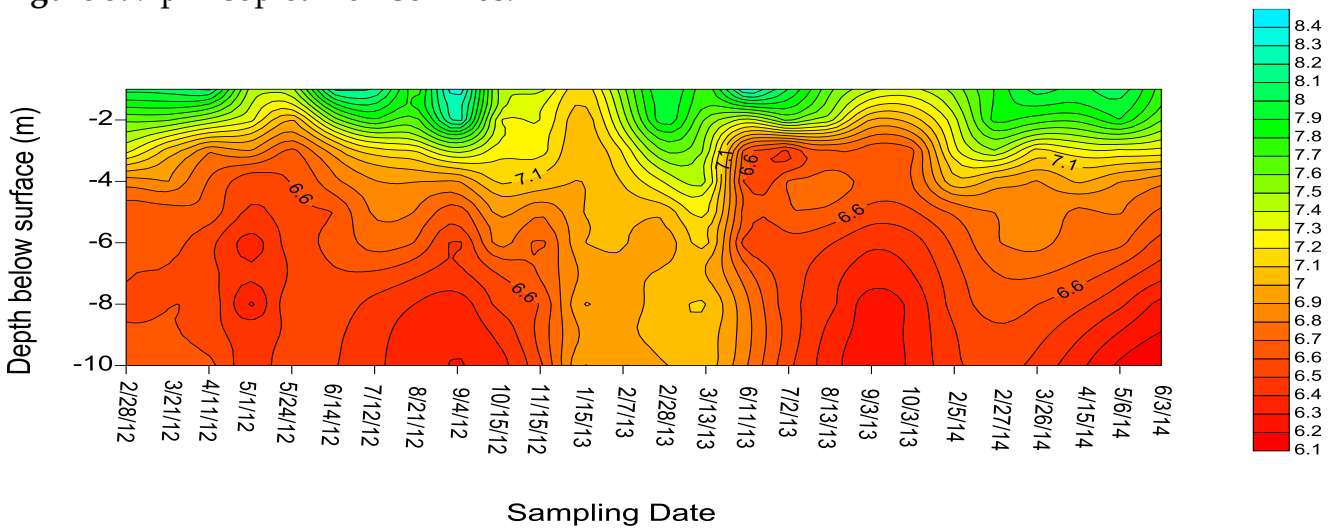


Figure 40: pH isopleth for Cidra.

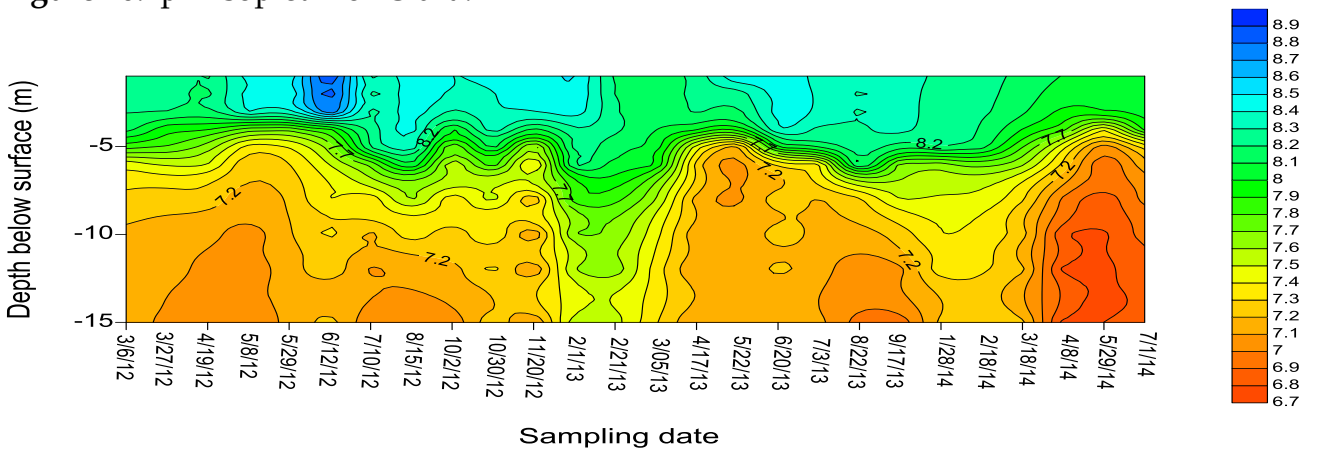


Figure 41: pH isopleth for Guajataca.

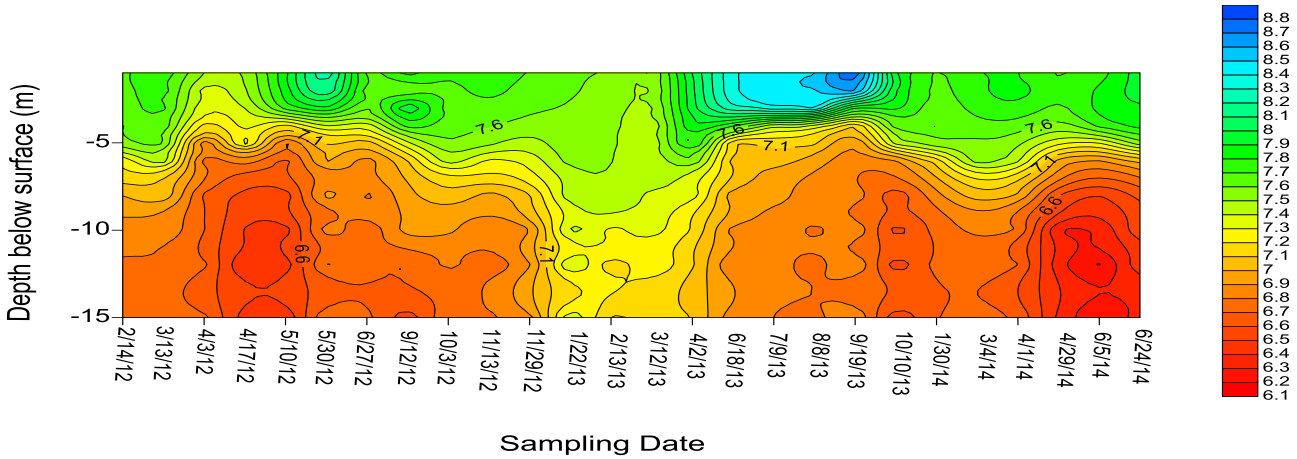


Figure 42: pH isopleth for Patillas.

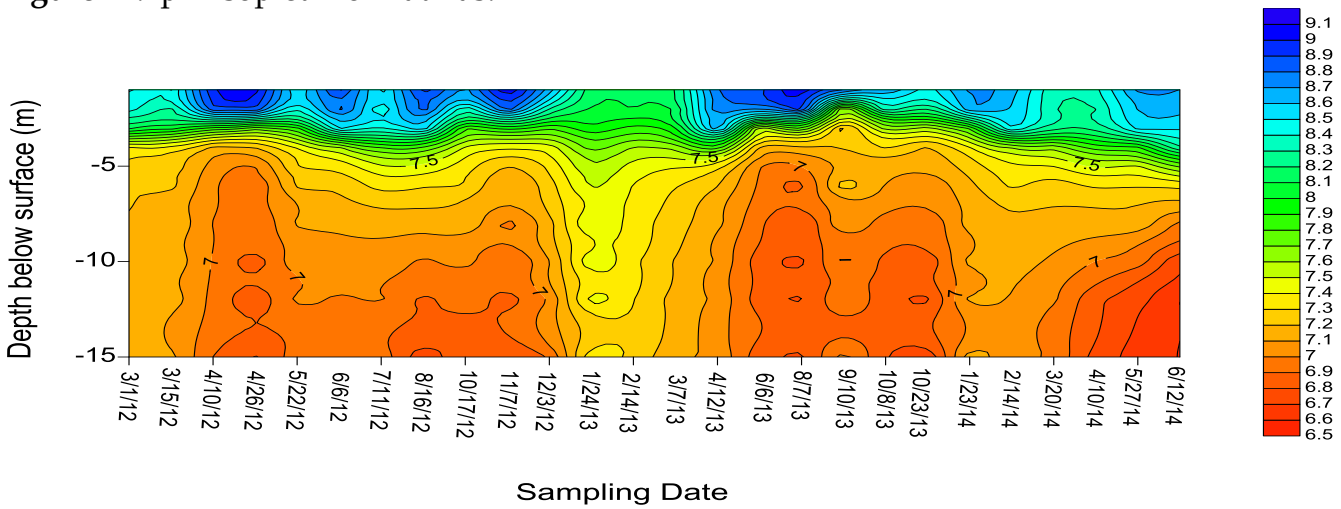


Figure 43: pH isopleth for La Plata.

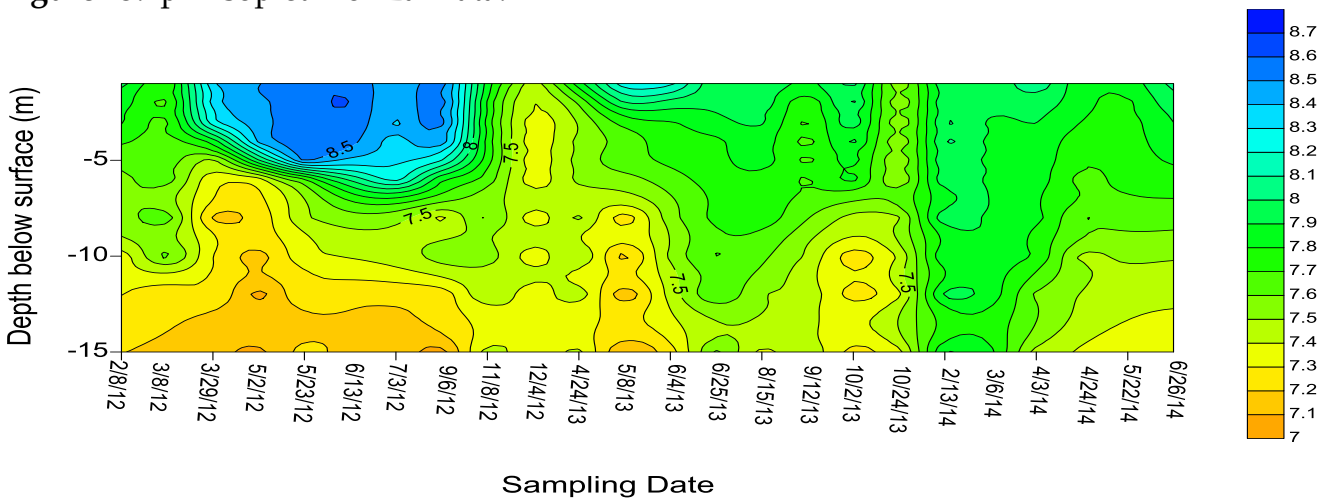


Figure 44: pH isopleth for Toa Vaca.

5.3. Dissolved Oxygen

Dissolved oxygen (DO) is essential to the metabolism of all aerobic aquatic organisms. Photosynthetic activities contribute to the accumulation of dissolved oxygen at the photic zone, whereas its counterpart process (i.e., respiration) results on its consumption.

Tropical waters are more susceptible to oxygen depletion than their temperate counterparts. This is a result of the reduced solubility of oxygen in warm waters coupled with higher rates of microbial metabolism experienced at higher temperatures (Townsend, 1996, 1999). Those effects are compounded by long periods of stratification commonly experienced by some tropical water-bodies. As a result, a significant oxycline, high DO values at the surface with a sharp transition to low or negligible values in hypolimnion waters is usually observed in tropical reservoirs. Since respiration involves the oxidation of organic matter the extent of hypolimnion anoxia has historically been related to trophic status. Eutrophic and hypertrophic reservoirs are more prone to developing hypolimnion anoxia than oligotrophic reservoirs, which has prompted this parameter to be used, at least in temperate regions, as an index of impairment. Certainly in our case, reservoirs with higher nutrient status (particularly, Cidra and La Plata) exhibited greater relative volume of anoxic waters than reservoirs of lower nutrient status (i.e., Patillas, and Cerrillos). The effects of thermal stratification are also evidenced on the definition and establishment of the oxycline. At Cerrillos, mixing of the water column during the winter months prevents the establishment of an oxycline and promotes the uniform distribution of dissolved oxygen throughout the water column (Figure 45). The effects were more dramatic during the 2012-2013 "winter" season as stated previously. Once stratified, Cerrillos exhibited DO values greater than 5 mg/L above a depth of 6 meters. The median DO value at the surface (1m) for this reservoir was 8.12 mg/L and 1.37 mg/L at depths equal to or greater than 10 meters. At Cidra, the effects of the 2012-1013 "winter" season were also evident (Figure 46). However, an oxycline can be generally observed at this reservoir during the rest of the year. During stratification, values greater than 5 mg/L occur generally above a depth of 3-4 meters. The median DO value at the surface (1m) for this reservoir was 7.53 mg/L and 0.15 mg/L (below sensor sensitivity) at bottom (≥10m) waters. An oxycline can be generally observed Guajataca during most part of the year (Figure 47). During stratification, values greater than 5 mg/L occur generally above a depth of 5 meters. The median DO value at the surface (1m) for this reservoir was 8.37 mg/L and 0.17 mg/L (below sensor sensitivity) at bottom (≥10m) waters. Patillas exhibited DO values greater than 5 mg/L above a depth of 5-7 meters during stratification (Figure 48). The median DO value at the surface (1m) for this reservoir was 7.80 mg/L and 0.24 mg/L (below sensor sensitivity) at bottom waters. La Plata exhibited DO values greater than 5 mg/L above a depth of 3-4 meters during stratification (Figure 49). The median DO value at the surface (1m) for this reservoir was 9.63 mg/L and 0.14 mg/L (below sensor sensitivity) at depths equal or

greater than 10 meters. Finally, at Toa Vaca the median DO values at the surface (1m) were 7.17 mg/L and 1.67 mg/L at bottom (≥10m) waters (Figure 50).

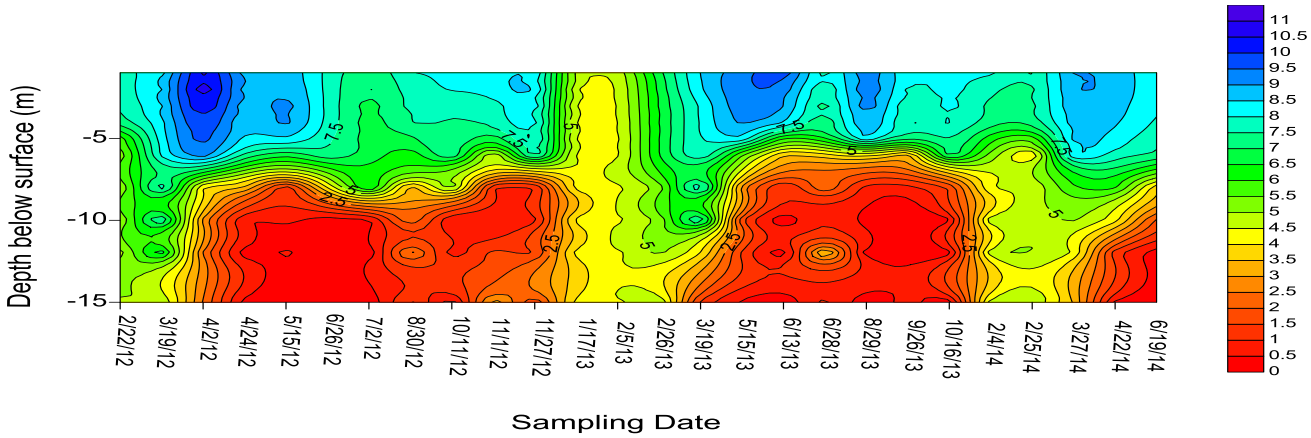


Figure 45: DO isopleth for Cerrillos.

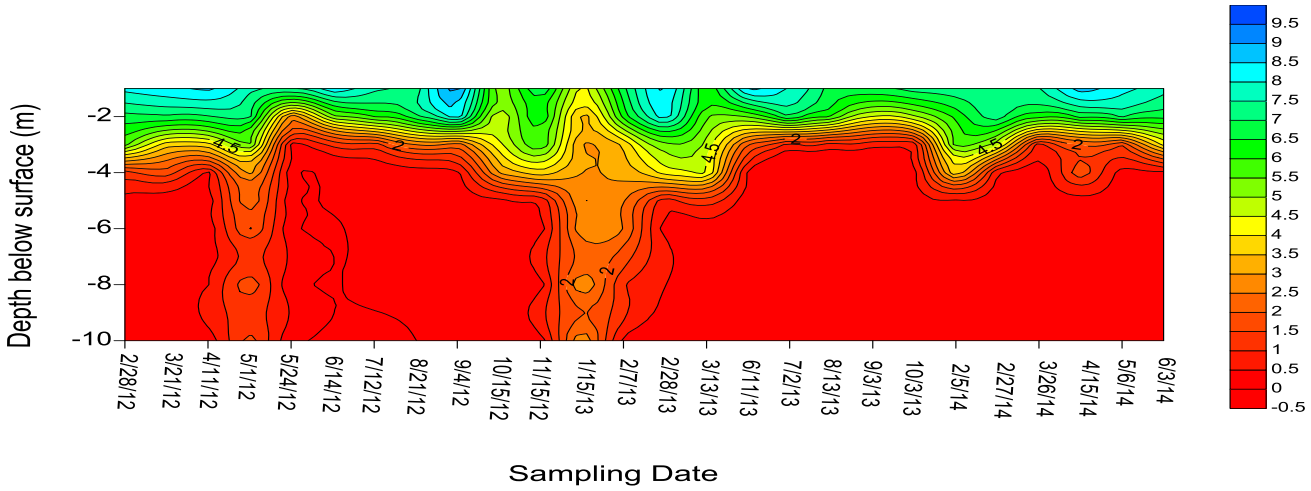


Figure 46: DO isopleth for Cidra.

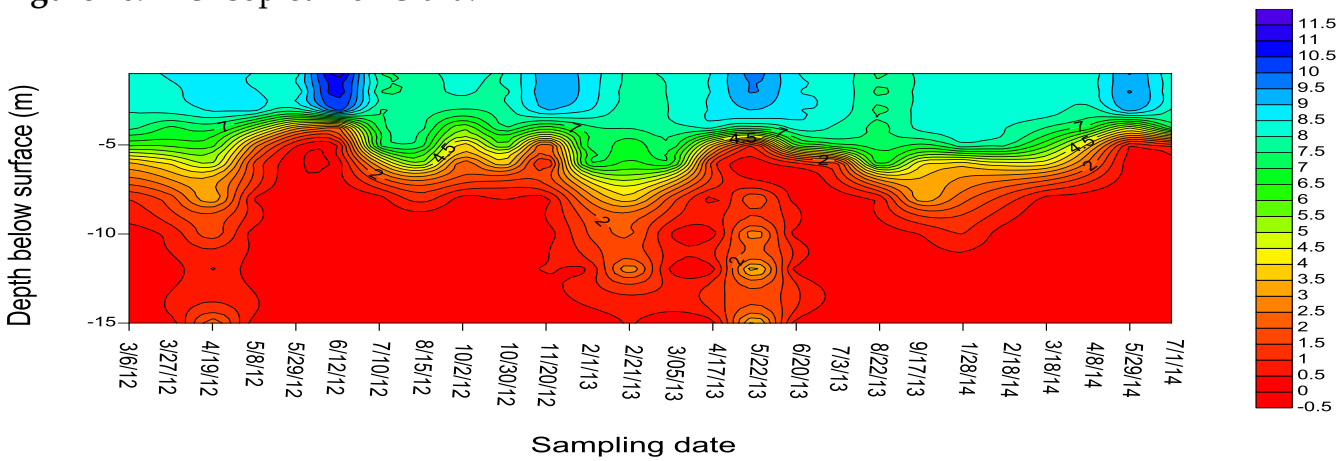


Figure 47: DO isopleth for Guajataca.

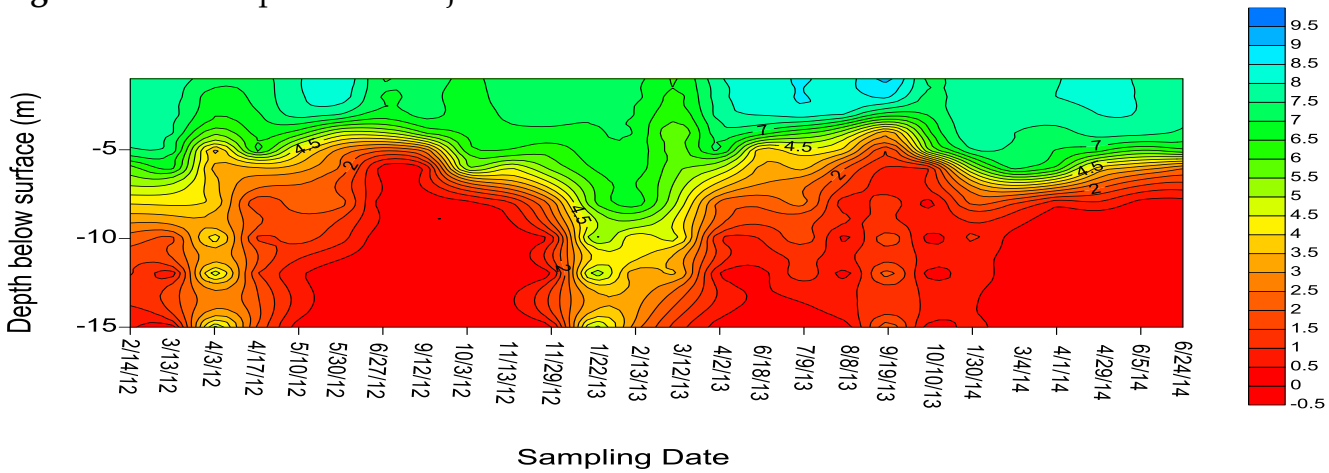


Figure 48: DO isopleth for Patillas.

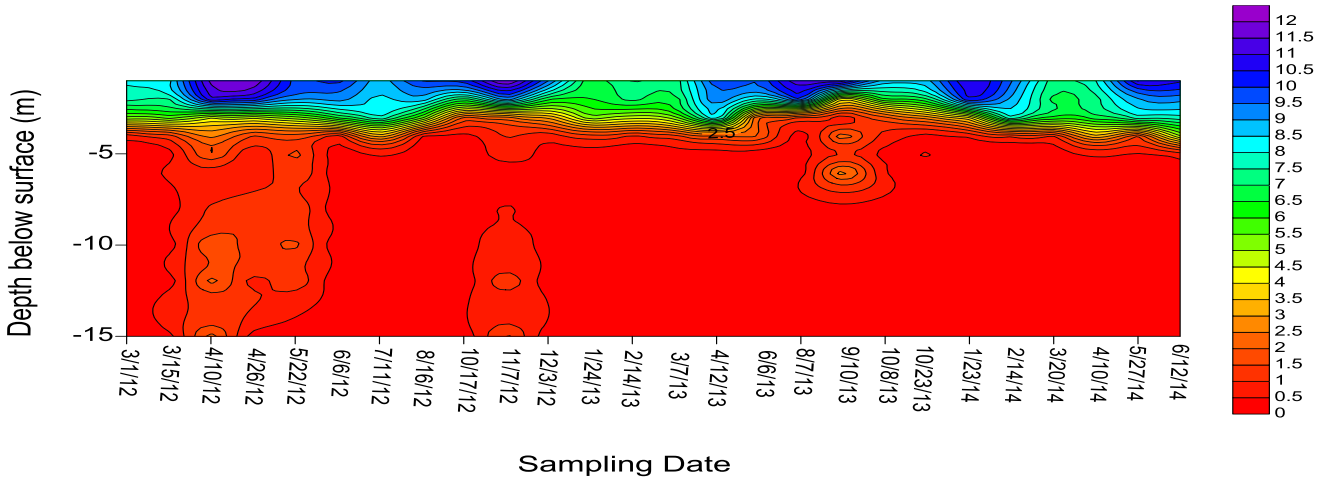


Figure 49: DO isopleth for La Plata.

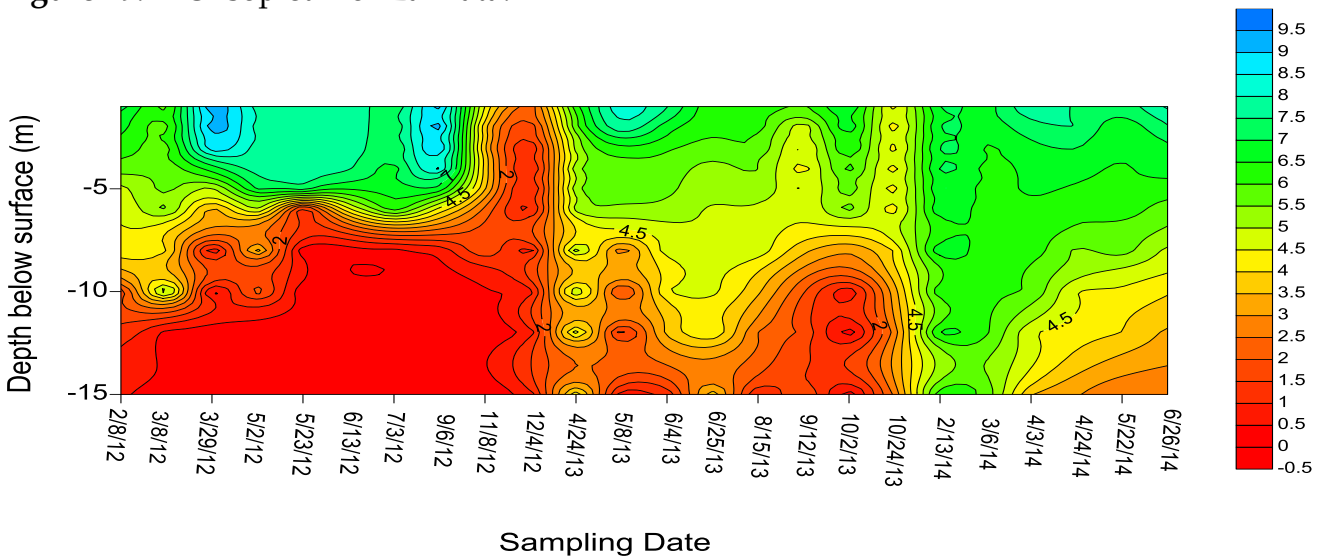


Figure 50: DO isopleth for Toa Vaca.

5.4 Oxidation Reduction Potential (ORP)

The oxidation reduction potential is a measure of the relative concentrations of oxidized to reduced members in a particular reaction. In general, reductive transformations bring about a considerable degradation in water quality, and intensive treatment is required for transformation into potable uses. Reducing conditions (i.e. <50 mV to negative potentials) bring about the conversion of nitrate (NO_3^-) to ammonia (NH_3), the reduction of iron (ferric (Fe^{3+}) to ferrous (Fe^{2+})), manganese (Mn^{4+} to Mn^{2+}), and sulfur (sulphate (SO_4^{2-}) to sulphide (S^{2-})), as well as the production of methane. These transformation reactions have a significant impact on the dynamics of nutrient and contaminant release from bottom sediments into the water column.

Strong reducing conditions have not been observed at Cerrillos within the first 15 meters during the monitoring period (Figure 51). The median ORP value at a depth greater than 10 meters was 343.32 mV. At Cidra, except for some brief periods during our "winter" season when the loss of thermal stratification promotes the complete mixing of the water column and thus the oxygenation of bottom waters, strong anoxic conditions prevail at the hypolimnion (Figure 52). The median ORP value at bottom (≥10m) waters was -137 mV for the monitoring period. At Guajataca and Patillas, median ORP values at bottom waters are -19.78 mV and 219.42 mV respectively (Figures 53 and 54). At La Plata, the effects of mixing, either as a result of rainfall events or as a result of the loss of thermal stratification, are evident in the ORP isopleths (Figure 55). In the absence of said events strong reducing conditions develop at the hypolimnion. The median ORP value at depths equal to or greater than 10 meters for this reservoir was -94 mV for the monitoring period. At Toa Vaca the median ORP value at bottom waters is 194.1mV (Figure 56).

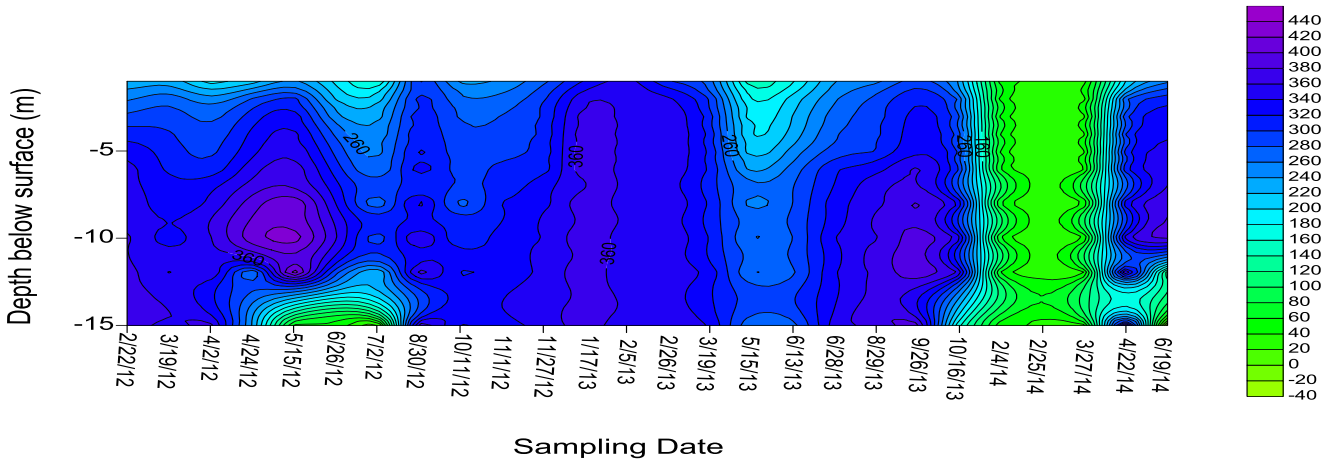


Figure 51: ORP isopleth for Cerrillos.

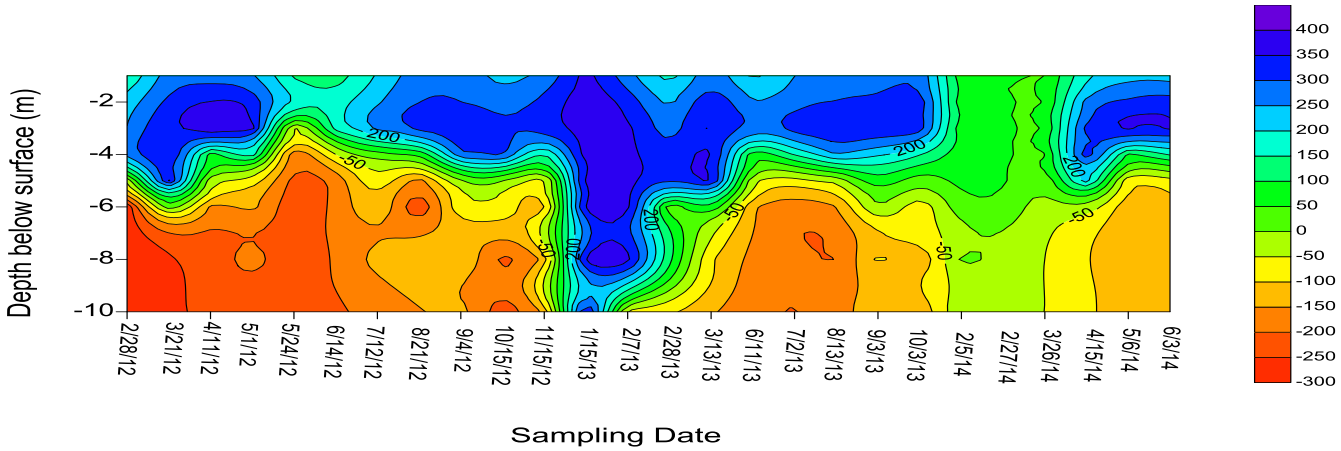


Figure 52: ORP isopleth for Cidra.

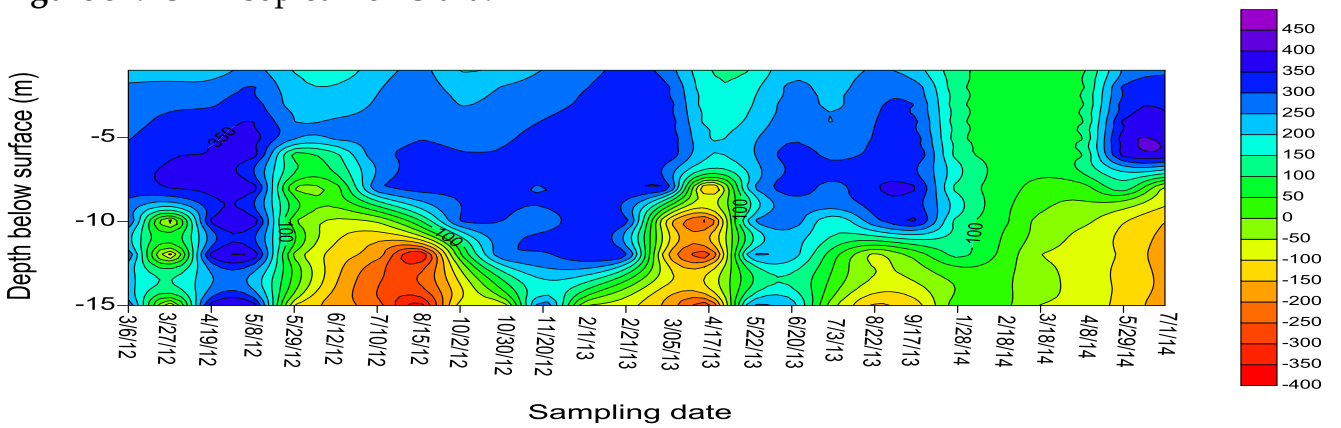


Figure 53: ORP isopleth for Guajataca.

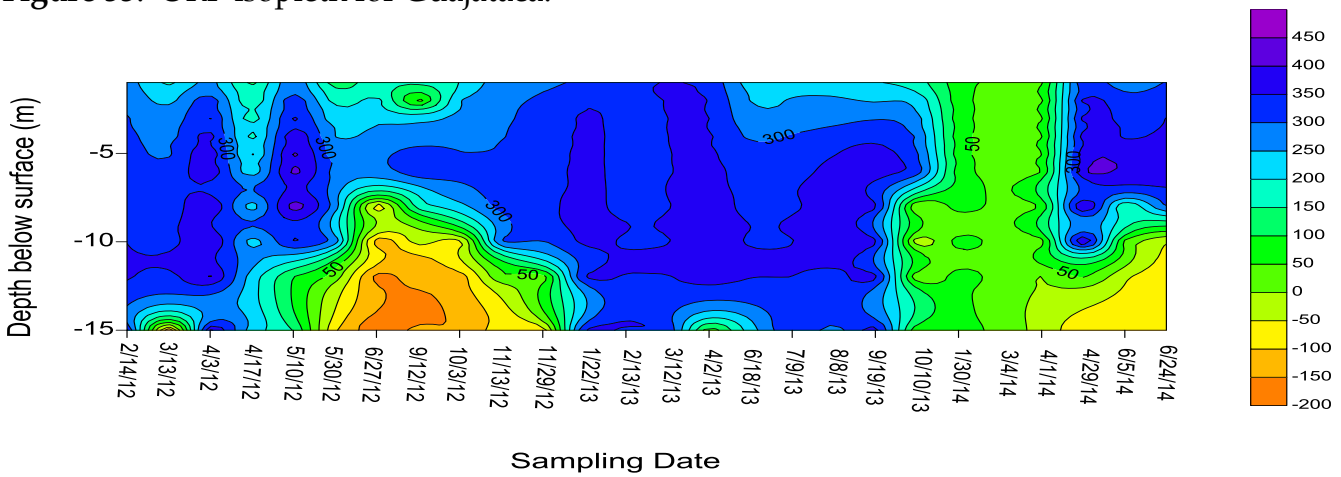


Figure 54: ORP isopleth for Patillas.

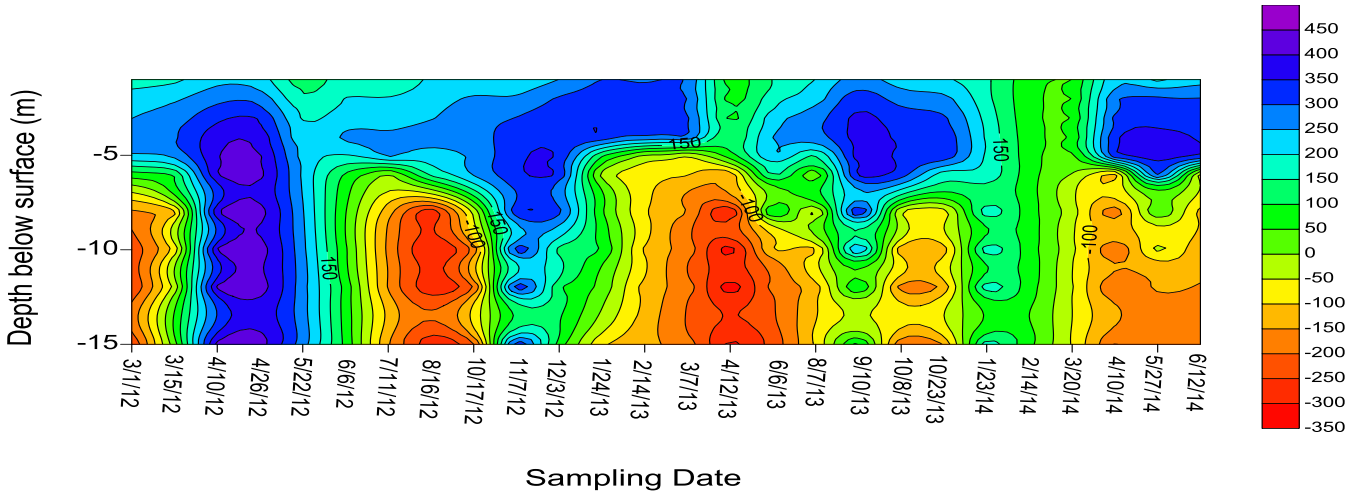


Figure 55: ORP isopleth for La Plata.

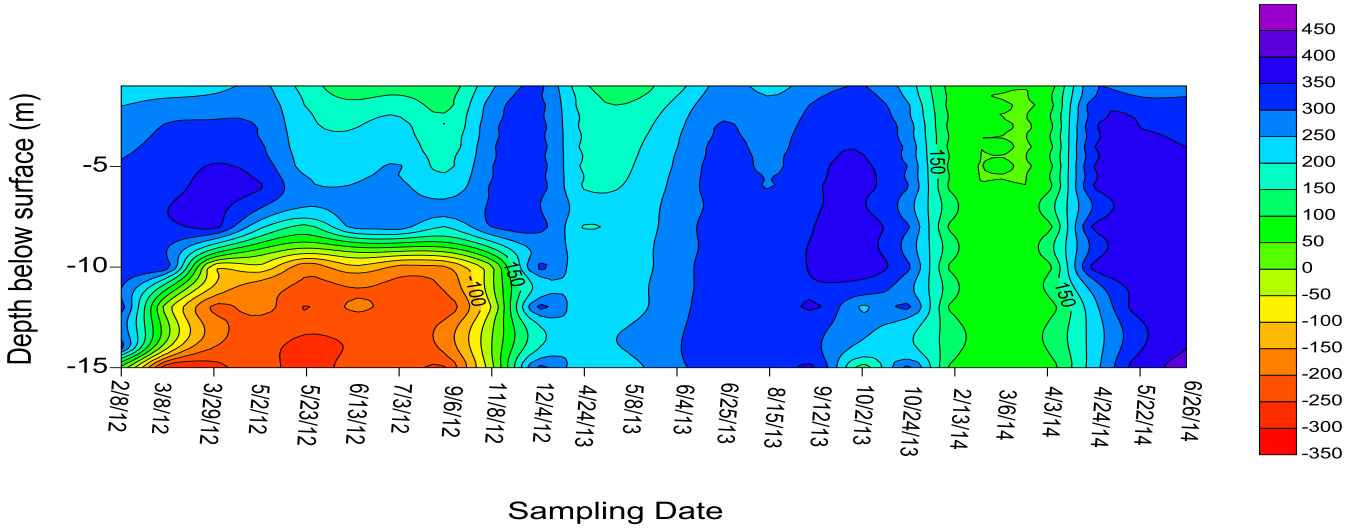


Figure 56: ORP isopleth for Toa Vaca.

6.0. Elemental composition of reservoir waters

The chemical composition of reservoir waters influences primary productivity and largely determines the quality of the resource for potable purposes. Some of the elements, particularly metals, are strongly influenced by several parameters that are intrinsically tied to the productivity of the systems (e.g., pH, DO, redox). On the interest of gaining additional information on the reservoirs being sampled we submitted samples obtained from each reservoir at different depths of the water column to a basic chemical characterization. The analyses included elements (basically cations) that our research team considers important to either primary productivity or to water quality for potable purposes. These analyses are not part of the project objectives and have been conducted only with the purpose of complimenting our analyses. The analyses were conducted by the Soil, Plant and Water Laboratory of the University of Georgia. Table 17 shows a

summary of the basic statistics for each element of interest. Figures 57 to 60 show the distribution of the concentration of the primary ions of interest in surface (1m) and hypolimnion (15m) waters. Depth-time isopleth for each element with concentration over detection limits are included in Appendix F.

The concentration of aluminum in hypolimnion waters of La Plata (mean- 43.90 µM) was significantly higher than concentrations reported at any other lake-depth combination (Figure 57). This result may be linked to the lithology and the nature of the soils that predominate in La Plata basin. Similarly, the results for calcium suggest a link between the observed results and the geologic substrate at each basin. Calcium concentrations at Guajataca (located in a calcareous basin) were significantly higher those of the other reservoirs (Figure 58). Concentrations at Cerrillos and Toa Vaca (both basins with significant calcareous intrusions) were next in decreasing order and were significantly higher than values at La Plata, Cidra and Patillas. The latter reservoirs are located in predominantly volcanic basins and placed lower in terms of calcium concentrations. In the case of iron the hypolimnion waters of Cidra (mean- 93.94 µM) exhibited significantly higher concentrations than those of the other reservoirs (Figure 59). No statistical difference was observed between other reservoir-depth combinations despite a relatively high mean value observed at the bottom waters of La Plata (35.16 µM). In terms of manganese, all reservoirs exhibited significantly higher concentrations at the hypolimnion than in surface waters, except for Cerrillos and Guajataca (Figure 60). In general, the concentrations of manganese and iron exhibited significant negative correlations with pH, dissolved oxygen and redox potential. Said results are expected due to the relevance of reductive dissolution reactions on the fate of these elements in nature.

Table 17: Summary statistics of concentrations of selected elements in reservoirs waters (all depths combined).

Reservoir	Element (units)	N	Average	Median	Min. Value	Max. Value
La Plata	Al (µM)	69	14.51	1.85	1.85*	123.04
	Ca (mM)	69	0.64	0.63	0.14	1.07
	Fe (µM)	69	15.28	7.22	0.90*	79.19
	Mg (mM)	69	0.45	0.44	0.07	0.76
	Mn (µM)	69	5.86	3.37	0.46*	35.04
Cidra	Al (µM)	54	4.37	1.85*	1.85*	28.40
	Ca (mM)	54	0.31	0.29	0.08	0.64
	Fe (µM)	54	66.41	5.51	0.90*	491.71
	Mg (mM)	54	0.26	0.28	0.06	0.38
	Mn (µM)	54	14.65	10.28	0.46*	50.15

Table 17 (cont):

Patillas	Al (µM)	68	2.88	1.85*	1.85*	17.09
	Ca (mM)	68	0.20	0.20	0.07	0.28
	Fe (µM)	68	3.70	0.90	0.90*	25.69
	Mg (mM)	68	0.19	0.19	0.03	0.26
	Mn (µM)	68	2.60	1.04	0.46*	19.00
Toa Vaca	Al (µM)	61	2.13	1.85*	1.85*	14.16
	Ca (mM)	61	0.82	0.80	0.42	1.06
	Fe (µM)	61	2.68	0.90*	0.90*	13.82
	Mg (mM)	61	0.41	0.40	0.18	0.56
	Mn (µM)	61	4.14	0.46	0.46*	23.52
Cerrillos	Al (µM)	64	1.85*	1.85*	1.85*	1.85*
	Ca (mM)	64	0.77	0.82	0.17	0.88
	Fe (µM)	64	0.90*	0.90*	0.90*	0.90*
	Mg (mM)	64	0.24	0.26	0.05	0.28
	Mn (µM)	64	0.59	0.46*	0.46*	3.62
Guajataca	Al (µM)	68	2.01	1.85*	1.85*	5.95
	Ca (mM)	68	1.12	1.22	0.28	1.43
	Fe (µM)	68	4.12	0.90*	0.90*	25.81
	Mg (mM)	68	0.15	0.16	0.04	0.18
	Mn (µM)	68	1.67	0.46*	0.46*	8.01

*- value reported is ½ the detection limit for the respective element

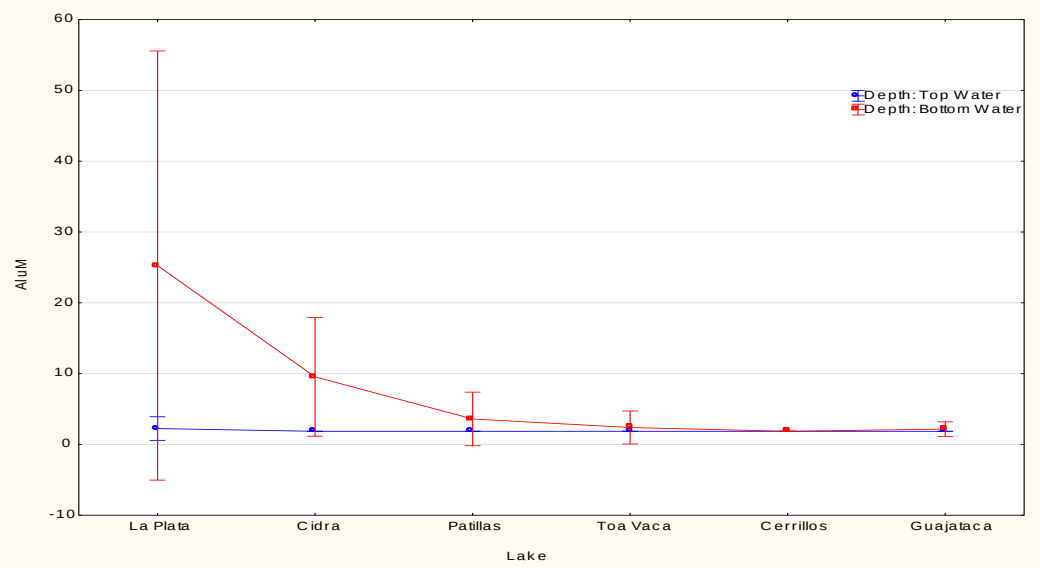


Figure 57: Distribution of aluminum concentration (µM) at the surface (1m) and hypolimnion (15m) waters of reservoirs being monitored.

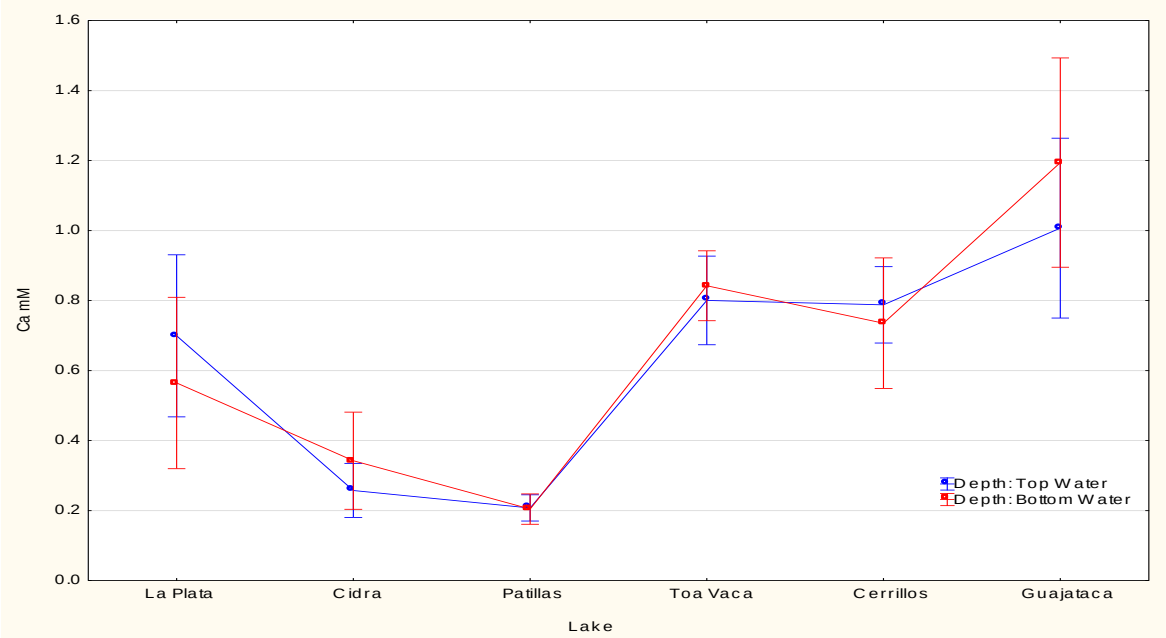


Figure 58: Distribution of calcium concentration (mM) at the surface (1m) and hypolimnion (15m) waters of reservoirs being monitored.

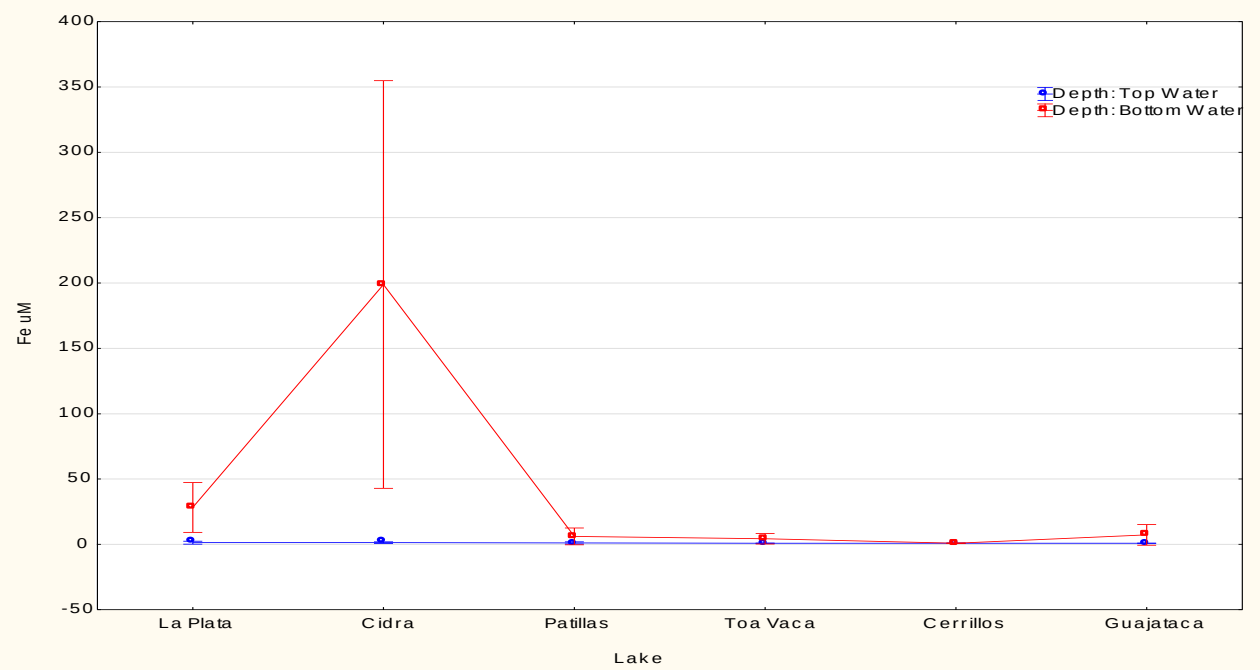


Figure 59: Distribution of iron concentration (µM) at the surface (1m) and hypolimnion (15m) waters of reservoirs being monitored.

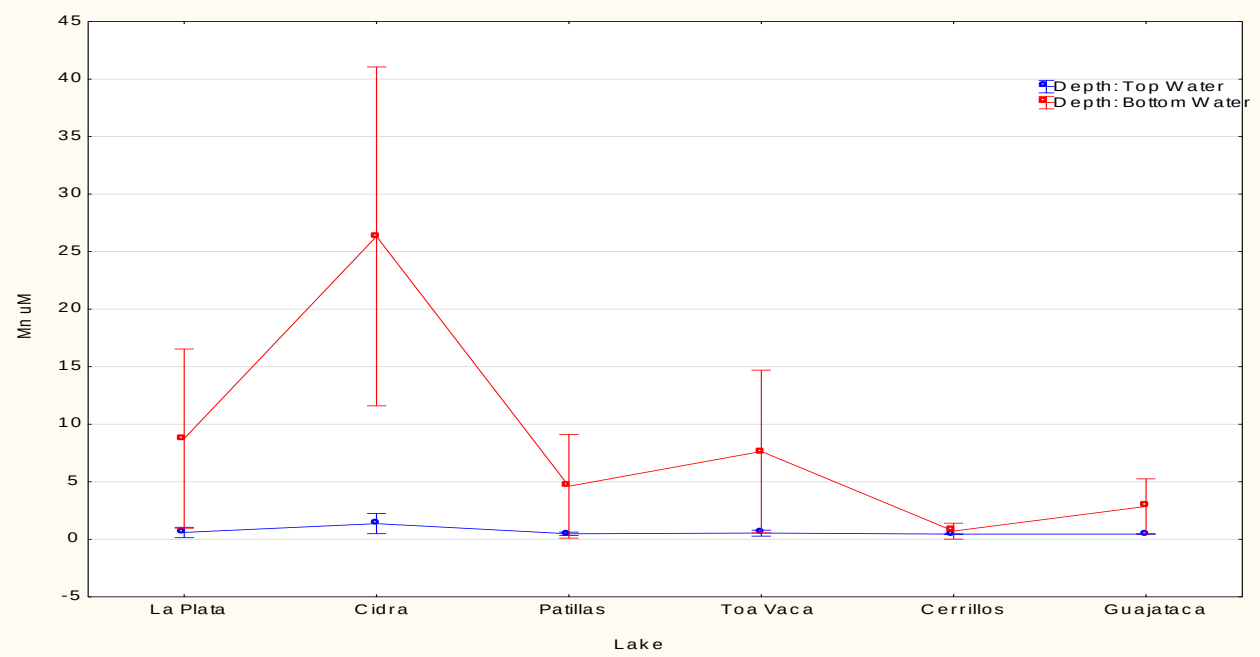


Figure 60: Distribution of manganese concentration (µM) at the surface (1m) and hypolimnion (15m) waters of reservoirs being monitored.

7.0 Phytoplankton Characterization at the Selected Reservoirs (First two years, 2012-2013, and first sampling of 2014)

7.1 Methodology

7.1.1 Storage of samples

Two 1-L bottles, one for each bongo net, from each studied reservoir were received in the Aquatic Biology Laboratory for plankton analysis. An additional 10 mL of formalin 10% were added to each bottle when these arrived to the laboratory. Each sample was split into two 500 mL bottles, labeling one bottle of each sample as for diatom protocol and all bottles with the information established in the S.O.P. Samples with less than 1L were not split and all the volume was maintained in the same bottle. All samples were stored in the refrigerator at 4°C.

7.1.2Determination of sample volume required for soft phytoplankton analysis

A determined volume from each bongo net bottle sample was set in the Utermöhl sedimentation chamber for 24 hours. The bottom of the chamber was covered with a slide and observed under a Fisher Scientific inverted microscope at a total magnification of 400X. Each sample was examined to determine the volume needed for further plankton enumeration. For volumes smaller than 5 mL, the settling time was shorter than 24 hours. Dense subsamples with a lot of organisms, that do not allow the proper counting and preliminary identification, were discharged and more diluted subsamples

were set in the chamber for another 24 hours. Tables 18, 19 and 20 indicate the volumes used for the analysis during 2012, 2013 and 2014, respectively.

Table 18. Volume determined for the preliminary phytoplankton analysis of each studied reservoir (2012).

Reservoir	Sample volume		
	1st sample 2012	2nd sample 2012	3rd sample 2012
Cerrillos	0.1 ml	0.5ml	10ml
Cidra	5 ml	5ml	10ml
Guajataca	5 ml	2.5ml	10ml
La Plata	10 ml	5ml	10ml
Patillas	10 ml	5 ml	10ml
Toa Vaca	10 ml	10 ml	10ml

*A water sample for this purpose was not collected during the first 2013 sampling event in Toa Vaca Reservoir. The first 2013 phytoplankton sample from Toa Vaca was collected afterwards.

The total number of organisms, the preliminary identification by phylum, and in some cases the identification to genera or species-level, were determined for each sample. During the first year of study (2012), a minimum of 110 organisms were counted in the preliminary analyses to verify if there were enough organisms for the subsequent enumeration analysis. A modification to the 2012 protocol was introduced by employing a horizontal count-strip across the center of the chamber, instead of counting a minimum 110 entities, to increase the number of organisms and increase and standardize the observed area in the slide. Colonial forms were counted as a unit cell; for example, members of *Pediastrum*, *Dinobryon*, *Microcystis*, *Staurastrum* and *Scenedesmus*. Non-overlapping field counts were made and we avoided starting at the edge, top and bottom parts of the chamber. The information from the preliminary count was recorded in a data form.

Table 19. Volume determined for the phytoplankton analysis for each reservoir studied during the second year of monitoring (2013).

Reservoir	Sample volume		
	1st sampling	2nd sampling	3rd sampling
Cerrillos	10 ml	1 ml	20 ml
Cidra	10 ml	5 ml	4 ml
Guajataca	1 ml	5 ml	20 ml
La Plata	10 ml	1 ml	4 ml
Patillas	20 ml	20 ml	20 ml
Toa Vaca	-----*	40 ml	50 ml

Table 20. Volume determined for the phytoplankton analysis for each reservoir studied during the third year of monitoring (2014).

Reservoir	Sample volume		
	1st sampling	2nd sampling	3rd sampling
Cerrillos	200 µl	**5 ml**	Pending
Cidra	5ml	**5 ml**	Pending
Guajataca	10 ml	**10 ml**	Pending
La Plata	5 ml	**10 ml**	Pending
Patillas	10 ml	**10 ml**	Pending
Toa Vaca	10 ml	**2 ml**	Pending

7.1.3. Soft Phytoplankton Analysis

In most of the cases, the samples utilized for the preliminary counts were also used for the final phytoplankton analyses. The number of organisms were counted and identified to the lowest taxonomic rank possible, except diatoms. Because we could not identify diatoms to species-level under light microscopy, we refer to this portion of the study as the "soft phytoplankton analysis". Nevertheless, diatoms were numerically accounted and classified as living or empty and as pennate or centric, for future analyses.

Live forms of the members of the soft phytoplankton (rest of the algal community; although including loricated algae like other ochrophytes and thecate dinoflagellates) observed in the samples were counted, identified and measured at 400X using a Leica DM3000B inverted microscope, equipped with a Micrometrics© camera (5 megapixels) and Micrometrics© SE Premium software Version 2.9. Pictures were taken for reference and measurements of 10 individuals from the dominant species were made for cell size calculations. For colonial forms, the number of cells was counted and most of the cells in the colony were measured to get an average of cell size. Colonial forms were counted as one individual for the calculations of cells per mL. As previously indicated, the diatoms were counted and enumerated as empty pennate, live pennate, empty centric and live centric. Empty lorica of Ochrophyta, like *Dinobryon,* were counted and measured. For Euglenozoa, only active forms (not empty lorica) were counted and measured. For Myzozoa, like *Peridiniopsis*, the live specimens were measured, but empty and live lorica were counted and enumerated separately. The taxonomy of each species was verified with Algaebase®, an internet data base.

A minimum of 250 units were counted during the first year of phytoplankton analysis with a 12.65% of counting error. The analysis was made using the center strip of the slide, as explained in the previous section, increasing the total of organisms counted and reducing the counting error to less than 12.5% (table 21). Counting error was calculated as indicated by the Standard Methods for the Examination of Water and Wastewater (1992) in the part 10200.

$$\frac{2}{\sqrt{N}}\ (100\%)$$

Table 21. Counting error for each reservoir during the second year of sampling (2013).

Reservoir	Sampling period					
	1st sampling		2nd sampling		3rd sampling	
	Bongo A	Bongo B	Bongo A	Bongo B	Bongo A	Bongo B
Cerrillos	12%	9.9%	7%	7.5%	6.3%	7.6%
Cidra	8.2%	9.2%	9.2%	10.2%	7%	6.3%
Guajataca	11.3%	9.9%	5.3%	9%	8.3%	
La Plata	5.0%	4.6%	10.7%	10.6%	11%	
Patillas	12.8	5.4	7.2	9.6	7.5	
Toa Vaca	----	----	11%	10%	10%	

Also, non-overlapping field counts were used as in the preliminary count analysis. The information from the analysis was recorded in a data form that included number of organisms (richness), cells per mL of volume sample settled and cells per mL of volume in the towing sample for each bongo per reservoir. Species, including unknown morphotypes, were organized by phylum.

7.1.4. Calculation of cells per mL of each identified organisms of the soft phytoplankton

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

$$\frac{\boldsymbol{cell}}{\boldsymbol{mL}} = \frac{\boldsymbol{C\ x\ \{TA/(A\ x\ F)\}}}{\boldsymbol{V}}$$

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 (490.87 mm^2) and the area of the field of view at 400X was used to calculate the number of cells of each taxon identified per milliliter of volume settled. The area of the field of view of the inverted microscope Fisher Scientific© was 0.159 mm^2, for Leica© DM3000B and Nikon Eclipse TS100it was 0.2206 mm^2. 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 mean 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 = $1/3\ \pi r^2 h \rightarrow 1/3\pi(0.1525m)^2(1.7m) = 0.0414m^3$
where:

r^2 = (radio of net)^2
h = length of net

Towing distance = B_v x T_{NT}
where:

B_v = velocity of the boat as miles/minutes
T_{NT} = net towing time in minutes

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

Sampled volume, mL = (Net volume x # nets in the distance traveled)(1,000,000)

7.1.5. Preliminary zooplankton analyses

For the preliminary zooplankton analyses, an additional 10 mL of formalin were added to each bottle when these arrived to the laboratory. After homogenization of the samples, three 1-mL aliquots per Bongo net were observed individually in a Sedgewick-Rafter chamber at 40X. Number of taxa and individuals per volume of sample was based upon the volume dispensed in the Sedgewick-Rafter chambers. Since each reservoir was sampled on three occasions, and 6 mL were observed per sampling, the mean density values reported herein correspond to the means after scanning the content of 18 chambers per reservoirs. Also, number of taxa per reservoir corresponds to the cumulative richness after scanning that many chambers. Species were identified using published keys and Santos-Flores (2001).

7.1.6. Triplot analysis of the soft phytoplankton functional groups and environmental variables for sampling year one (2012)

Morphological traits are relatively simple to measure and their relation to physiology is usually well defined (Reynolds, 1997 in Kruk et al., 2010). Specific growth rates, resource-uptake and light-interception properties are all dependent upon organismic dimensions, volumes and surface/volume ratios (Reynolds, 1988; Kirk, 1996). Therefore, Kruk et al. (2010) constructed a classification based on morphological traits, generating a "morphologically based functional classification" scheme, abbreviated to MBFC, and the resulting clusters are referred to as "morphologically based functional groups" of MBFG's (table 22). The functional groups (MBFG) scheme does not invalidate other, more complex, ecological classifications of phytoplankton (Reynolds, 1988). MBFG offers a classification that does not require as many traits that typically are not easy to obtain for all species. Also, MBFG's might prove to be easier to understand by modelers and water-quality managers.

Table 22. Main "morphologically based functional groups" (MBFG) proposed by Kruk et al. (2010).

MBFG	Morphology	Ecological traits
I= G1	Small organisms with high surface/volume ratio	Fast individual growth rate and high numerical abundance; well adapted to rapid resource acquisition and diminished sinking losses; recover rapidly from intensive grazing. Some are toxic cyanobacteria.
II= G2	Small flagellated organisms with siliceous exoskeletal structures	This group includes only Chysophyceae (Ochrophyta in part). They avoid sinking and have capacity to forage for nutrients. Flagella and siliceous scales might reduce loss by grazing. Facultative mixotrophy imparts them a tolerance to low nutrient concentrations.
III= G3	Large filaments with floating organelles (aerotopes)	Slow growth, but high surface/volume ratio gives them tolerance to limiting light conditions. Some species fix nitrogen and produce resistant propagules (hormogonia and akinetes); therefore, they have tolerance to low nutrient levels. Some are toxic, bloom-forming cyanobacteria.
IV= G4	Organisms of medium size lacking specialized traits	The species have moderate tolerances to limiting resources and low to moderate sinking rates. Usually prone to grazing. Many are chlorophytes and none is a nuisance species.
V= G5	Unicellular flagellates of medium to large size	Phylogenetically diverse. Their moderate size and surface/volume ratio, together with the possession of flagella, reduce sinking. High motility and the production of cysts help them survive under low nutrient conditions. Species rarely posse serious threats to water quality.
VI= G6	Non-flagellated organisms with siliceous exoskeletons	This group only contains diatoms, which rarely posse serious threats to water quality. Silica is usually a limiting factor.
VII= G7	Large mucilaginous colonies	Mucilage, along with lipids and aerotopes, provides them controllable buoyancy and protection against grazing. However, large size and volume, and low surface/volume ratio make them susceptible to low nutrient conditions.

Using the phytoplankton and water quality data from sampling year one (2012), a triplot analysis was performed with the aid InfoStat® and employing "Partial Least Squares" method. The rationale behind this analysis is that functional groups (G1-G7) tend to be most present and relatively abundant in the sites (reservoirs) that are located (plotted) near them (or in the direction of their projection) in ordination space. Length of environmental vector indicates its importance to the ordination. Direction of the vector indicates its correlation with each of the axes. Angles between vectors indicate the correlation between the environmental variables themselves. A similar analysis is in progress, but using the phytoplankton and water quality data from sampling year two (2013).

7.1.7. Diatoms assessment in the six reservoirs

Diatoms are one of the most diverse and widely used groups of freshwater organisms in limnological monitoring (Stoermer & Smol, 1999; Battarbee et al., 2001). These organisms are usually non-flagellated (except for their reproductive stages) and have siliceous exoskeletons; therefore they comprise the **morphologically-based functional group 6** (sensu Kruk et al., 2010). Diatoms are frequently used as indicators of eutrophication in temperate systems. In Europe, there is a long history of using biological indicators to monitor the integrity of fresh water ecosystems. However, little is known about their application in tropical and subtropical systems.

A major difficulty in employing diatoms as bioindicators is that identification often requires clearing the frustules (exoskeletons) under harsh acidic and/or oxidizing conditions and the subsequent observation under oil immersion (>1000X) or Scanning Electron Microscopy (S.E.M.).

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

Cellular contents of diatoms obscure the wall markings on which the taxonomy is based; therefore, the organic matters inside the cell must be removed (oxidized) prior to identification. The net-plankton samples collected over year 2012 (500mL per sampling) were concentrated by sedimentation. In a 50mL centrifuge tube, each concentrated sample (approximately 10mL; thereafter called the subsample) was oxidized with 10mL of $KMnO_4$ (10%). The subsamples were horizontally placed in a shaker and homogenized in darkness during 24 hours, then 1mL of concentrated H_2SO_4 (30%) was added to each subsample in a chemical hood. Immediately, >30% H_2O_2 was added until the subsamples turned colorless. To eliminate excess acid the subsamples were cleaned by removing the supernatant and the volume replaced with water; this procedure was done at least six times. These cleaned subsamples were used to prepare diatom slide for SEM analysis. Final volume depended 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).

7.1.7.2. Identification of Diatoms: Frustule Morphology

Basic identification of diatoms was done based on features of their silicified cell walls: the frustules. The frustules are composed of two valves fitting one over the other (the epivalve and the hypovalve) (Ehrlich, 1995). The valve shape, planes of symmetry and valve ends can be determined in unprocessed cells. Other 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. All these features were considered 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).

7.2 Results

7.2.1. Soft phytoplankton analyses for the first year of study (2012)

7.2.1.1. First sampling of 2012

Twenty-two taxa were found during the phytoplankton enumeration analysis of the six reservoirs that included organisms from the phyla Charophyta, Chlorophyta, Cyanobacteria, Euglenozoa and Ochrophyta. Seventeen were identified to genus or, in some cases, to species, and five organisms were classified to phylum, family or possible genera. The phylum with more organisms was Chlorophyta, with 10 taxa, followed by Myzozoa with 5 taxa including some unknown species. The species of the dinoflagellate genus *Peridiniopsis* were the only species found in all six reservoirs, followed by the euglenozoan *Trachelomonas* observed in 5 of the 6 reservoirs. Also, the genera *Euglena*, *Pandorina*, *Pediastrum*, *Dinobryon* and the species *Tetraedron minimum* were observed in four of the 6 reservoirs. The reservoir with the highest number of taxa was Cidra, with 15, followed by Guajataca, La Plata and Patillas, all with nine species (table 23). Five species were observed in Toa Vaca and Cerillos reservoirs, being the latter the one with the lowest number of identified soft phytoplankton, with only 4 taxa.

Ochrophyta was the most abundant phylum in all the reservoirs during the first sampling, and this group includes the pennate diatoms, the centric diatoms and *Dinobryon*. Diatoms were quantified during the analysis of the soft phytoplankton as pennate diatoms or centric diatoms and as mentioned above are part of the phylum Ochrophyta, but are not included in the table 20. Subsamples (500mL) for diatom analyses were treated by acidic and oxidative digestions; the diatoms identities are pending upon completion of the Scanning Electron Microscopy (SEM) analysis.

The second most-abundant phylum varied per reservoir. For example, for Cerrillos and La Plata it was the phylum Myzozoa, with the dinoflagellates of the genus *Peridiniopsis*. In Cidra and Guajataca, it was the phylum Chlorophyta with the species *Pediastrum simplex* and *Tetraedron minimum.* In Patillas, it was the phylum Chlorophyta with *Pediastrum simplex* and an unknown colonial species. The Cyanobacteria were the second most abundant group in the Toa Vaca reservoir.

Table 23. Taxa found in the six reservoirs during the first sampling of 2012

Taxon	Cerillos	Cidra	Guajataca	La Plata	Patillas	Toa Vaca
Cosmarium sp.		X		X	X	
Colonial cyanobacterium						X
Dinobryon sp.		X	X		X	
Euglena sp.		X	X		X	X
Pandorina sp.		X	X	X		X
Pediastrum duplex		X				
Pediastrum simplex		X	X	X	X	
Pediastrum simplex var. *echinulatum*		X			X	
Pediastrum simplex var. *ovalatum*	X					
Peridiniopsis sp.	X	X	X	X	X	X
Peridinium sp.		X				
Phacus cf. *longicauda* var. *insecta*		X				
Phacus cf. *pleuronectes*		X				
Platydorina sp.				X		
Scenedesmus sp.			X	X		
Staurastrum sp.		X	X		X	
Tetraedron minimum	X	X	X			X
Trachelomonas sp.	X	X	X	X	X	
Unknown cf. *Pleodorina* sp.					X	
Unknown dinoflagellates cf. Peridiniaceae		X				
Unknown round yellow-brown sp.				X		
Unknown cf. *Treubaria*				X		
Total Richness	4	15	9	9	9	5

7.2.1.2. Second sampling of 2012

A total of 28 organisms of the soft phytoplankton were identified within five different phyla that include Cyanobacteria, Charophyta, Chlorophyta, Euglenozoa and Ochrophyta (table 24). Twelve of those were classified to genus-level, 5 to species and 11 are unknown speciesa. The reservoir with highest species richness was Guajataca with 19, followed by Cidra with 14, Patillas and Toa Vaca both with 9, and Cerrillos and La Plata with 6 (table 24). The predominant phylum during this sampling was Chlorophyta, with 10 taxa, followed by Euglenoza with 6 taxa. *Peridiniopsis* was observed in the six reservoirs and the species *Tetraedron minimum*, *Pediastrum simplex* and a possible cyanobacterium (straight filamentous growth) were found in five of them. Another possible Cyanobacteria (filamentous screw-like curved) growth was observed in 4 of the reservoirs. Also, the genera *Pandorina* and *Staurastrum* were identified in four of the six reservoirs.

Again, the phylum Ochrophyta was the group with the highest number of organisms in the Cerrillos and Patillas lakes during the second sampling. The genus *Dinobryon* was found only in Patillas. The phylum Myzozoa, with *Peridiniopsis*, and the phylum Chlorophyta, with *Pediastrum simplex*, were the second-most abundant taxa in Cerrillos

and Patillas, respectively. In Cidra, *Peridiniopsis* and *Peridinium* (Myzozoa) were the dominant taxa, followed by the phylum Chlorophyta, with *Pediastrum simplex* and *Tetraedron minimum*. These species were also found in Guajataca during the second sampling but at higher numbers, being the Chlorophyta the dominant phylum and Cyanobacteria as second-most abundant. Also, Cyanobacteria were found in higher quantities in La Plata and Toa Vaca but the dominant forms were filamentous in La Plata, while filamentous and colonial in Toa Vaca.

Table 24. Taxa found in the six reservoirs during the second sampling of 2012

Taxon	Cerillos	Cidra	Guajataca	La Plata	Patillas	Toa Vaca
Ankistrodesmus sp.			X			
Cosmarium sp.			X			
Crucigenia tetrapedia			X			X
Colonial cyanobacterium			X			X
Filamentous, screw like, curved cyanobacterium	X		X	X	X	
Filamentous, straight cyanobacterium	X	X	X	X		X
Dinobryon sp.		X	X		X	
Euastrum sp.			X			
Euglena acus		X				
Euglena sp.			X	X		X
Pandorina sp.		X	X	X		X
Pediastrum simplex		X	X	X	X	
Pediastrum simplex var. *echinulatum*		X	X			
Peridiniopsis sp.	X	X	X	X	X	X
Peridinium sp.		X			X	
Phacus longicauda var. *insecta*		X				
Scenedesmus sp.		X	X			
Staurastrum sp.	X	X	X		X	
Strombomonas sp.		X				
Tetraedron minimum	X	X	X	X		X
Trachelomonas sp.		X				X
Unknown cf. *Pleodorina* sp.					X	
Unkown cf. T*rachelomonas*			X			
Unknown cyanobacterium cf. Merismopediaceae	X					
Unknown dinoflagellates cf. Peridiniaceae					X	X
Unknown round yellow-brown sp. 3					X	
Unknown cf. Volvaceae			X			
Unknown cf. Chlorophyta			X			
Total	6	14	19	6	9	9

7.2.1.3. Third sampling of 2012

Thirty-one species were observed during the soft phytoplankton analysis of Cerrillos, Guajataca, Patillas and Toa Vaca reservoir during the third sampling (Table 25). Most of the organisms are Chlorophyta and second Euglenozoa. Dominant taxa was *Tetraedron minimum* classified in the phylum Chlorophyta, was observed in the six reservoirs analyzed. Other taxa like unknown filamentous cyanobacteria, the genera *Euglena*, *Peridiniopsis*, *Trachelomonas* and the species *Pediastrum simplex* were found in five of the six reservoirs. Patillas was the reservoir with the highest number of taxa (16), followed by Guajataca and La Plata, both with 15. Once again, Ochrophyta was the phylum with the highest number of individuals, when the diatoms were included in the analysis, followed by Myzozoa in Cerrillos reservoir. Also, Ochrophyta dominated in Guajataca but its composition includes diatoms and *Dinobryon* sp. In the case of La Plata and Toa Vaca, cyanobacteria were the main group observed. The phylum Chlorophyta dominated in the water samples of Cidra and Patillas.

Table 25. Taxa found in the six reservoirs during the third sampling of 2012

Taxon	Cerillos	Cidra	Guajataca	La Plata	Patillas	Toa Vaca
Ankistrodesmus sp.			X		X	
Cosmarium sp.	X		X	X		
Colonial cyanobacterium				X		X
Filamentous, screw-like, curved cyanobacterium	X	X	X	X		X
Filamentous, straight cyanobacterium		X	X	X		X
Dinobryon sp.		X	X		X	X
Euglena acus		X				
Euglena sp.		X	X	X	X	X
Microcystis sp.			X			
Pandorina cf. *morum*					X	
Pandorina sp.				X	X	X
Pediastrum simplex	X	X	X	X	X	
Pediastrum simplex var. *echinulatum*		X			X	
Pediastrum simplex var. *ovalatum*						
Peridiniopsis sp.	X	X	X	X	X	X
Peridinium sp.		X			X	
Phacus sp.		X			X	
Platydorina sp.		X				
Scenedesmus sp.				X		
Staurastrum sp.	X	X	X	X		
Strombomonas sp.				X		
Tetraedron minimum	X	X	X	X	X	X
Trachelomonas sp.		X	X	X	X	X
Unknowndinoflagelates cf. Peridiniaceae			X			
Unknown cf. *Coelastrum cambricum*					X	
Unknown cf. *Coelastrum* sp.					X	
Unknown cf. *Eudorina* sp.					X	

Unknown cf. *Treubaria*				X		
Unknown cf. *Golenkinia*			X			X
Unknown cf. Chlorophyta			X			
Unknown cf. *Monoraphidium griffithii*				X	X	X
Total	6	14	15	15	16	11

7.2.1.4. Overall results for the soft phytoplankton analysis during the first year of study (2012)

A total of 41 taxa, excluding diatoms, were identified during the first year of monitoring the six reservoirs. Fifthteen organisms were identified to genera, seven to species, six to phylum, three to family, six to possible genera and four to possible species. These were distributed within six phyla: Cyanobacteria with 5, Charophyta with 4, Chlorophyta with 19, Euglenozoa with 8, Myzozoa and Ochrophyta with 1 (Fig. 61). However, diatoms were included as part of the Ochrophyta in the phytoplankton quantitative analyses (as live pennate, empty pennate, live centric and empty centric).

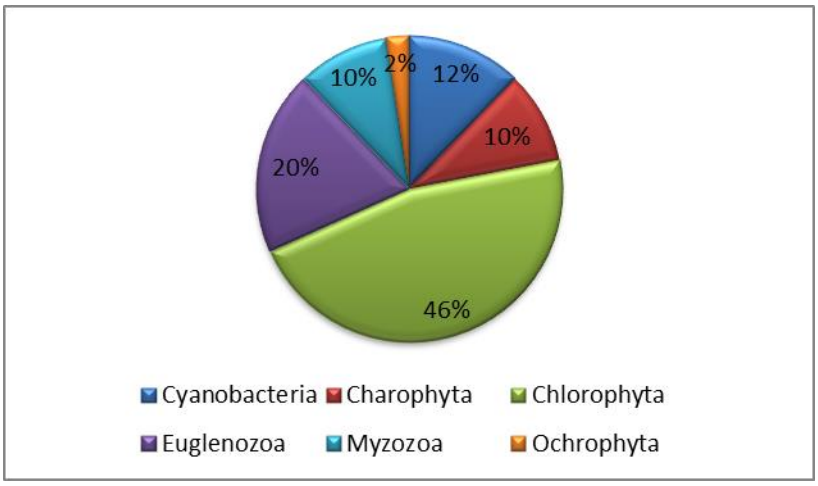


Figure 61. Percentage of the phyla of algae collected during the first year of monitoring in six reservoirs (combined).

The dominant organism across all the samplings was the dinoflagellate *Peridiniopsis* sp., found in all artificial lakes, followed by the chlorophyceans *Tetraedron minimum* and *Pediastrum simplex*. *Peridiniopsis* was observed in the 18 samplings and *T. minimum* and *P. simplex* were found in 15 and 14 samplings, respectively. About thirteen organisms were observed once over the year and from those, seven were classified as unknowns. The freshwater phytoplankton of Puerto Rican reservoirs has some undescribed species. Patillas and Guajataca were the reservoirs with the highest richness, with 22 taxa, followed by Cidra with 21. Samples from Guajataca had 20 taxa and the dominant species were *Dinobryon sp.*, *Euglena* sp., *P. simplex*, *Peridiniopsis* sp., *Staurastrum* sp. and *T. minimum*, all observed in the three samplings (Fig. 62). Cidra was also the reservoir with the highest diversity per sampling (15 species for the first sampling, 14 for the second

sampling and 4 for the third; Fig. 61) and Guajataca with 9, 19 and 15, respectively. The dominant species in Toa Vaca were colonial cyanobacteria, *Pandorina* sp., *Peridiniopsis* sp. and *T. minimum*. Cerrillos was the reservoir with the lowest number of taxa, with 10, and two species dominated there over the year: *Peridiniopsis* sp. and *Tetraedron minimum* (Fig. 63).

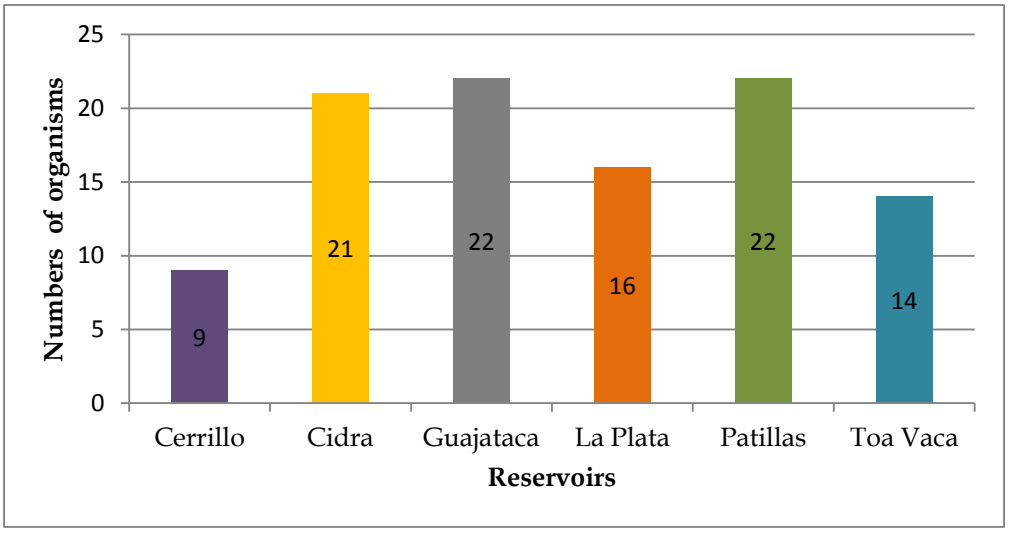


Figure 62. Number of taxa identified in each reservoir during the first year of monitoring.

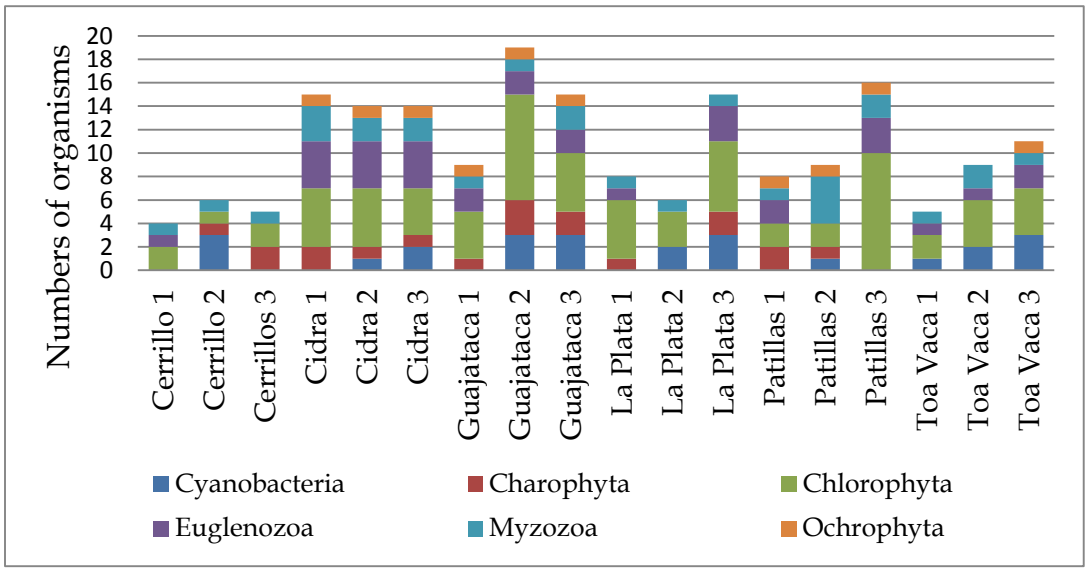


Figure 63. Phyla observed in the six reservoirs per sampling during the first year.

During the first year of study (2012), similar diversity indexes were obtained between and within reservoirs (Table 26). Changes in diversity (H') throught the year were site specific (Table 26). The highest similarity (Jaccard) was observed between Guajataca

and Toa Vaca. Both have 13 taxa in common with a Jaccard Index of 0.62 similarities (Table 27).

Table 26. Shannon Index (H') of diversity in the six reservoir in the first year of monitoring (2012)

Samples	Cerrillos	Cidra	Guajataca	La Plata	Patillas	Toa Vaca
1	1.26	2.28	2.31	1.70	2.06	2.04
2	2.35	2.77	2.88	1.43	1.25	2.54
3	2.20	2.52	2.82	2.92	2.37	2.39

Table 27. Jaccard Similarity Index for the six reservoir in the first year of monitoring (2012)

Reservoirs	Cerrillos	Cidra	Guajataca	La Plata	Patillas	Toa Vaca
Cerrillos	1	0.33	0.32	0.44	0.28	0.32
Cidra	0.33	1	0.45	0.52	0.47	0.38
Guajataca	0.32	0.45	1	0.32	0.41	**0.62**
La Plata	0.44	0.52	0.32	1	0.36	0.5
Patillas	0.28	0.47	0.41	0.36	1	0.38
Toa Vaca	0.3	0.38	0.62	0.5	0.38	1

7.2.1.5. Preliminary zooplankton analyses for first two sampling years (2012 & 2013)

The mean and cumulative species richness, the mean density and species identities for the zooplankton analyses are shown in Appendix L. This appendix includes six tables, one per reservoir.

Tables 28 and 29 summarize the species richness and/or diversity of the zooplankton groups. For the rotifers, emphasis was set on the genus *Brachionus* as it was the most common and diverse genus, with five widely distributed species. The rotifers dominated numerically in most samples and there is a strongly significant correlation between rotifers and chlorophyll-a concentrations (r = 0.88; 2012 data). Rotifers were numerically followed by Copepoda if nauplii are considered (data no shown), but tables 28 and 29 included only mature copepods which indeed were not diverse. Most copepods were in juvenile stages during the 2012 samplings.

ANOVA results suggest that the preliminary zooplankton densities within two major groups [rotifers and crustaceans (= Copepoda + Cladocera)] varied among sampling dates (p= 0.003; 2012 data) but not between Bongo nets used simultaneously (p = 0.21; 2012 data).

The cladoceran *Bosmina longirostris* was present in most samples, in all reservoirs, and its density laso correlated positively with median values of chlorophyll-a (r= 0.61; p = 0.015; 2012 data). Although results are preliminar in nature and only the first sampling year has

been explored, the density of this cladoceran species, as well as the rotifers' attributes (species richness and densities) seem promising surrogate metrics as indicators of eutrophication in local reservoirs. These zooplankton metrics are strongly correlated with high chlorophyll-a concentrations and low water transparencies.

Table 28. Cumulative richness and mean density of the zooplankton in the selected reservoirs in 2012.

Lake	ROTIFERA SPECIES	Brachionus spp. (ind./ml)	CLADOCERA SPECIES	CLADOCERA (ind/ml)	COPEPODA (ind/ml)	COPEPODA SPECIES
Cidra	27	66.6	12	38	28	3
La Plata	25	61.8	11	21	16	3
Toa Vaca	16	20.8	4	6	43	4
Guajataca	16	23.4	8	21	74	6
Cerrillos	16	25	10	15	33	6
Patillas	14	37.2	7	41	21	5

Table 29. Cumulative richness of the zooplankton in the selected reservoirs in 2013.

Lake	ROTIFERA	CLADOCERA	COPEPODA
Cerrillos	13	7	4
Cidra	21	10	4
Guajataca	13	8	5
La Plata	17	11	3
Patillas	13	5	6
Toa Vaca			

7.2.1.6. Triplot analysis for the phytoplankton and environmental parameters in the six reservoirs for first year of sampling (2012)

Figure 64 shows the output of triplot analysis of the phytoplankton functional groups (G1-G7), reservoirs and water quality parameters. As previously stated, the rationale behind this analysis is that functional groups (G1-G7) tend to be most present and relatively abundant in the sites (reservoirs) that are located (plotted) near them (or in the direction of their projection) in ordination space. Figure 64 was based on the relative abundances of the phytoplankton functional groups as based on their morphologies

(MBFG's). This required the application, for the first time, of the MBFG concept to many subtropical species.

The analysis nearly explained 60% of the variation (sum of both axes), and separated the six reservoirs into two groups, with three members each. One group was made up by Toa Vaca, Patillas and La Plata reservoirs; while the other group included Cerrillos, Cidra and Guajataca reservoirs. The first group of reservoirs included two traditionally eutrophic reservoirs and an oligotrophic-mesotrophic reservoir: Patillas. Within this group, there is a high correlation among nutrient levels (TN and TP), high chlorophyll- and chlorophyll-a. On the other hand, the second group included two traditionally mesotrophic reservoirs and a eutrophic site: Cidra. Even when Cidra had the high mean values for nutrients (TP, TN) in our study, within this site when TN decreased also concomitantly did TP and chlorophyll-a. The first group of reservoirs was typified by unicellular flagellates (functional group 5). Meanwhile group 2 (G2), mainly composed of chrysophytes, related positively to nitrate, and showed strong negative relationships with TN, TP, oxygen and chl-a, as predicted by a previous model.

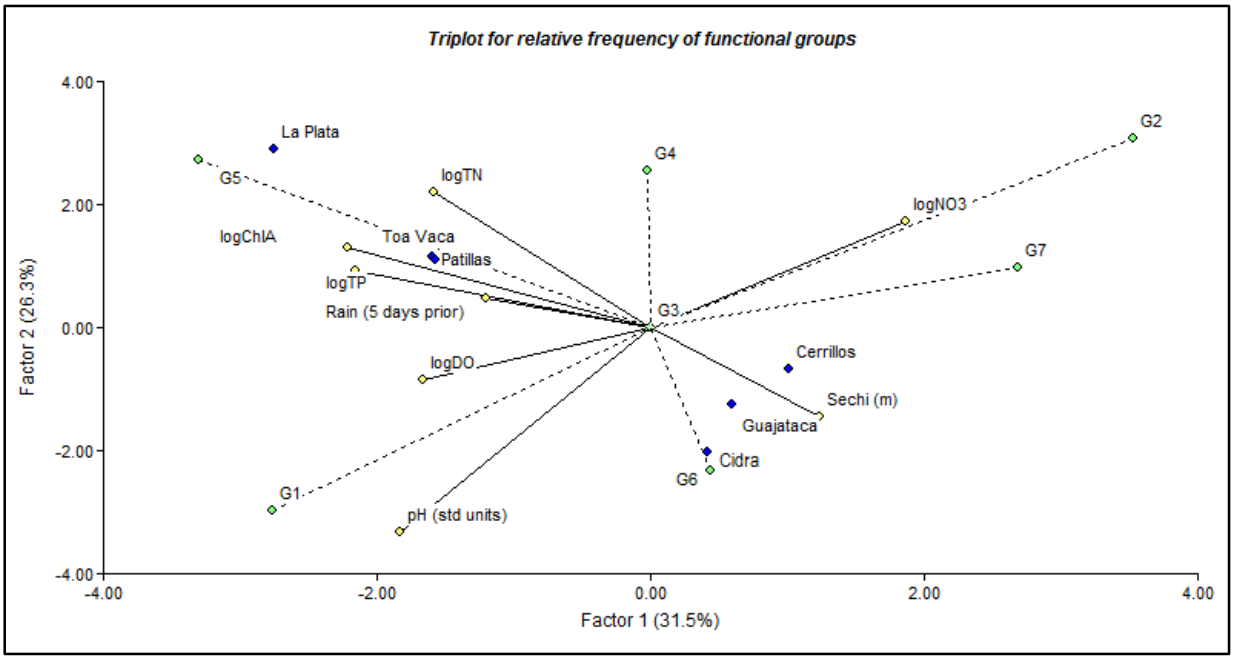


Figure 64. Triplot analysis of the phytoplankton functional groups (G1-G7), reservoirs and water quality parameters for the first year of sampling (2012).

Interestingly, the phytoplankton group 5 (G5), which is known (*sensu* Kruk et al., 2010) to survive low nutrient conditions, seems to be exploiting the surface of the reservoirs with high nutrient content (Toa Vaca, Patillas, La Plata). This discrepancy could due to

their high motility (flagella), which will favor them in systems with reduced transparency (low Secchi depth). They can move close to ther surface to overcome light deprivation. Group 6 (diatoms) showed a negative relation to nitrogen (TN). Although group 2 (G2), mainly chrysphytes, related positively to nitrate, they showed strong negative relationships with TN, TP, oxygen and chl-a, as predicted by the MBFG model (Kruk et al., 2010). Facultative mixotrophy might be imparting members of group 2 a tolerance to low nutrient concentrations.

The center or centroid of the triplot analysis was group 3 (G3), which includes important toxic, bloom-forming cyanobacteria. All other groups were depicted by their relative relations to G3. Future triplods, with data from sampling years two (2013) and three (2014) will be conducted with a different group as the triplot's centroid.

7.2.1.7. Overall results for the analysis of diatoms during the first year of study (2012).

7.2.1.7.1. Summary of diatom diversity per reservoir

A total of 31 taxa were identified, belonging to 13 genera, of which 25 were identified to species level. Dominant taxa present in "oligo-mesotrophic" (based on Carlson's Index) reservoirs were *Achnanthidium minutissimum*, centric diatoms, *Navicula,* and *Ulnaria acus.* For "mesotrophic" reservoirs, the dominant taxa were *Achnanthidium minutissimum, Ulnaria acus* and *Ulnaria ulna.* In eutrophic reservoirs, centric diatoms, *Ulnaria acus* and *Ulnaria ulna* were the dominant species.

The reservoirs with the highest values of dominance were Cerrillos and La Plata, the ones with the lowest values were Guajataca and Toa Vaca. *Ulnaria ulna* was the most common and abundant species, present in all six reservoirs, always above 20% of relative abundance. A summary of the diversity indexes in each reservoir is given below, under each locality.

CERRILLOS

Ulnaria ulna had 93% relative abundance in the first sampling event. For the second sampling *Ulnaria acus* was also dominant, with a relative abundance of 87%, while in the last sampling *Achnanthidium minutissimum* had a 68% of abundance. Shannon and Margaleff indexes were 0.35 and 1.03, respectively. A total of 14 taxa were registered for Cerrillos.

CIDRA

Ulnaria ulna had 92% of relative abundance in the first sampling, but it decreased to 27% for the second sampling. In the last sampling centric diatoms were 27% abundant. Shannon and Margaleff indexes were 0.29 and 1.28, respectively. A total of 13 taxa were registered for Cidra.

GUAJATACA
Navicula sp. had 52% of abundance in the first sampling and 34% in the second sampling. *Ulnaria acus* had 21, 39 and 30% of relative abundance, respectively, for each sampling event. For the third sampling, *Achnanthidium minutissimum* had a 50% of relative abundance. Shannon and Margaleff indexes were 0.41 and 1.11, respectively.
A total of 10 taxa were registered for Guajataca.

LA PLATA
Ulnaria ulna had 93 and 95% of relative abundance in the first and second, respectively. In the third sampling *Ulnaria acus* had 57% of relative abundance. Shannon and Margaleff indexes were 0.37 and 1.08, respectively. A total of 16 taxa were registered for La Plata.

PATILLAS
Ulnaria ulna had 79% of relative abundance in the first sampling and 33% in the third sampling. *Ulnaria acus* had 54% of relative abundance in the second sampling event. For the third sampling, *Navicula* sp. had a 44% of relative abundance. Shannon and Margaleff indexes were 0.40 and 1.28, respectively. A total of 18 taxa were registered for Patillas.

TOA VACA
Ulnaria ulna had 82, 36 and 34% of relative abundance, respectively, in each sampling event. Shannon and Margaleff indexes were 0.36 and 1.13, respectively. A total of 16 taxa were registered for Toa Vaca.

7.2.1.7.2. Details on selected taxa

Achnanthidium minutissimum
This species was abundant in the third sampling of the Cerrillos and Guajataca reservoir with 68 and 60% of abundance respectively. *A. minutissimum* is found to be sensitive to trophic conditions (oligo-mesotrophic to mesotrophic in indices like Trophy D, TDI and Rott), pH, oxygen, saprobity (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 colonizer (Biggs *et al*., 1998; Rimet *et al*., 2007). Has high tolerance of several environmental factors (Ponader & Potapova, 2007), occurs under a wide range of ecological conditions, being found in oligo to hypereutrophic systems (Luttenton & Lowe, 2006). A trophic scaling of indifferent might thus be the best.

Cyclotella meneghiniana
It is a centric diatom regarded globally as the most common species of the group, 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 (1991), 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 & 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*., 2004), 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 acus

This species was present above 20% of relative abundance 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%); and the third sampling event in Guajataca (30%). This species is eutrophication tolerant and has been associated with eutrophication in tropical streams.

Ulnaria ulna

This species was also present above 20% of relative abundance 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%). Van Dam and Mertens (1993) have accepted *U. ulna* as an indicator for eutrophic lakes.

OTHER SPECIES WITH BIOINDICATOR VALUES

Pinnularia sp. and *Sellaphora pupula*.- Eutrophication tolerant (Van Dam et al., 1994).
Gomphonema parvulum.- Cosmopolitan, widely distributed in inland waters, and considered as indicators of eutrophic conditions (Van Dam et al., 1994).
Gomphonema gracile.- Associated with eutrophication (Biggs et al., 1998; Passy, 2007).
Melorisa varians.- Pollution tolerant (Szczepocka & Szulc 2009).
Nitzchia palea.- Eutrophication tolerant.

7.2.1.7.2. Diatom diversity per samplings and localities

The relative abundances and the diversity indexes determined for the diatoms are shown in figures 65 and 66, respectively.

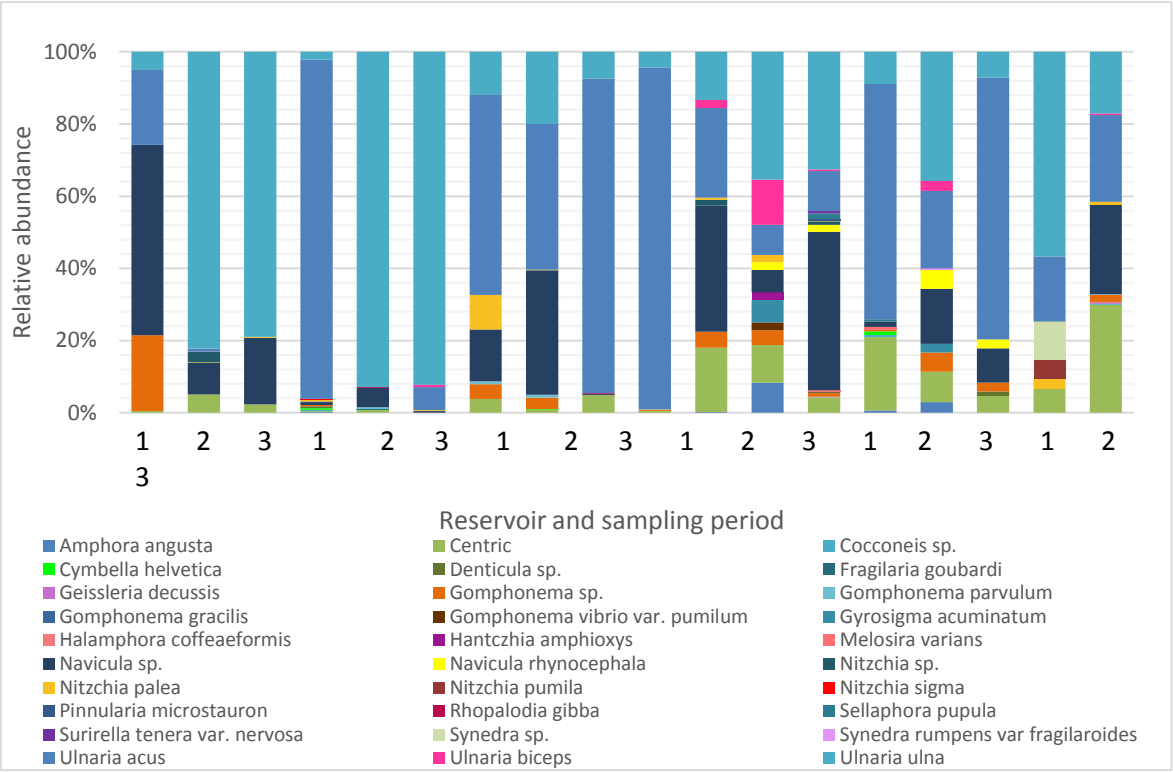


Figure 65. Relative abundances of diatom species in each sampling event.

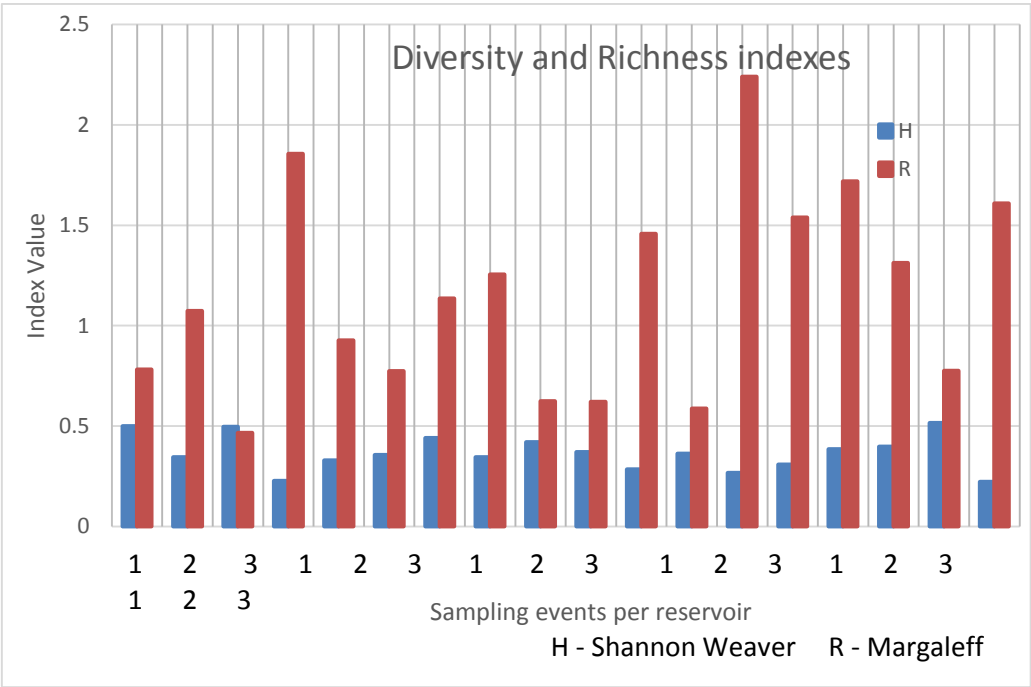


Figure 66. Diversity and richness indexes for the diatoms in each sampling event.

Table 30. Taxa present in relative abundances higher than 0.25 in a single sampling event.

	Cerrillos	Cidra	Guajataca	La Plata	Patillas	Toa Vaca
Achnanthidium minitissimum	0.679	0.597				
Centric diatoms		0.265				
Navicula sp.		0.524	0.336	0.44		
Ulnaria acus	0.872	0.393	0.296	0.933	0.949	0.545
Ulnaria ulna	0.927	0.923		0.567	0.79	0.326

Table 31. Average concentrations and range of total nitrogen (TN), total phosphorous (TP), chlorophyll-*a* and Secchi disc depth, and number of lakes in which each diatom taxon was present.

Diatom taxa	TN (mg/L)			TP(mg/L)			Chl-a (ug/L)			Secchi (m)			# *lakes*
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	
Achnanthidium minutissimum	0.405	0.088	1.039	0.029	0.002	0.086	22.806	0.703	124.175	2.130	0.703	4.025	6
Amphora angusta	0.465	0.465	0.088	0.049	0.009	0.086	27.215	2.305	106.420	2.043	0.813	3.075	4
Centric diatoms	0.411	0.088	1.039	0.033	0.002	0.089	20.122	0.703	124.175	2.089	0.703	4.025	6
Cocconeis sp.	0.503	0.088	0.888	0.046	0.009	0.086	48.701	2.305	124.175	1.642	0.850	3.075	3
Cymbella helvetica	0.337	0.088	0.532	0.037	0.009	0.086	6.811	1.733	19.623	2.036	1.000	3.075	4
Denticula sp.	0.418	0.304	0.532	0.050	0.015	0.086	11.834	4.045	19.623	2.513	1.000	4.025	2
Fragilaria goubardi	0.190	0.190	0.190	0.009	0.009	0.009	2.008	2.008	2.008	3.750	3.750	3.750	1
Geissleria decussis	0.544	0.201	0.888	0.022	0.002	0.042	62.439	0.703	124.175	0.776	0.703	0.850	2
Gomphonema sp.	0.450	0.088	1.039	0.036	0.002	0.089	26.115	0.703	124.175	1.856	0.703	4.025	6
Gomphonema parvulum	0.401	0.190	0.888	0.021	0.002	0.042	26.441	0.703	124.175	1.874	0.703	3.750	5
Gomphonema gracilis	0.404	0.404	0.404	0.039	0.039	0.039	3.585	3.585	3.585	2.478	2.478	2.478	1
Gomphonema vibrio var. pumilum	0.272	0.261	0.282	0.026	0.009	0.043	3.478	2.813	4.143	2.675	2.500	2.850	2
Gyrosigma acuminatum	0.316	0.261	0.404	0.030	0.009	0.043	3.513	2.813	4.143	2.609	2.478	2.850	2
Halamphora coffeaeformis	0.282	0.282	0.282	0.009	0.009	0.009	2.813	2.813	2.813	2.500	2.500	2.500	2
Hantczhia amphioxys	0.404	0.404	0.404	0.039	0.039	0.039	3.585	3.585	3.585	2.478	2.478	2.478	1
Melosira varians	0.088	0.088	0.088	0.009	0.009	0.009	2.305	2.305	2.305	3.075	3.075	3.075	1
Navicula sp.	0.423	0.088	1.039	0.028	0.002	0.086	19.497	0.703	124.175	2.117	0.703	4.025	6
Navicula rhynocephala	0.349	0.261	0.493	0.030	0.009	0.045	4.961	2.813	10.220	2.771	2.000	4.025	3
Nitzchia sp.	0.550	0.282	1.039	0.050	0.009	0.086	28.532	2.813	106.420	1.758	0.813	2.500	4
Nitzchia palea	0.513	0.201	1.039	0.042	0.002	0.089	28.418	0.703	124.175	1.603	0.703	2.850	5
Nitzchia pumila	0.366	0.366	0.366	0.056	0.056	0.056	11.995	11.995	11.995	1.750	1.750	1.750	1
Nitzchia sigma	0.532	0.532	0.532	0.086	0.086	0.086	19.623	19.623	19.623	1.000	1.000	1.000	1
Pinnularia microstauron	0.282	0.282	0.282	0.009	0.009	0.009	2.813	2.813	2.813	2.500	2.500	2.500	1

Rhopalodia gibba	0.236	0.190	0.283	0.007	0.005	0.009	1.839	1.670	2.008	3.338	2.925	3.750	1
Sellaphora pupula	0.288	0.088	0.493	0.021	0.009	0.045	5.113	2.305	10.220	2.525	2.000	3.075	3
Surirella tenera var. nervosa	0.346	0.261	0.493	0.032	0.009	0.045	5.725	2.813	10.220	2.450	2.000	2.850	2
Synedra sp.	0.366	0.366	0.366	0.056	0.056	0.056	11.995	11.995	11.995	1.750	1.750	1.750	1
Synedra rumpens var fragilaroides	0.333	0.261	0.404	0.041	0.039	0.043	3.864	3.585	4.143	2.664	2.478	2.850	1
Ulnaria acus	0.463	0.088	1.039	0.036	0.002	0.089	22.481	0.703	124.175	1.872	0.703	4.025	6
Ulnaria biceps	0.624	0.261	1.039	0.041	0.009	0.070	43.929	2.813	124.175	1.748	0.813	2.850	3
Ulnaria ulna	0.437	0.088	1.039	0.033	0.002	0.089	20.251	0.703	124.175	2.028	0.703	4.025	6

7.2.2. Second year of sampling (2013)

7.2.2.1. First sampling of soft phytoplankton in 2013

Some 42 organisms were identified from five reservoirs during the first sampling of 2013; these included 3 genera of Charophyta, 13 genera and one morphotype of Chlorophyta, 4 morphotypes of Cyanobacteria, 4 genera of Euglenozoa, 2 genera of Myzozoa and 1 genus of Ochrophyta (table 32). Most of the taxa belonged to Chlorophyta and Euglenozoa (fig. 67). Organisms of the genus *Peridiniopsis* and *Staurastrum* and the species *Pediastrum simplex* were observed in four of the five reservoirs (table 32). The most species-rich reservoirs were Guajataca and Cerrillos, with 15 and 8 taxa, respectively, and both were dominated by chlorophytes (fig. 68 & 69). Diatoms were quantified during the analysis of the soft phytoplankton as pennate diatoms or centric diatoms and are part of the phylum Ochrophyta, but were not included in the table 32. Ochrophyta was the dominant group in Cerrillos and La Plata reservoirs, and the second most abundant in Guajataca Reservoir. *Pediastrum simplex* was the most abundant taxon in Guajataca.

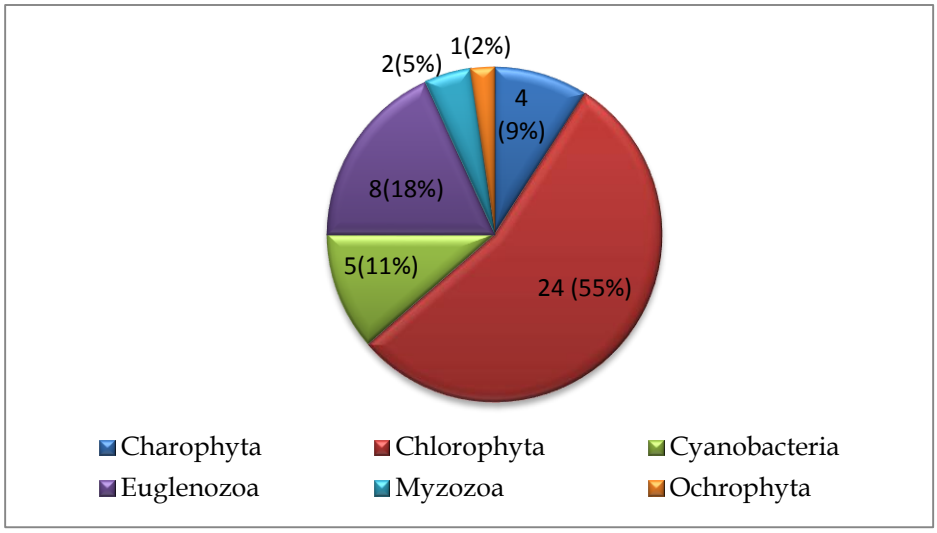


Figure 67. Percentage contribution of each phylum to total richness during the first sampling of the second year of study (2013).

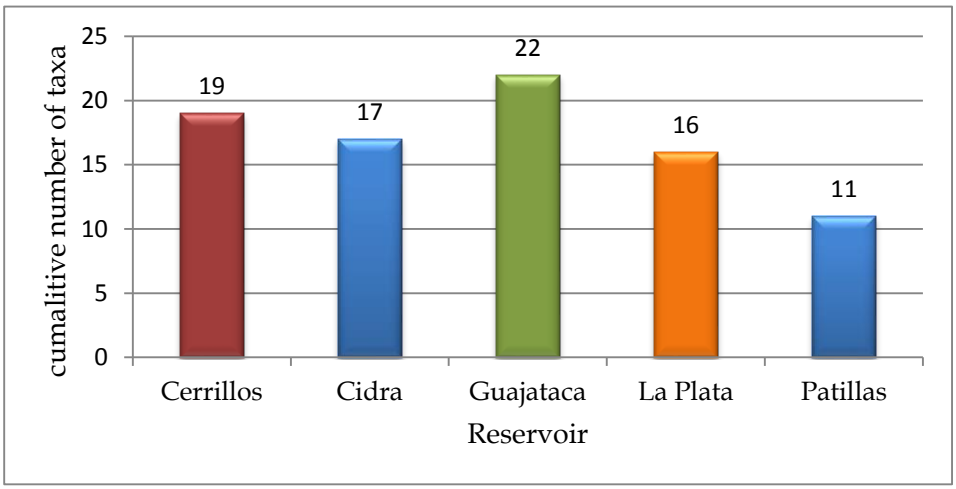


Figure 68. Number of taxa identified per reservoir in the first sampling of the second year of study (2013).

Table 32. Phytoplankton found in the first sampling of the second year of study in five reservoirs.

Taxon	Cerrillos	Cidra	Guajataca	La Plata	Patillas
Ankistrodesmus falcatus var. *mirabilis*					X
Coelastrum sp.		X			
Coelastrum cambricum		X	X		
Coelastrum cf. *pseudomicroporum*				X	
Cosmarium sp.	X		X	X	X
Crucigenia tetrapedia	X	X			
Colonial cyanobacteria	X		X		
Filamentous, screw-like, curved cyanobacteria			X		X
Filamentous, straight cyanobacteria	X				X
Dinobryon sp.		X	X	X	X
Euastrum sp.	X				
Euglena acus		X			
Euglena sp.		X	X	X	
Golenkinia sp.			X		
Golenkinia radiata					X
Micractinium sp.			X		
Micractinium pusillum			X		
Monoraphidium sp.			X	X	
Oocystis sp.		X			
Pandorina sp.				X	
Pediastrum simplex	X	X	X	X	X
Pediastrum simplex var. *echinulatum*	X		X	X	
Peridiniopsis sp.	X	X	X	X	X
Peridinium sp.	X	X		X	
Phacus sp. (small)		X			
Phacus cf. longicauda var. *insecta*		X		X	
Platydorina cf. caudata				X	
Polyedriopsis spinulosa			X		
Scenedesmus cf. brasiliensis	X				
Scenedesmus cf. denticulatus	X		X		
Scenedesmus cf. quadricauda	X		X	X	
Scenedesmus cf. quadricauda var. *parvus*			X		
Staurastrum sp.	X	X	X	X	X
Staurastrum sp. 2	X				
Strombomonas sp.		X			
Treubaria cf. schmidlei				X	
Treubaria triappenduculata			X		
Tetraedron minimum	X	X	X		X
Trachelomonas sp.		X			
Trachelomonas sp.(dark lorica)	X	X			

Trachelomonas sp. (small)	X			X	
Unknown cyanobacteria	X				
Merismopediaceae					
Unknown Chlorophyta	X		X		X
Chroococcus sp.			X		
Total	**19**	**17**	**22**	**16**	**11**

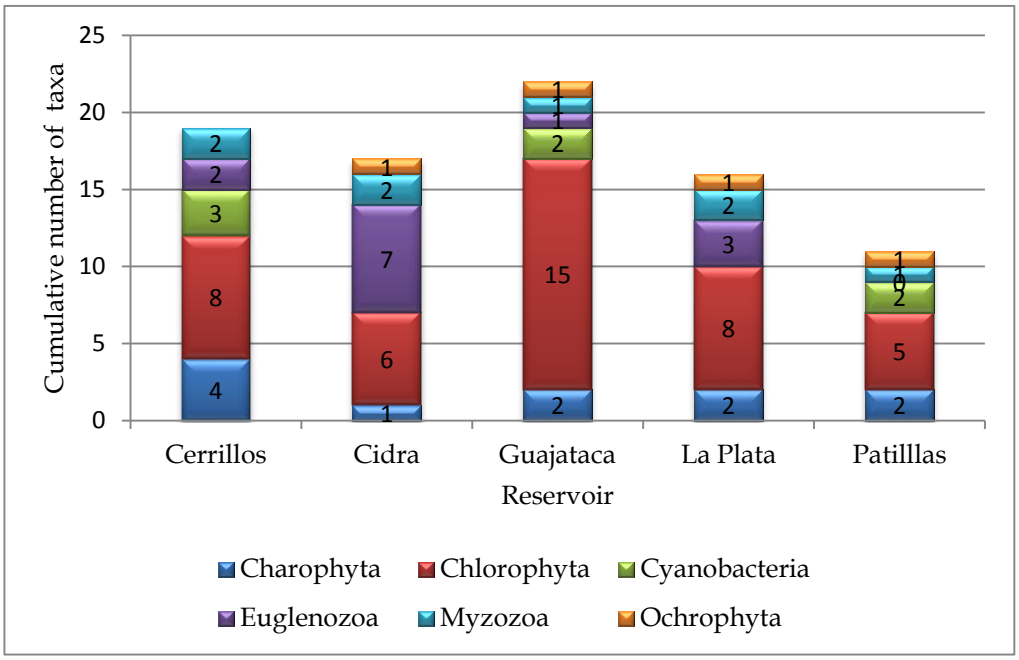


Figure 69. Composition and cumulative number of species per phyla in each reservoir for the first sampling of year 2013.

7.2.2.2. Second sampling of soft phytoplankton for 2013

A total of 47 taxa were identified in the second sampling of the six reservoirs. These included specimens of the phyla Charophyta, Chlorophyta, Cyanobacteria, Ochrophyta, Euglenozoa and Myzozoa (table 33). Chlorophyta was dominant in five of the six reservoirs, with 57% of the total specimens observed (fig. 70). Also, it had the highest number of taxa per phylum for the sampling, with 27 taxa. Some 18 taxa were classified to genus and 19 taxa to species, including 4 varieties. Also, 5 unknown species were recorded. The highest values for cumulative richness were observed in Guajataca and Cidra, with 32 and 25 species, respectively (fig. 71). *Pediastrum simplex* and *Peridiniopsis* sp. were found in all six reservoirs (table 33).

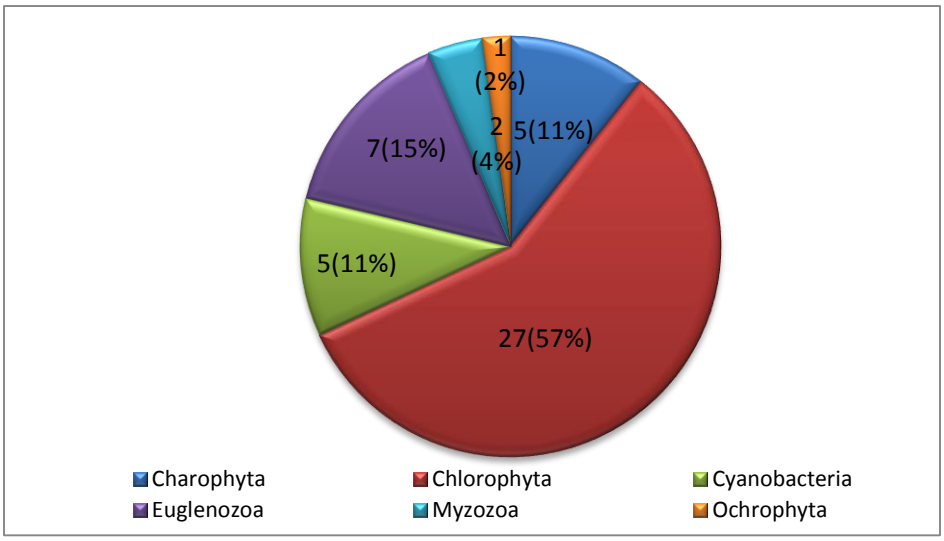


Figure 70. Number of taxa per phylum during the second sampling of the second year of study (2013).

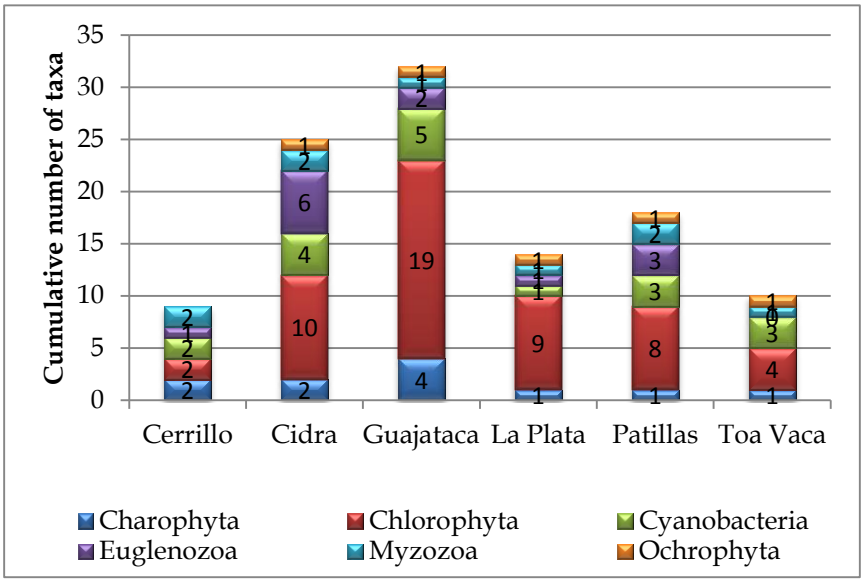


Figure 71. Number of taxa identified per phylum in each reservoir during the second sampling of the second year of study (2013).

Table 33. Soft phytoplankton identified during the second sampling of the second year of study (2013) in six reservoirs.

Taxon	Cerrillos	Cidra	Guajataca	La Plata	Patillas	Toa Vaca
Anabaena sp.		X	X		X	
Ankitrodesmus falcatus var. *mirabilis*					X	
Coelastrum sp.	X	X	X			
Cosmarium sp.1	X	X	X	X		X
Crucigenia tetrapedia			X			X
Colonial cyanobacterium	X	X	X			X
Filamentous, screw-like curved cyanobacteria		X	X	X	X	X
Filamentous, straight cyanobacteria	X	X	X		X	X
Dictyosphaerium sp.			X			
Dinobryon sp.		X	X	X	X	X
Euastrum sp.			X			
Eudorina sp.					X	
Euglena sp.			X		X	
Golenkinia radiata		X	X	X		
Lagerheimia citriformis			X			
Microcystis sp.			X			
Monoraphidium griffithii		X				
Pandorina sp.		X	X	X		X
Pandorina morum			X	X		
Pediastrum simplex	X	X	X	X	X	X
Pediastrum simplex var. *echinulatum*		X	X	X	X	
Peridiniopsis sp.	X	X	X	X	X	X
Peridinium sp.	X	X			X	
Phacus cf. *longicauda* var. *insecta*		X				
Platydorina cf. *caudate*				X		
Pleurotaenium sp.					X	
Polyedriopsis spinulosa			X	X		
Scenedesmus cf. *armatus*		X				
Scenedesmus cf. *denticulatus*			X			
Scenedesmus cf. *quadricauda*		X	X			
Scenedesmus sp.			X			
Staurastrum sp.	X	X	X			
Strombomonas sp.		X			X	
Treubaria cf. *schmidlei*			X	X		
Treubaria triappenduculata			X	X		
Tetraedron caudatum var. *longispinum*			X			
Tetraedron trigonum		X				
Tetraedron minimum		X	X		X	X
Tetrastrum sp.					X	
Trachelomonas sp.	X	X				
Trachelomonas sp.(dark lorica)		X				
Trachelomonas sp. (small)		X				
Unknown Chlorophyta					X	
Unknown Euglenozoa		X	X	X	X	

Cosmarium sp.2			X			
Tetraedron sp.			X			
Unknown circular solitary chlorophyte			X		X	
Total	**9**	**25**	**32**	**14**	**18**	**10**

7.2.2.3. Third soft phytoplankton sampling of 2013

Some 46 taxa were found during the analysis of the third series of samplings. These included 27 genera in 7 phyla and several unknown organisms grouped into 5 phyla. Chlorophyta was the phylum with more taxa, followed by Euglenozoa (fig.72). Also, it was the first time that a yellow-green alga (Phylum Heterokonthophyta/Ochrophyta in part; Class Xanthophyceae) was observed and it was identified as *Goniochloris* sp. (table 34). The highest cumulative number of taxa was observed in Cidra reservoir and the lowest number was recorded in Toa Vaca reservoir (fig 73). *Peridiniopsis* and two cyanobacterial morphs were observed in all six reservoirs. The chlorophytes *Pediastrum simplex* and *Tetraedron minimum* were found in 5 of the six reservoirs (table 34).

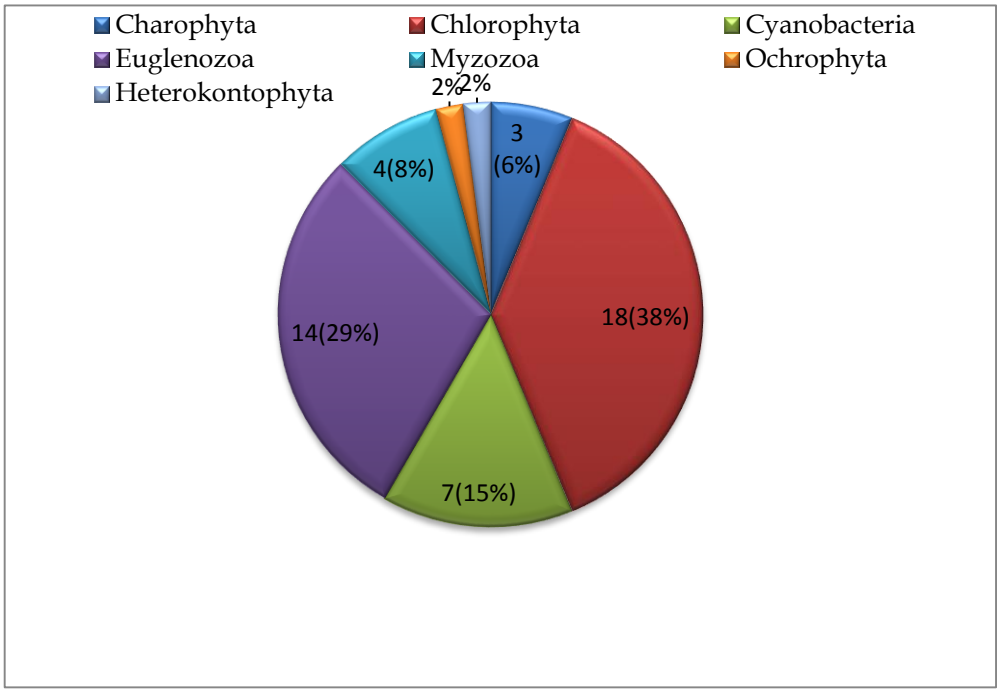


Figure 72. Percentage composition of phyla and cumulative number of taxa found during the third sampling of the second year of study (2013).