The Influence of Environmental Variables and an Invasive Freshwater Bivalve on the Phytoplankton Community of Two Tropical Reservoirs

By

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Abstract

Reservoirs of Puerto Rico are an important source of drinking water. The phytoplankton community is a large determinant of water quality, as some species are toxic. Thus, understanding the phytoplankton community structure is important to ensure clean reservoirs. Both field and mesocosm studies were completed using two reservoirs to identify the factors impacting the phytoplankton community structure. In the field study, reservoirs were sectioned into zones and sampling was conducted in "dry" and "wet" seasons to evaluate spatial and temporal differences in the phytoplankton and environmental parameters. Mesocosm experiments examined the direct impact of *Corbicula fluminea* on nutrients and the phytoplankton community. Results indicate parameters influencing the phytoplankton community structure differ depending on initial trophic status, as temperature and total phosphate played a major role in the mesotrophic system while specific conductance was the most important in the eutrophic system. Within each reservoir, a spatial gradient existed from the dam to riverine zone for several parameters, including secchi depth. However, temporal environmental patterns had the largest influence on the phytoplankton community structure. In the mesocosms, *C. fluminea* influenced phytoplankton dynamics but had no effect on nutrient concentrations; however, sediment was found to elevate total phosphate concentrations. Both studies show the reservoirs to be complex systems with several factors influencing their water quality.

Resumen

Los embalses de Puerto Rico son fuentes importantes de agua potable. La comunidad del fitoplancton en los embalses es un factor que puede impactar la calidad del agua, ya que algunas especies son tóxicas. Por ende, necesitamos entender qué componentes influencian al fitoplancton para asegurar embalses saludables. Se hicieron estudios de campo y laboratorio, usando dos embalses, para identificar los factores que influyen en la estructura de la comunidad de fitoplancton. En el estudio de campo, los embalses estaban divididos en zonas y se tomaron muestras en épocas de "lluvia" y de "sequía" para buscar diferencias en tiempo y espacio en el fitoplancton y en los parámetros ambientales. Los experimentos de laboratorio sirvieron para examinar el impacto directo de *Corbicula fluminea* en nutrientes y en la comunidad de fitoplancton. Los resultados indican que los parámetros que influyen el fitoplancton dependen del estatus trófico inicial en el embalse. El fósforo total y temperatura tuvieron más influencia en la sistema mesotrófico, pero el conducto específico fue más importante en el sistema eutrófico. En cada embalse existe un gradiente espacial, pero los patrones ambientales tuvieron la mayor influencia en la estructura de la comunidad de fitoplancton. En los experimentos de laboratorio, *C. fluminea* tuvo un impacto en la dinámica del fitoplancton pero no en la concentración de nutrientes. El sedimento aumentó la concentración de fosfato total. Ambos estudios demuestran que los embalses son sistemas complicados con muchos factores que tienen un impacto en la calidad de agua.

Dedication

To the women who taught me I could do anything:

Kathy Chappell

Denise Dougan

Deborah Dougan

Evelyn Chappell

Barbara Dougan

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Chapter 1

Introduction to the Thesis

There are 19 reservoirs in Puerto Rico which are principally used for water consumption, irrigation, and energy. Consumption however, is their major use as 70% of potable water in Puerto Rico originates from reservoirs (Ortíz-Zayas et al. 2004). In addition to these services, the reservoirs serve as a recreational area for fishing and boating. Due to the high interaction between people and reservoirs on the island, water quality should be a public health concern. Water quality is impacted by nutrient concentrations and the phytoplankton community; however, little research has been conducted on either parameter. Nutrient criteria used to identify the trophic status of Puerto Rican reservoirs were established less than 10 years ago (Martinez et al. 2005), while only a handful of studies have examined the phytoplankton community (Santos-Flores 2001; Martinez et al. 2005; Pantoja-Agreda et al. 2009). Thus, more research needs to be conducted in order to better understand the water quality within the reservoirs and the interaction with the phytoplankton community.

The phytoplankton community must be better understood as phytoplankton has the ability to impact several of the services the reservoirs provide. For example cyanobacteria are known to produce cyanotoxins which have been identified as a serious health threat for humans and wildlife when consumed at elevated concentrations (Araoz et al. 2009).The presence of cyanotoxins in Puerto Rico may be important as *Microcystis aeruginosa*, a cyanotoxin producing cyanobacteria, has been identified in 19 reservoirs throughout the island (Martinez et al. 2005). As the cyanotoxin level in drinking water is unregulated (World Health Organization 2004), the factors allowing the population of *M. aeruginosa* to increase need to be understood so large blooms can be avoided.

The conditions that favor cyanobacteria are high temperatures, a low N/P ratio, a low dissolved CO_2 level, and a low pH level (Jacoby et al. 2000). No one has examined the relationship between environmental variables and cyanobacteria in Puerto Rico, however, as the phytoplankton community within these reservoirs has been understudied. An initial survey of the phytoplankton was conducted by Santos-Flores (2001), who found phytoplankton richness within the reservoirs to have tripled in the past 50 years. Additionally, Martinez et al. (2005) examined the phytoplankton community and found phytoplankton genera to be associated with

nitrogen and phosphate concentrations. Pantoja-Agreda et al. (2009) concluded there are spatial and temporal differences in the phytoplankton abundance and diversity within the reservoirs, demonstrating that despite the reservoirs' relatively shallow and small area, they are still complex ecosystems.

These initial results provide a sound base for this phytoplankton study, which will examine the impact of the trophic status on the phytoplankton community within two reservoirs. For example, the species richness of phytoplankton taxa has been suggested to decrease with increasing eutrophication (Dodson et al. 2000), implying an overall decrease in biodiversity in lakes with a higher initial nutrient concentration. Thus, the phytoplankton community structure will vary depending on the trophic status of the system.

Eutrophication is an additional threat to the health of the reservoirs in Puerto Rico, as Martinez et al. (2005) found the majority of reservoirs surveyed to be eutrophic based on total phosphorous concentrations. In addition to causing decreased phytoplankton diversity, eutrophication can also increase total algae biomass including that of the toxic cyanobacteria (Smith et al. 1999). Not only can cyanobacteria blooms be toxic, but they can also disrupt recreational activities (Paerl 1988). Eutrophication occurs due to a high nutrient concentration within the system, typically as a result of human activities including sewage runoff and fertilization application (Smith et al. 1999). However, other factors such as the presence of invasive bivalves have also been found to increase nutrient concentrations within a system.

The impact of invasive freshwater bivalves has been heavily studied in the United States as they can create large ecological problems (Sousa et al. 2009; Higgins and Vander Zanden 2010). There are several invasive bivalves, however most studies have focused on the zebra mussel due to its presence in the Great Lakes and its association with increased cyanobacteria blooms in low nutrient systems (Vanderploeg et al. 2001; Raikow et al. 2004; Sarnelle et al. 2005; Knoll et al. 2008). Although the zebra mussel has not been identified in Puerto Rico, a similar invasive bivalve has been spreading throughout the freshwater systems of the island since 1998: *Corbicula fluminea* (Williams et al. 2001). Though *C. fluminea* has not been found to directly promote cyanobacteria growth, it does have the ability to alter nutrient concentrations (Lauritsen and Mozley 1989) and the phytoplankton community (Cohen et al. 1984; Boltovskoy et al. 1995) within invaded systems.

This thesis examined the factors influencing the phytoplankton community structure within two Puerto Rican reservoirs with differing trophic statuses. Specifically, the impact of environmental variables, nutrient concentrations, *C. fluminea* presence, and the resulting spatial and temporal patterns in the phytoplankton community diversity and biomass were examined.

Thesis layout

This thesis is divided into two chapters. Each chapter describes a separate study with its own dataset and hypotheses that is intended to be submitted for publication to a peer-reviewed journal. The final chapter summarizes the conclusions of both studies and gives suggestions as to future projects which could be conducted.

Chapter 2

Identification of the Environmental Variables which Determine Phytoplankton Community Structure in Two Distinct Reservoirs

Introduction

Phytoplankton serves as the base of the trophic food web in lake systems, with several organisms depending on it as a food source (Carpenter et al. 1985). The phytoplankton community structure is known to change seasonally in freshwater lakes, often becoming dominated by certain taxonomic groups that take advantage of favorable conditions (Graham et al. 2004; Duarte et al. 2006; Grover and Chrzanowski 2006). Additionally, phytoplankton diversity can also experience seasonal shifts (Duarte et al. 2006; Lopes et al. 2009). Seasons in freshwater lakes have been identified by changes in parameters including temperature (Grover and Chrzanowski 2006), phytoplankton population structure (Graham et al. 2004), and rainfall (Nabout et al. 2006; Lopes et al. 2009). The differences between seasons, however, exist due to changes in environmental parameters such as water transparency and nutrient concentrations (Nabout et al. 2006; Lopes et al. 2009). Although environmental variables differ between seasons, they can also vary by year, which can cause the phytoplankton community to experience the same unpredictability (Nabout et al. 2006).

One factor that has been found to have a large impact on phytoplankton communities in all freshwater systems is nutrient concentration. Adding nutrients to a system has been shown to increase phytoplankton biomass and phytoplankton abundance (Gallegos et al. 1992; Brett and Goldman 1997). Additionally, nutrient levels have the ability to alter the phytoplankton community, as different components of the community are limited by different nutrients (Wetzel 2001; Reynolds 2006). Even though phosphorous has been shown to be the limiting nutrient in many freshwater systems, some tropical lakes have been found to be more limited by nitrogen (Hecky and Kilham 1988).

Although several variables have the potential to alter the phytoplankton community, rainfall is a major factor (Nabout et al. 2006; Lopes et al. 2009). Rainfall and runoff are related to factors such as total phosphorous, pH, light limitation, and oxygen concentration which have also been important determinants of phytoplankton species' distribution in reservoirs (Vanni et al. 2006; Dantas et al. 2008; Silva et al. 2010). Additional studies show that changes in the depth

of the mixing zone, which lead to varying degrees of disturbance, can also impact phytoplankton diversity (Lopes et al. 2009). Thus, it is clear many factors can determine the phytoplankton community structure within a reservoir.

Much of the work on seasonal shifts of phytoplankton communities in lakes has been completed in temperate environments (Reynolds 2006). The studies conducted in tropical lakes differ greatly in design and in the type of lake examined, making a predictable pattern of phytoplankton succession difficult to identify for tropical systems (Lewis 1978; Nabout et al. 2006; Lopes et al. 2009). The phytoplankton succession pattern within Puerto Rican reservoirs is largely unknown. In fact, the only studies examining phytoplankton within Puerto Rican reservoirs have focused on species identification and not on large scale temporal or spatial evaluations (Santos-Flores 2001; Martinez et al. 2005; Pantoja-Agreda et al. 2009). Thus, there is a need to relate the occurrence of groups within the phytoplankton community in Puerto Rico to environmental variables through time.

The objective of this study was to determine whether differences in the spatial and temporal phytoplankton community composition could be explained by environmental parameters including: water transparency, nutrient concentration, and temperature. Specifically, phytoplankton community composition, measured by diversity and abundance, was compared across a spatial gradient in dry and wet seasons for two subtropical Puerto Rican reservoirs. The two reservoirs differed in initial nutrient status, with one classified as eutrophic and the other as mesotrophic.

Methods

This study was carried out in two reservoirs of Puerto Rico: Guajataca and La Plata. The Guajataca reservoir (18° 22’ 39.0468” N, 66° 55’ 14.9082” W) is located in northwest Puerto Rico, between the municipalities of San Sebastian, Isabela, and Quebradillas (Figure 2.1, Ortíz-Zayas et al. 2004). The reservoir is 316 m long and 202 m above sea level (Ortíz-Zayas et al. 2004). The drainage area of Guajataca is approximately 79.8 km^2, with an average rainfall of 218 cm per year (Ortíz-Zayas et al. 2004). In January 1999, Guajataca was estimated to have a volume of 42,278,824m^3 and its water is renewed 2.5 times a year (Soler-López et al. 2000a). Additionally, Guajataca was found to have a sedimentation rate of 87,577.2 m^3/year, a surface

area of 3.4 km^2, and an average depth of 12.5 m (Soler-López et al. 2000a). The Guajataca reservoir was classified as mesotrophic in 2005 (Martinez et al. 2005).

The La Plata reservoir (18° 19" 39.8634" N, 66° 14' 4.4988" W) is located in north central Puerto Rico in the municipality of Toa Baja (Figure 2.1, Ortíz-Zayas et al. 2004). It has a drainage area of 468.8 km^2, is 236 m long, and is 47.2 m above sea level (Ortíz-Zayas et al. 2004). The average rainfall within the La Plata watershed is 188.0 cm per year (Ortíz-Zayas et al. 2004). La Plata was found to have a volume capacity of 35,458,902 m^3 in 1998, with a sedimentation rate of 197,974 m^3/year (Soler-López et al. 2000b). La Plata has a surface area of 3.3 km^2, an average depth of 10.7 m, and its water is renewed 8.2 times a year (Soler-López et al. 2000b). The reservoir was classified as eutrophic in 2005 (Martinez et al. 2005).

The Guajataca and La Plata reservoirs were selected based on their different initial nutrient concentrations in order to compare phytoplankton community composition at different nutrient concentrations. Both reservoirs were visited twice during the dry season and once during the wet season. However, as rainfall was highly variable between seasons (Table 2.2), each sampling period was classified by its relative rainfall and temperature. Samples from Guajataca were collected on February 22, 2010, August 9, 2010, and March 4, 2011 (cold wet, warm wet, and cold dry, respectively). La Plata samples were taken on March 9, 2010, August 16, 2010, and March 11, 2011 (cold wet, warm wet, and cold dry, respectively). On two of the sampling trips (August 9, 2010, and August 16, 2010), sampling was ended prematurely due to hazardous weather conditions and one transect from each reservoir was lost (2 samples per reservoir). Each reservoir was divided into 3 zones: the dam zone, the transition zone, and the riverine zone (Figure 2.1 and 2.2). This was done to determine whether there were spatial differences in the parameters examined. The sampling locations were selected through the use of transect lines established by previous studies (Soler-López et al. 2000 a,b). See appendix C for the latitude and longitude of all points sampled.

Three transects were randomly selected within each zone. On each transect, one sample was taken from both the middle reservoir area and the littoral area. Thus on each sampling trip, approximately 18 samples were taken. The following parameters were measured on site using a handheld YSI Pro Plus multisensor: temperature, specific conductance, pH, Secchi disc depth, and reservoir depth. Water samples were collected from within 1 m of the surface using a 4L Van Dorn bottle, and each sample was divided to evaluate the chlorophyll *a* level, the

phytoplankton community, and the nutrient concentration. The nutrient concentrations were determined at the Soil and Water Quality Laboratory located at the Rio Piedras Agricultural Experimental Station in Rio Piedras, Puerto Rico. The nutrients targeted were: total phosphorous (TP) and total Kjeldahl nitrogen (TKN).

Chlorophyll a Analysis

The chlorophyll *a* of each sample was determined using the protocol for identifying chlorophyll *a* in marine and freshwater algae through fluorescence originated by the U.S. Environmental Protection Agency (Arar and Collins 1997). A volume (150 mL) of the original water sample was filtered through a Whatman GF/F glass fiber membrane. Filters were stored in 50 mL amber centrifuge tubes below 0°C for no longer than 1 week. To extract the chlorophyll *a* from the filter paper, 5mL of a 90% acetone solution was added to the centrifuge tube. The filter paper was then mashed with a glass stirring rod. Once the paper reached a satisfactory consistency, the stirring rod was rinsed with 5mL of the 90% acetone solution into the tube, resulting in a total volume of 10 mL. The centrifuge tubes were then stored below 0°C for an additional 12-24 hours.

Next, the tubes were centrifuged at a speed of 5100 RPM for 5 minutes. The samples were then decanted into 20 mL amber tubes. 5 mL of the 90% acetone solution was then added to the 50 mL tube and the sample was again centrifuged. As before, the sample was decanted. An additional 5mL of 90% acetone was added and the process was repeated a final time. Thus, a total of 20 mL of 90% acetone was utilized to extract the chlorophyll *a*.

The chlorophyll *a* extract was then transferred to a clear glass vial to be analyzed by a TD- 700 Fluorometer (Version 2.0, Turner Designs). Samples which proved to have a high concentration of chlorophyll *a* were diluted with 90% acetone and the chlorophyll *a* levels were corrected afterward. The entire process, from the initial filtration to recording the chlorophyll *a* values, was completed in the dark.

The formula used to determine the final chlorophyll *a* concentration is:

$$\frac{\{\text{reading} * \text{extraction volume} * \text{dilution factor}\}}{\text{initial sample volume}}$$

In this formula,

reading= the initial reading given by the fluorometer
extraction volume = the amount of 90% acetone used to extract chlorophyll *a*
dilution factor = the amount by which the sample was diluted
initial sample volume = the volume of the sample taken from the reservoir

Although 20 mL of 90% acetone was added to extract the chlorophyll *a* concentration, not all of this volume was recovered in the decanting process as it was assumed that 5mL of acetone remained in the filter. Thus, the extraction volume was considered to be 15 mL.

Phytoplankton Identification and Biomass Calculation

Upon returning to the lab, each designated phytoplankton sample was preserved with a 1 % lugol solution (Lund et al. 1958). The phytoplankton community was analyzed through the utilization of a modified form of the Utermohl method (Utermohl 1958; Paxinos and Mitchell 2000). The sample was concentrated by a factor of 10 before 210 µL were pipetted into a Palmer Chamber with a cover slip. All phytoplankton within 50 square fields, each with an area of 62,500 μm^2, was identified to the genus level and counted for each sample at a magnification of 400 using a compound microscope (Paxinos and Mitchell 2000), to determine the number of cells per mL (Equations in Appendix B). The phytoplankton was identified to the genus level using several guides (Collins 1909; Smith 1950; Wehr and Sheath 2003). Many times it was not

possible to identify the phytoplankton to the species level and it was not necessary as the aim of the study was to examine the general structure of the phytoplankton community.

Additionally, cell measurements were taken of the first 9 individuals of each genus to determine the average cell biovolume of each genus per sample. The average cell biovolume was calculated using a variety of geometric formulas (Hillebrand et al. 1999). The formula for each genus was selected based on the shape of the organism (Appendix B). The average cell biovolume was then multiplied by the number of cells per mL to obtain the total biovolume for one genus per sample. To convert the biovolume to wet biomass, we assumed the phytoplankton to have a specific gravity of 1g/cm^3, giving biomass units of µg/L (Riemann et al. 1989; Knoll et al. 2008). The biomass of each genus could then be compared among samples across time and space. The biomass was not calculated for the genus *Heliozoa* because it does not have chloroplasts; however, it was included in calculating the Shannon Diversity Index.

Shannon Index Calculation

Additionally, the Shannon Diversity Index (H') was calculated for each sample. The formula used was:

$$H' = \sum \frac{n_i}{N} \ln\left\{\frac{n_i}{N}\right\}$$

In this formula,

H' = the Shannon Index value

n_i = the number of cells of each genus counted in the sample

N = the sum of all cells counted for all genera

Statistical Tests

The chlorophyll *a* concentration, TP, TKN, NO_3, physical parameters, phytoplankton biomass, and H' were compared using a 2 x 3 x 3 factor ANOVA in Infostat (version 2008). A complete model was run for all parameters with reservoir, zone, and season as factors. Post-hoc comparisons were done using the Bonferroni test statistic. A multiple regression analysis was

conducted in order to determine which nutrients were more influential on the chlorophyll *a* concentration.

In order to determine the relationship between the environmental variables measured and the phytoplankton community structure within the reservoirs, a canonical correspondence analysis (CCA) was completed using the program PC-ORD, version 6 (McCune and Mefford 1999). The independent variables used to complete the CCA were selected by completing a non-metric multi dimensional analysis (NMS). All variables measured throughout the study were included in the NMS; however, only variables with a high correlation to the axes in the ordination were selected to be used in the CCA analysis. CCA was chosen as it creates a direct relationship between the environmental variation and phytoplankton community variance (ter Braak 1986). The ordination allows a visual representation of how both the sites sampled and the distribution of the phytoplankton classes relate to gradients in the environmental variables.

Results

Differences between reservoirs

TP concentrations within La Plata (51.64 ± 8.54 µg/L) were higher than in Guajataca (15.64 ±25.93 µg/L) for all time periods (Figure 2.6, Appendix G). However, there was no difference in the TKN concentration between Guajataca (0.80 ± 0.82 mg/L) and La Plata (0.58 ± 0.24 mg/L) during any sample period (Appendix G). NO_3 concentrations were higher in La Plata (0.84 ± 0.74 mg/L) than Guajtaca (0.01 ± 0.01 mg/L) only during the warm wet sample period (Appendix G, H). Additionally, the NO_3 concentration only varied between zones in La Plata during the warm wet season (dam: 0.31 ± 0.04 mg/L, transition: 0.59 ± 0.23 mg/L, riverine: 2.01 ± 0.41 mg/L) (Appendix H). NO_3 concentrations within the Guajataca reservoir did not vary in time or space (Appendix G, H).

Specific conductance was found to be higher in La Plata (338.38 ± 23.49 µS/cm) than Guajataca (261.18 ± 23.49 µS/cm) for all time periods (Appendix G). The pH was higher in La Plata (8.60 ± 0.08) than Guajataca (8.04 ± 0.06) only during the cold wet sampling period (Appendix G). Secchi disc depth was greater in the Guajataca reservoir than in the La Plata reservoir for two sampling periods: cold wet and warm wet (Figure 2.4, Appendix G). There was no difference in temperature patterns between the two reservoirs.

Chlorophyll *a* was higher in La Plata (132.36 ± 129.49 µg/L) than in Guajataca (38.09 ± 8.26 µg/L) only during the cold dry season sampling (Figure 2.5, Appendix G). H' was also compared between La Plata and Guajataca; however, there was a 3- way interaction where H' varied between reservoirs and across season and zone with no clear identifiable pattern. Total phytoplankton biomass was found to be higher in La Plata (156.2 mg/L) than in Guajataca (12.5 mg/L), but only during the cold wet period (Appendix G).

Patterns within Reservoirs

Environmental parameters

The surface water temperature in the Guajataca reservoir ranged from 25.6 to 30.9°C, while the La Plata reservoir experienced temperatures ranging from 25.5 to 30.3°C (Table 2.1). The highest temperatures were recorded in the warm wet season for both Guajataca and La Plata (30.5°C ± 0.2 se and 29.6°C ± 0.5 se, respectively) (p= 0.05). Temperature did not vary spatially in either reservoir (Appendix G).

The lowest specific conductance in the Guajataca reservoir was recorded in the warm wet season (229.1 µS/cm) (p= 0.05) (Appendix G), which was a nearly 20% decrease from the cold wet season (Table 2.1). The La Plata reservoir had the highest specific conductance in the cold dry season (364.7 µS/cm) (p= 0.05) (Appendix G). The pH in the Guajataca reservoir ranged from 7.92 to 8.67 (Table 2.1). The highest pH was measured in the warm wet season (8.61 ± 0.05), and the lowest in the cold wet (8.04 ± 0.06) (p= 0.05). The pH in the riverine zone was higher than the dam zone in the cold dry season (p= 0.05) (Appendix G). In the La Plata reservoir, pH ranged from 8.12 to 8.86 and did not differ between seasons (Table 2.1). The pH of the riverine zone did not vary through the study; however, the pH of the dam and transition zones decreased from the cold wet to the cold dry season by less than 5% ($p<0.001$).

Secchi disc depth varied within the reservoirs by season. The warm wet season in the Guajataca reservoir had the largest secchi disc depth (clearest water) (2.47m ± 0.61) while La Plata's (1.34 m ± 0.34) was during the cold dry season (p= 0.05). Additionally, there was an interaction between zone and time. The secchi depth tended to be greater in the dam zone for both reservoirs while the riverine zone was lowest regardless of season. (Figure 2.4A,B).

Chlorophyll a

Chlorophyll *a* is considered an indicator of the phytoplankton biomass present, as well as of nutrient concentrations. Chlorophyll *a* did not vary over time or space within the Guajataca or La Plata reservoir, except for one time point. In the La Plata reservoir, the riverine zone during the cold dry season had the highest chlorophyll *a* (145.0 ± 161.4 µg/L). This was partially due to the inclusion of the highest chlorophyll *a* concentration measured for the entire study (492 µg/L). There was also a correlation between the log of chlorophyll *a* and the log of total biomass in the Guajataca reservoir (r^2= 0.52, p<0.001), but not in the La Plata reservoir (Appendix D).

Chlorophyll *a* and TP were positively correlated (r^2= 0.79, p<0.001). When the reservoirs were analyzed separately, the chlorophyll *a* and TP were found to be highly correlated in the Guajataca reservoir (r^2= 0.8, p=0) (Appendix D), which has been seen in other studies conducted in Guajataca (Pantoja-Agreda et al. 2009). In the La Plata reservoir, however, the relationship between chlorophyll *a* and TP was not as strong (r^2= 0.33, p=0.02) and the correlation was lower than the value suggested by Canfield and Bachmann (1981) for artificial lake systems (r^2= 0.57). A multiple regression analysis revealed that TP and TKN explained 32% of the variation in chlorophyll *a* concentrations in the La Plata reservoir (R^2=0.32, p<0.001), while TP alone explained 55% of the variation seen in the Guajataca reservoir (R^2= 0.55, p<0.001).

Nutrient Concentrations

TP concentration in the Guajataca reservoir did not differ between zones or across seasons (Figure 2.6A). In the La Plata reservoir, TP was highest in the riverine zone (77.1 µg/L) (Figure 2.6B). Temporally, the warm wet season had the highest TP (60.1 ± 3.3 µg/L), the cold dry season was the lowest (46.0 ± 3.0 µg/L), and the cold wet was intermediate (59.0 ± 3.0 µg/L). There was no clear spatial or temporal patterns in the TKN concentration in either reservoir. NO_3 did not vary in Guajataca over time or space. In La Plata, however, the warm wet season was found to have the highest NO_3 concentration (0.84 ± 0.74 mg/L) (Appendix H). Within this season, spatial differences existed as well with the riverine zone having the highest concentration (2.01 ± 0.05 mg/L) while the dam zone had the lowest (0.31 ± 0.04 mg/L).

Phytoplankton Community Composition

The phytoplankton community was identified to the genus level, which was the lowest classification possible. However, the different genera of the centric diatoms were not able to be differentiated due to the limitations of the microscope used. Based on previous research, we know the two most common centric diatoms in Puerto Rican reservoirs are *Melosira* and *Cyclotella* but their relative frequencies are unknown (Santos-Flores 2001). H' is determined by both species richness and evenness, thus a high H' value indicates high species richness, high species evenness, or both. In the Guajataca reservoir H' did not differ spatially or temporally (Figure 2.8A). However, although H' did not differ over time in La Plata, there were spatial differences. The riverine zone had the highest H' in the cold wet season (1.60), while the dam zone had the highest H' in the cold dry season (1.37) ($p= 0.05$) (Figure 2.8B) (Appendix G).

The phytoplankton biomass was calculated for each genus separately, but the genera were then divided into 6 classes for statistical analysis as some genera had low biomass. This classification was based on Nabout et al. (2006). Each class is made up of the following genera: Bacillariophyceae- *Navicula*, *Syneda*, Centric diatoms. Dinophyceae- *Peridinium*. Cyanophyceae- *Spirulina*, *Merismopedia,* unknown cyanobacteria, *Anabaena*. Chlorophyceae- *Oocystis*, *Crucigenia*, *Coelastrum*, *Scenedesmus*, *Tetraedron, Treubaria, Pediastrum*, *Pandorina*, *Eudorina*, filamentous algae, unknown green algae. Zygnematophyceae- *Starastrum*, Desmid. Euglenophyceae- *Trachelomonas*.

The biomass of the Guajataca reservoir did not vary spatially or temporally; however, the phytoplankton classes did follow spatial patterns. Chlorophyceae and Dinophyceae comprised over 50% of the phytoplankton community in the dam zone of each season sampled. However, Dinophyceae alone contributed 50% of the total biomass in the riverine zone of the cold wet season (Figure 2.9A). Temporal trends were also observed within the phytoplankton classes. In the warm wet season, Dinophyceae dominated the phytoplankton community in both the transition and riverine zones, making up over 85% of each zone's biomass ($p<0.001$) (Figure 2.9 B,C). However, in the cold dry season Chlorophyceae and Bacillophyceae tended to be the most prevalent throughout the Guajataca reservoir, comprising 65-90% of the phytoplankton biomass depending on zone (Figure 2.9).

In the La Plata reservoir, there was no difference in the phytoplankton biomass over time. However, a spatial difference was observed during the cold wet season as the riverine zone was

found to have the largest biomass (19,239 µg/L) (p= 0.05). As in the Guajataca reservoir, the phytoplankton classes followed spatial patterns in La Plata as well. Chlorophyceae dominated the La Plata reservoir in the cold wet season, as it comprised a majority of the biomass in the transition and riverine zones (45% and 67%, respectively) (Figure 2.10 B,C), and was also a large percentage of the biomass in the dam zone (35%), second only to Cyanophyceae (39%). However, in the warm wet season and cold dry season, Dinophyceae dominated the La Plata reservoir, making up over 70% of the biomass in each zone (Figure 2.10).

In order to determine the variables to be used in the canonical correspondence analysis (CCA), a non-metric multidimensional analysis (NMS) was first run using the data collected from both reservoirs. The number of phytoplankton cells found were added across transects and the environmental variables were averaged to compensate for the low values found for some classes. The NMS was run using the program PCORD, which uses the Sorensen index as a measurement of distance in order to plot the sample points. The stress found for the NMS was 3.421 (p=0.004). A 2D ordination was recommended. Examining this ordination, the following parameters were selected based on their strong relationship to one of the 2 axes: specific conductance, pH, secchi disc depth, and TP.

Axis 1 of the CCA explained 13% of the variance, while axis 2 explained 2.9% of the variance. The specific conductance, TP, and pH were highly correlated with axis 1 (-0.627, 0.496, and -0.366 respectively), while secchi disc depth was correlated with axis 2 (0.485). One important factor that can be noticed upon examining the ordination is that the season and reservoir sampled tended to be a large factor in determining the placement of the sample points (Figure 2.11). Thus, the reservoirs were analyzed separately.

An NMS was again run to determine whether the environmental parameters used for each CCA would differ depending on the reservoir. For Guajataca, pH, specific conductance, temperature, TP, and the season sampled were the variables selected; however, the season sampled and specific conductance were discarded due to their high correlation to at least one of the remaining variables. The stress found was 10.15 (p=0.02) and a 2D ordination was recommended. In La Plata, specific conductance, pH, chlorophyll *a*, TP, NO_3, TKN, and the season sampled were the variables selected; however, the season sampled, TKN, and chlorophyll *a* were removed due to a high correlation with at least one of the remaining variables. The stress was 4.54 (p=0.004) and, again, a 2D solution was recommended.

In the CCA of the Guajataca reservoir, temperature and pH were correlated to axis 1 (0.893 and -0.392, respectively) but TP was correlated with axis 2 (-0.527). Axis 1 explained 29.5% of the variance (P= 0.005; Monte Carlo permutation test with 999 permutations), while axis 2 explained 7.5%, meaning a total of 37% of the variance in phytoplankton community composition between samples is explained by the variables selected (Figure 2.12). After running the CCA for La Plata, axis 1 was correlated with specific conductance, pH, and NO_3 (-0.794, -0.296, and -0.341, respectively). Axis 2 was correlated with TP (-0.253). Axis 1 was found to explain 68.8% of the variance (P=0.001; Monte Carlo permutation test with 999 permutations), while axis 2 explained 8.5%, giving a total of 77.3% of the variance observed in the phytoplankton community structure between samples being explained by the variables selected (Figure 2.13). A high explanation of the variance was probably found due to the small number of phytoplankton classes used in the analysis.

Discussion

The phytoplankton community structure varies spatially across both reservoirs and through time (Figures 2.9 and 2.10). Each reservoir seems to have its own unique community composition with La Plata having a higher phytoplankton biomass than Guajataca. Season appears to influence community composition in both reservoirs due to the variation in environmental variables observed across seasons (Figure 2.11). In the mesotrophic Guajataca reservoir, temperature and TP seem to be the most influential in determining the phytoplankton community structure, while specific conductance and NO_3 impacts the community in the eutrophic La Plata reservoir.

Differences between Reservoirs

Guajataca and La Plata always differed in the concentration of TP, with Guajataca having lower levels of TP than La Plata (Figure 2.6 A,B). This is not surprising since the reservoirs were selected based on their classification and TP is the main factor used to determine the nutrient classification of freshwater systems (Smith et al. 1999). The Guajataca reservoir is classified as mesotrophic, while the La Plata reservoir is classified as eutrophic (Martinez et al 2005).

TKN concentrations are also expected to vary between mesotrophic and eutrophic systems, but no difference was found between Guajataca and La Plata in any season. However,

NO_3 concentrations were higher and varied across zones in La Plata during the warm wet season. It is unclear why high NO3 concentrations were only observed in La Plata during this season; however, it could be due to the combination of La Plata being strongly stratified and an intermediate level of rainfall (Table 2.2).

Although most freshwater systems are thought to be phosphorous limited (Wetzel 2001), some have also been found to be nitrogen limited as well (Martinez et al. 2005). This seems to be the case for eutrophic La Plata and not mesotrophic Guajataca, based on the N/P ratio. The Guajataca reservoir was found to have a higher N/P ratio (79.86 ±18.00) than the La Plata reservoir (11.36 ± 18.10) ($p=0.009$). A low N/P ratio signifies nitrogen is limiting within the system. This conclusion is supported by the fact that chlorophyll *a* is dependent on TKN in La Plata but not in Guajataca (see results). Thus, the phytoplankton community in La Plata is limited by nitrogen whereas the community in the Guajataca reservoir lacks phosphorous.

Stratification typically occurs in the summer or rainy season in tropical systems (Lewis 1978; Sotomayor et al. 2008), due to higher surface water temperatures, while mixing occurs during the winter or dry season (Ramberg 1987; Calijuri et al. 2002; Lopes et al. 2009). Stratification must be taken into consideration as it has been shown to impact the phytoplankton community structure by influencing water transparency, nutrient concentration, phytoplankton biomass, and phytoplankton diversity (Ramberg 1987; Calijuri et al. 2002; Hubble and Harper 2002; Sotomayor et al. 2008; Lopes et al. 2009). As rainfall has also been found to be an important factor in other systems (Nabout et al. 2005; Lopes et al. 2009), it was considered here as well. However, individual rainfall collection stations were not available for each individual sampling point and thus comparisons can only be made between seasons. In order to estimate the amount of precipitation falling in the watershed of each reservoir, the total rainfall from two separate rainwater collection stations used by the USGS were combined for 10 days prior to the sampling trips (Table 2.2). Although stratification varied somewhat between reservoirs, rainfall patterns did not seem to differ between reservoirs.

Examining the temperature profiles (Martinez unpublished data), it is clear that stratification only occurred in the warm wet season of the Guajataca reservoir, which is supported by the highest surface temperatures and greatest water transparency being measured in this season. Increased water transparency indicates decreased turbidity and, thus, a stable water column (Ramberg 1987; Lopes et al. 2009; Pantoja-Agreda et al. 2009), while increased

temperatures indicate the water column is stable through thermal stratification (Calijuri et al. 2002). However, some parameter measurements cannot be explained by stratification in the warm wet season, such as a low specific conductance and high pH level, but could be a result of increased water flow into the system due to intense rainfall and runoff. The geology of Guajataca is calcareous, meaning water flowing into the system interacts with the $CaCO_3$ substrate. $CaCO_3$ has been shown to increase the pH and decrease the conductivity in the runoff of other systems (Gilley et al. 2007).

La Plata was not only stratified in the warm wet season, but also in the cold wet season (Martinez unpublished data); however, this may have been a rare event due to the fact that it is usually dry during the cold season. Although the highest temperatures were measured in the warm wet season, the greatest water transparency was observed during the cold dry season. The lack of high water transparency during the stratified seasons could be due to increased runoff (Ramberg 1987). Additionally, Pantoja-Agreda et al. (2009) suggested turbidity has a biogenic origin in another Puerto Rican reservoir, thus other parameters should be examined in order to determine the cause of low water transparency. The specific conductance and pH varied widely in the La Plata reservoir without exhibiting a seasonal pattern. As the geology of the La Plata reservoir is mostly volcanic, there are many ions within the system which may vary differently depending on whether mixing within the reservoir is occurring. Additionally, this variation could be due to fluctuations in the rainfall observed over the three seasons sampled.

La Plata was also found to have a higher specific conductance than Guajataca in every sample period. This is most likely due to the different characterization of each reservoir basin. However, the variation in stratification may account for the higher pH level found in La Plata in the cold wet season, as La Plata was stratified and Guajataca was not. Secchi disc depth is another parameter which has been found to vary with the classification of freshwater systems (Smith et al. 1999); however, it was only found to be greater in Guajataca in the cold wet and warm wet seasons. The higher secchi disc depth observed in La Plata during the cold dry season could be due to the decrease in runoff, while a lower secchi disc depth in Guajataca was most likely measured due to mixing.

Chlorophyll *a* has been found to be typically higher in eutrophic systems (Smith et al. 1999); however, in this study the chlorophyll *a* was only higher in La Plata during the cold dry season. The lack of a difference in the other seasons sampled could be due to the variation of

chlorophyll *a* measured and the limited number of samples. Although diversity within a phytoplankton community is typically thought to increase with increasing nutrient concentrations (Hutchison 1961), this was not found between Guajataca and La Plata as there was a large amount of variation. However, the overall H' in the Guajataca reservoir was higher than in the La Plata reservoir (1.21 ± 0.05 and 0.92 ± 0.05, respectively) ($p<0.001$). Eutrophic systems have also been found to have a higher phytoplankton biomass than mesotrophic systems (Wetzel 1983). In this study, eutrophic La Plata was only found to have a higher phytoplankton biomass than Guajataca in the cold wet season. The high biomass found could be the result of stratification and increased runoff.

Spatial Variance within Reservoirs

A gradient between the dam and riverine zone was found for the environmental parameters in both the Guajataca and La Plata reservoirs, as expected (Martinez et al. 2005; Pantoja-Agreda et al. 2009). In Guajataca and La Plata, water clarity tended to be higher in the dam zone which agrees with the findings of a previous study (Pantoja-Agreda et al. 2009). However, no other trends existed in the Guajataca reservoir. In the La Plata reservoir TP was found to be higher in the riverine zone, with NO_3 also higher in the riverine zone during one sample season.

High nutrient concentrations in the riverine zone in the La Plata reservoir were most likely due to the La Plata River feeding into this zone. The river contains water from a large drainage basin that includes both urban and agricultural land. These types of watersheds typically have a high nutrient load. The lack of a spatial nutrient gradient in the Guajataca reservoir could be due to the variation of TP and TKN measured within the sample period (Figures 2.6A and 2.7A). As there is a nutrient gradient within the La Plata reservoir, higher chlorophyll *a* concentrations in the riverine zone can be explained by the high nutrient concentration, as a relationship was found to exist between chlorophyll *a* and the TP concentration (see results). Additionally, the high chlorophyll *a* level in the riverine zone most likely explains the decreased water transparency, as organic matter was found to have a larger impact than suspended solids on water clarity in a previous study (Pantoja-Agreda et al. 2009).

In La Plata, a high H' was observed in the riverine zone in the cold wet season. High phytoplankton diversity occurring at high nutrient concentrations is not uncommon and, due to

its counter-intuitive nature, is known as the paradox of the plankton (Hutchison 1961). This holds true for La Plata, where the highest diversity was found with the highest nutrient concentration. This pattern was not observed in Guajataca, however, where no difference in the nutrient concentration or phytoplankton diversity was observed between zones.

In addition to examining phytoplankton diversity, the phytoplankton community composition was also examined between zones. The biomass within the Guajataca reservoir did not vary between zones (1,729 µg/L), nor did the biomass of the different classes. This suggests the phytoplankton community did not vary spatially within the Guajataca reservoir. In the La Plata reservoir, however, the riverine zone had the highest biomass in the cold wet season. The high biomass in the riverine zone is most likely due to the higher nutrient concentration within the zone as well and contributes to the difference in H'. As the phytoplankton community composition does not vary between zones, the high H' may be the result of a higher overall phytoplankton biomass in the riverine zone.

Examining how parameters differ spatially within a reservoir or lake is an analysis typically not undertaken, as a majority of studies choose instead to focus on changes over time and depth at one sample point (Grover and Chrzanowski 2006; Lopes et al. 2009). Though previous studies conducted in Puerto Rican reservoirs have recognized a spatial difference between the dam and riverine zones, the transition zone has been relatively unexamined (Martinez et al. 2005; Pantoja-Agreda et al. 2009), which seems to be justified as this zone contains parameters similar to both the dam and riverine zones. In order to reduce sampling effort for future studies, the dam and riverine zones should be emphasized as the transition zone was typically found to have intermediate values for both the environmental parameters and the phytoplankton biomass.

Temporal Variance within Reservoirs

Although the zones have different characteristics, differences in the environmental parameters and phytoplankton communities were observed between seasons as well. The shifting values observed were most likely due to the variable stratification and rainfall patterns seen throughout the sampling period.

TP and TKN concentrations in the Guajataca reservoir did not vary through time. High TP and TKN concentrations were expected to be found in the wet season based on previous

studies (Lopes et al. 2009; Pantoja-Agreda et al. 2009), due to increased runoff entering the system. The fact the nutrient level did not vary between sampling seasons in Guajataca could due to the limited number of seasonal samples taken and the variable rainfall observed. Although TKN concentrations in La Plata did not vary, the highest TP and NO_3 concentrations were found in the warm wet season. As this was a wet season, it is possible increased runoff elevated TP and NO_3 concentrations.

As environmental parameters shift between seasons, it is expected that the phytoplankton community will change as well (Lewis 1978). Although phytoplankton diversity within both Guajataca and La Plata was variable, there was no pattern between seasons. Additionally, the range of H' values observed in both Guajtaca (0.45-1.82) and La Plata (0.29- 1.75) included values that were lower than the H' range found by a previous Guajataca study (1.67-2.21) (Pantoja-Agreda et al. 2009). The low H' values found in this study are most likely a result of Dinophyceae dominating the phytoplankton community (Hubble and Harper 2002; Graham et al. 2004; Duarte et al. 2006).

The highest diversity was found in the zone with the highest biomass for the La Plata reservoir. This is probably due to a high species evenness. The lowest diversity in La Plata was observed during the warm wet season, which was most likely the result of a low phytoplankton biomass (Figure 2.10). Mittelbach et al. (2001) found there is a continuum of responses in productivity of different ecosystems depending on species diversity. It seems that the reservoirs reflect this complexity, exhibiting patterns that often seem contradictory. More sampling would need to be done to adequately address this question within the reservoirs of Puerto Rico.

The biomass within the La Plata reservoir did not differ consistently between seasons. However, the highest biomass was observed during the cold wet season in the riverine zone, during a period of stratification. The lowered biomass measured during the warm wet season is likely due to an increase in the amount of suspended particles, which limits the light penetration depth and decreases the overall phytoplankton biomass (Diehl et al. 2002) (Figure 2.4B).

Seasonal shifts in the phytoplankton are known to occur within lakes, and previous research shows the phytoplankton succession pattern in tropical lakes is similar to that observed in temperate ones (Lewis 1978). The phytoplankton succession pattern begins once mixing has ended and the water column has stabilized. The pattern moves from diatoms to green algae, followed by blue-green algae and finally dinoflagellates (Lewis 1978). Although this succession

pattern was not observed within these reservoirs, most likely due to limited sampling effort, it is possible snapshots of the cycle were sampled. For example, sampling during the cold wet season most likely occurred during the green algae phase, as a large biomass of Chlorophyceae was found in both reservoirs. Additionally, the reservoirs were most likely in the final stage of the phytoplankton succession pattern during the warm wet season sampling, as Dinophyceae was found to dominate both phytoplankton communities.

Different classes of phytoplankton were able to dominate during different seasons within both reservoirs. In the cold seasons, Chlorophyceae tended to have one of the highest biomass in Guajataca and La Plata. Chlorophyceae is a C- strategist, as defined by Reynolds (1988), and is thus typically associated with high nutrient and light levels. Although nutrient levels were not high during these sampling seasons, mixing was occurring, which has also been found to favor Chlorophyceae (Calijuri et al. 2002). Bacillariophyceae, an additional C-strategist, was found to have a large biomass in the Guajataca reservoir during the cold dry season and has also been associated with turnover events in other systems (Ramberg 1987; Hecky and Kling 1981). However, due to their rapid sinking rate, diatoms typically do not persist within the water column once mixing stops unless a resuspension event occurs (Nabout et al. 2006).

Dinophyceae seemed to constantly dominate both the Guajataca and La Plata reservoirs, especially during the warm wet season (Figure 2.9 and 2.10). Dinophyceae is classified as an S-strategist (Reynolds 1988), due to its enhanced resistance to sinking and ability to store nutrients. Additionally, Dinophyceae is able to survive in low nutrient environments and avoid zooplankton predation, which may allow it to become more dominant in the summer as observed in other systems (Vanni and Tempte 1990; Graham et al. 2004; Duarte et al. 2006). Zooplankton predation was not likely an issue in these reservoirs, as zooplankton did not seem to be abundant (personal observation); however, their ability to photosynthesize and digest matter, known as mixotrophy, may greatly aide them in these Puerto Rican reservoirs (Reynolds 2006). Mixotrophy is common in many dinoflagellates, though it is typically studied in marine species (Stoecker 1999). This would allow Dinophyceae a competitive edge for resources within the phytoplankton community.

In order to attempt to predict phytoplankton community composition, a CCA was conducted. Initially, the reservoirs were analyzed together as the same environmental variables were expected to impact the phytoplankton similarly in both reservoirs (Figure 2.11). However,

improved relationships were observed when the reservoirs were analyzed separately. This allowed us to see that there are actually different variables determining the phytoplankton community composition in the two reservoirs (Figures 2.12 and 2.13).

In the Guajataca reservoir, temperature and pH seem to have a large impact on community composition, with TP playing a smaller role. TP is known to influence phytoplankton communities, as it is highly correlated with chlorophyll *a* and certain classes, including cyanobacteria (Dillon and Rigler 1974; Calijuri et al. 2002). Although TP typically has a large impact on phytoplankton communities, its importance may have been reduced due to the small range of occurrences TP was measured. Additionally, temperature has been documented to play a large role in phytoplankton community composition, though normally in temperate systems (Grover and Chrzanowski 2006). However, pH is not thought of as having a major influence on the phytoplankton community, though it can be an indicator of dissolved oxygen concentrations and phytoplankton activity. It is likely both temperature and pH were more a factor of the season sampled, which still played a large role in determining the phytoplankton community even within reservoirs.

The environmental variables which influence community composition were different in La Plata. Although TP and pH were also found to be important determinants of the phytoplankton community, specific conductance was the variable with the most influence. As specific conductance is not typically thought of as an important factor, it could be a signal of a larger process occurring within the reservoir, such as mixing. Another possibility is the phytoplankton within La Plata is highly dependent on the concentration of certain ions which were not measured in this study. Some ions which have been found to be important to most phytoplankton species include calcium and silicon (Reynolds 2006). As the specific conductance varied with the sample season, it is likely ions which are important to phytoplankton also vary seasonally. Thus, ions in addition to phosphorous and nitrogen may also be limiting to phytoplankton growth in the La Plata reservoir.

The fact that different variables had a larger impact on determining the phytoplankton community composition in the two reservoirs could be a result of the initial different trophic statuses. Thus, the findings of this study suggest the environmental parameters which influence the phytoplankton structure could vary with trophic status.

Constraints and Conclusions

The total number of genera found during the entire sampling period was 20 for the Guajataca reservoir and 19 for the La Plata reservoir. Although these values agree with a previous study (Pantoja-Agreda et al. 2009), they are low compared to other tropical lakes and reservoirs (Table 2.3). However, there are some major differences between those studies and this one. One such difference is sampling frequency. Most other studies sampled more intensively than ours, ensuring that more phytoplankton genera would be identified. Another sampling difference is the fact that only the surface water was targeted, while other studies examined a vertical profile. This limited the number of phytoplankton genera found, as several phytoplankton species vary in their vertical distribution (Ganf 1974). Additionally, in some cases the phytoplankton genera could not be identified. Thus it is possible rare genera were missed due to identification limitations. In order to achieve a more accurate evaluation of the genera present in the phytoplankton in both reservoirs, a more thorough sampling protocol should be employed.

The reservoirs in Puerto Rico seem to undergo constant change, where no two seasons are alike. Additionally, a spatial gradient seems to exist in both reservoirs. The initial trophic status of the reservoir has a major impact in determining which environmental variables influence the phytoplankton community structure. Rainfall is highly variable within the system, which seems to have a large impact on several parameters. Thus, long term studies should be conducted which take into account the complexity of these systems. Only with more frequent sampling over a longer time period will we understand how the phytoplankton community responds to environmental variables.

Table 2.1. Physical parameters found in each reservoir as influenced by zone[1] and season. Each value is the mean calculated from the 6 measurements taken within each zone, although DZ in the cold wet season for Guajataca was based on 5 measurements. The standard deviation is also given for each value. Parameters were measured within 1m of the reservoir surface.

	Cold Wet Season			Warm Wet Season			Cold Dry Season		
Parameters	**DZ**	**TZ**	**RZ**	**DZ**	**TZ**	**RZ**	**DZ**	**TZ**	**RZ**
				Guajataca					
Temperature (°C)	26.7 ± 0.1	27.2 ± 0.2	26.9 ± 0.3	30.4 ± 0.1	30.7 ± 0.2	30.4 ± 0.3	25.7 ± 0.1	26.4 ± 0.4	26.4 ± 0.2
pH	8.04 ± 0.08	8.08 ± 0.02	7.99 ± 0.02	8.61 ± 0.03	8.59 ± 0.06	8.64 ± 0.04	8.21 ± 0.04	8.31 ± 0.06	8.38 ± 0.03
Specific Conductance (µS / cm)	282.6 ± 1.82	283.43 ± 1.26	264.68 ± 1.57	229.68 ± 0.57	229.37 ± 2.4	228.16 ± 1.32	278.56 ± 0.16	278.30 ± 0.64	277.45 ± 0.85
				La Plata					
Temperature (°C)	28.7 ± 0.5	28.1 ± 0.4	27.5 ± 0.3	29.7 ± 0.3	29.7 ± 0.3	29.6 ± 0.9	25.9 ± 0.5	26.1 ± 0.2	26.2 ± 0.1
pH	8.59 ± 0.11	8.62 ± 0.06	8.59 ± 0.07	8.80 ± 0.05	8.72 ± 0.07	8.57 ± 0.12	8.20 ± 0.05	8.43 ± 0.10	8.64 ± 0.05
Specific Conductance (µS / cm)	313.02 ± 1.48	318.80 ± 4.49	337.40 ± 2.13	317.28 ± 7.56	337.22 ± 3.64	322.03 ± 8.29	365.10 ± 0.49	362.58 ± 1.37	366.52 ± 5.56

[1] The zones are labeled as follows: DZ= dam zone, TZ= transition zone, RZ= riverine zone.

Table 2.2. Total rainfall occurring 5, 7, and 10 days prior to sampling within the watersheds of the Guajataca and La Plata reservoirs. The two stations used for the Guajataca watershed were: USGS 50010800 (located at the Guajataca dam) and 50010500 (located at the Guajataca River in Lares). The two stations used for the La Plata watershed were: 50045000 (located at the La Plata dam) and 50043800 (located at the La Plata River in Comerio). These rainfall measurements were taken from the USGS website (www.usgs.gov).

		Guajtaca Reservoir		**La Plata Reservoir**	
Season	**Day**	**Dam (in)**	**River (in)**	**Dam (in)**	**River (in)**
Cold Wet	10	2.76	1.14	3.63	1.82
	7	2.76	1.14	0.23	0.24
	5	2.76	1.14	0.08	0.16
Warm Wet	10	0.03	0.46	2.25	1.9
	7	0.03	0.46	1.17	1.31
	5	0	0.11	1.16	0.53
Cold Dry	10	0.02	0	0.37	0.02
	7	0.02	0	0.37	0.02
	5	0.02	0	0.32	0.02

Table 2.3. The number of species observed within several reservoirs and lakes worldwide. Note some studies did not distinguish between cold and warm season species counted.

Lake/ Reservoir Name, Location	Source	Number of Species in Cold Season	Number of Species in Warm Season
Guajataca Reservoir, Puerto Rico	Current Study	19 genera	15 genera
La Plata Reservoir, Puerto Rico	Current Study	17 genera	12 genera
Guajataca Reservoir, Puerto Rico	Pantoja et al. 2009	22 species	
IAG Reservoir, Southeast Brazil	Lopes et al. 2009	19-26 species	18-32 species
Floodplain lakes of the Araguaia River, Central Brazil	Nabout et al. 2006	292 species	
Barra Bonita Reservoir, Sao Paulo State, Brazil	Calijuri et al. 2002	112 species	79 species
Lake Kariba (man made), Southern Africa	Ramberg 1987	82 species	
Lake Naivasha, East Africa	Hubble and Harper 2002	170 species	
Lake Batata, Brazil	Melo and Huzar 2000	203 species	
Lake Tanganyika, Burundi	Hecky and Kling 1981	103 species	
Lake Lanao, Phillipines	Lewis 1978	70 species	
Crystal Bog Lake, Wisconsin	Graham et al. 2004	96 species	

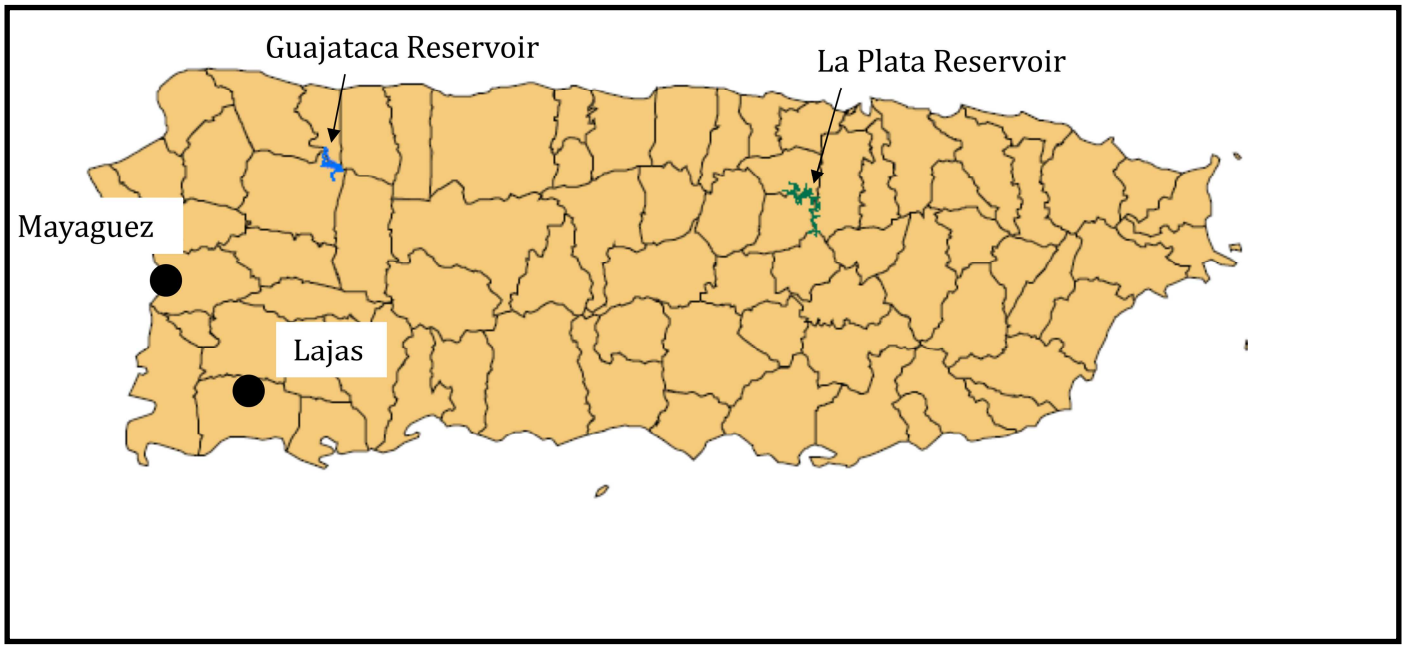

Figure 2.1. Map depicting the location of Guajataca and La Plata reservoirs used in the study. Mayaguez is where the mesocosm experiments were carried out. Lajas is where the Lajas Experimental Agricultural Station is located, which is where the Asian clams used in the mesocosm experiment originated (see chapter 3).

Figure 2.2. Map depicting the three zones sampled in the Guajataca Reservoir. The three transect lines that were sampled in each zone are identified with lines. Two samples were collected on each transect (littoral and middle) **(●)**. For the geographic coordinates of each sample site, see Appendix C.

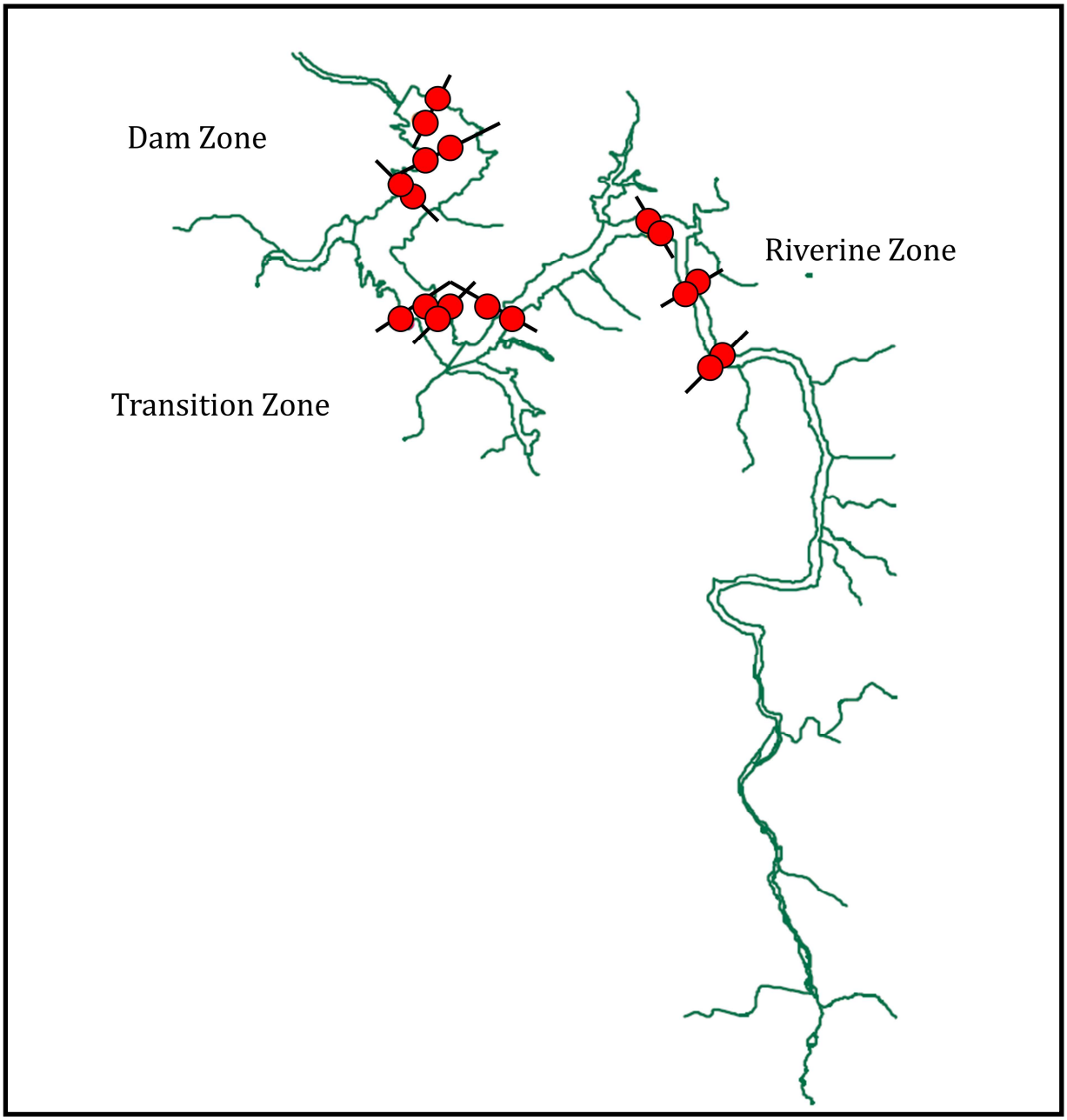

Figure 2.3. Map depicting the three zones sampled in the La Plata Reservoir. The three transects that were sampled in each zone are identified with lines. Two samples were collected on each transect (littoral and middle) (●).For the geographic coordinates of each sample site, see Appendix C.

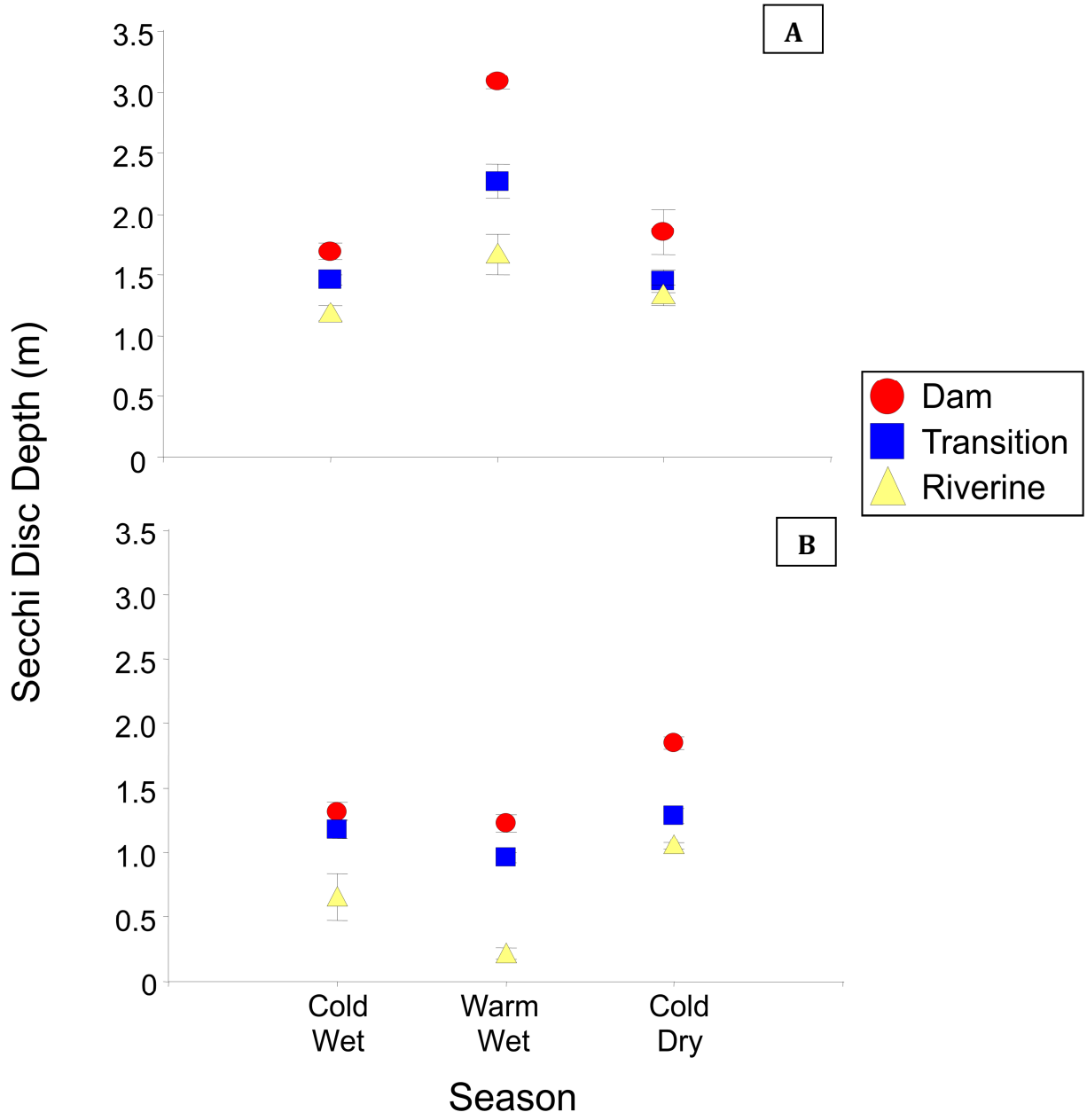

Figure 2.4. Mean secchi disc depth measurement in each zone for (A) Guajataca and (B) La Plata. The average values are shown for each zone and the bars associated with each point represent the standard error. If no bars are visible, it indicates a small standard error.

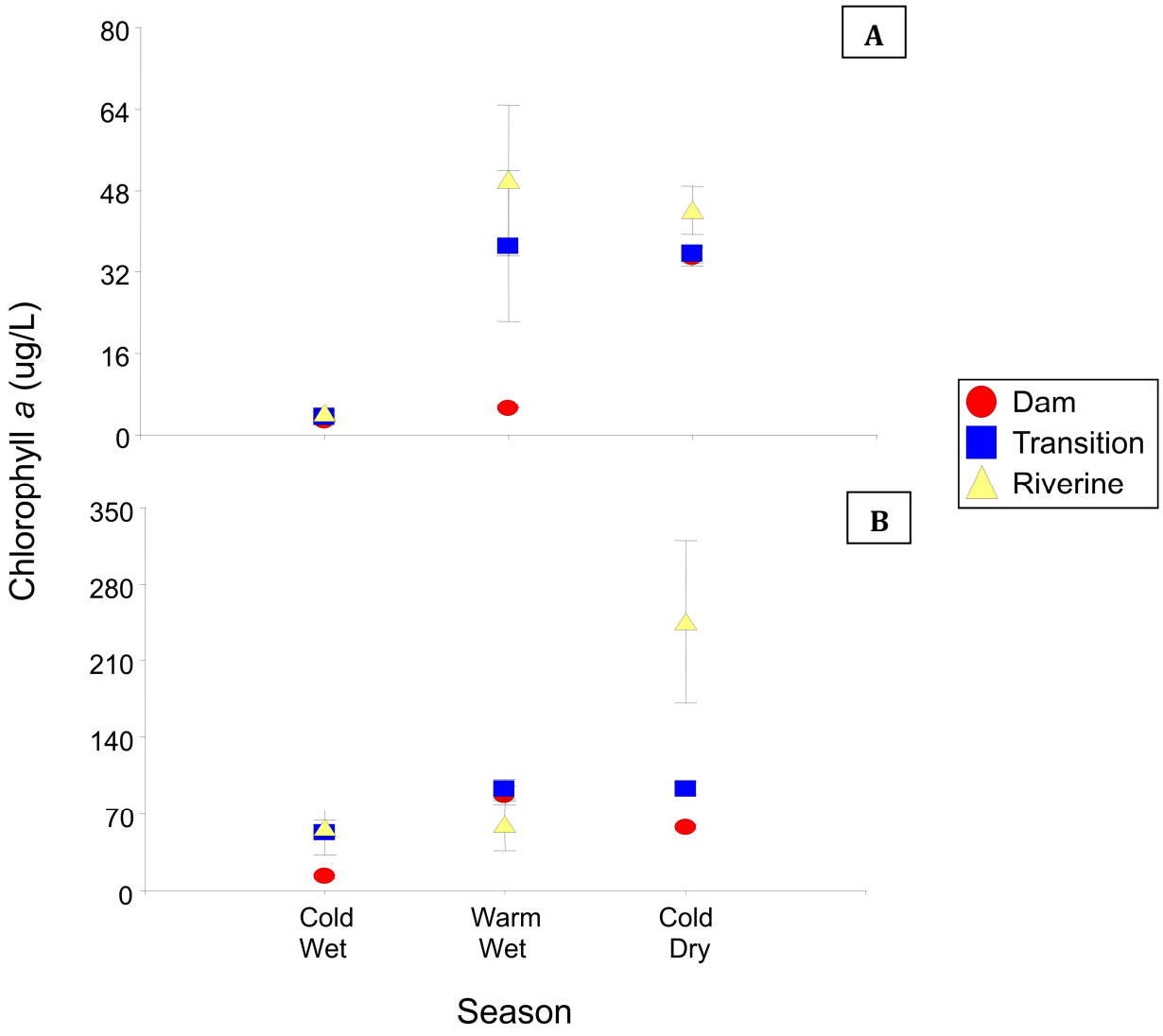

Figure 2.5. Mean chlorophyll *a* concentration measured in each zone for (A) Guajataca and (B) La Plata. The values are divided by zone, with the bars representing the standard error. If no bars are visible, it indicates a small standard error. Note that the y axis of each graph is not the same.

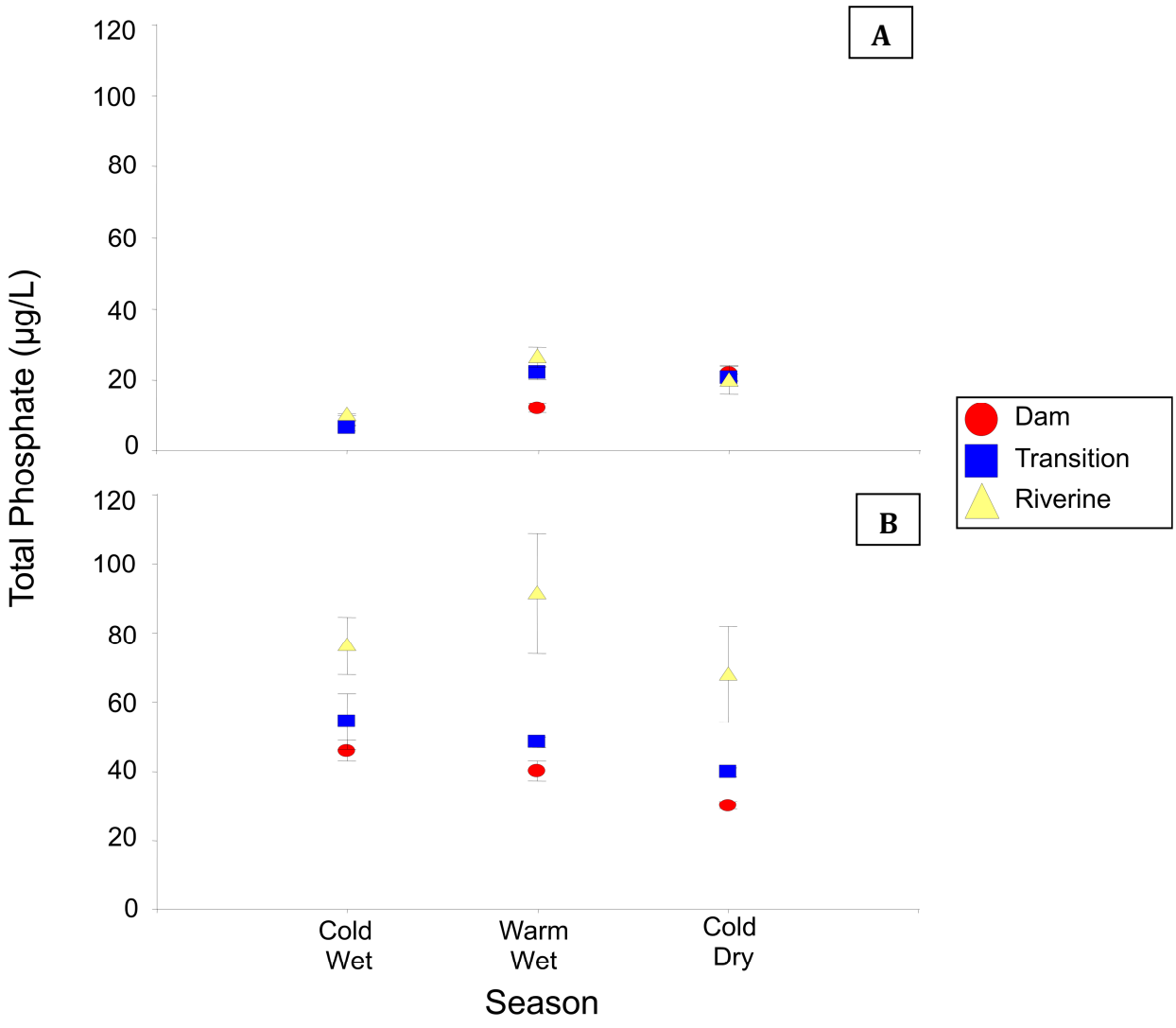

Figure 2.6. Mean total phosphate concentration found in each zone for (A) Guajataca and (B) La Plata. The bars associated with each point represent the standard error. If no bars are visible, it indicates a small standard error.

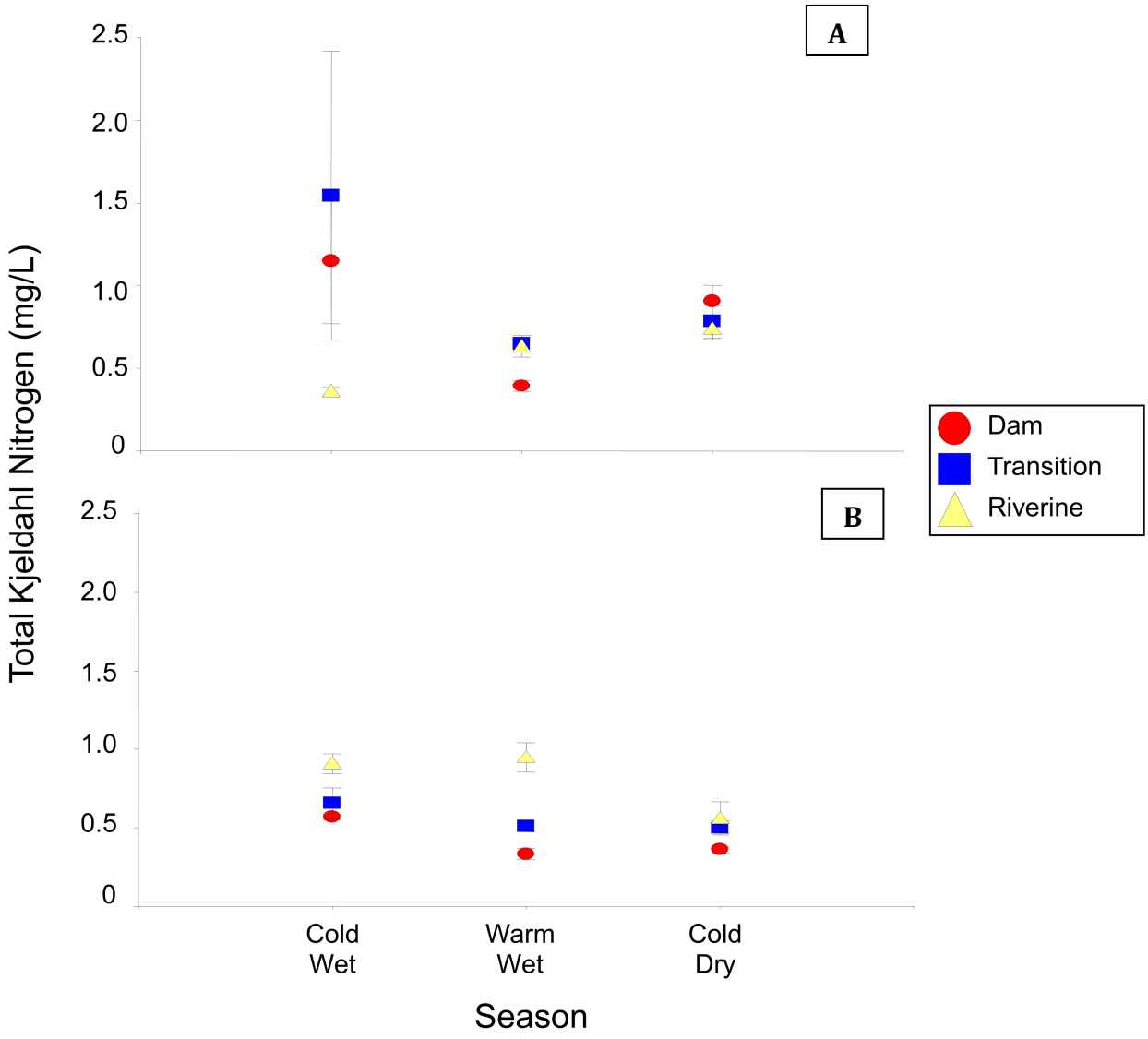

Figure 2.7. Mean concentration of total Kjeldahl nitrogen found in each zone for (A) Guajataca and (B) La Plata. The bars associated with each point represent the standard error. If no bars are visible, it indicates a small standard error.

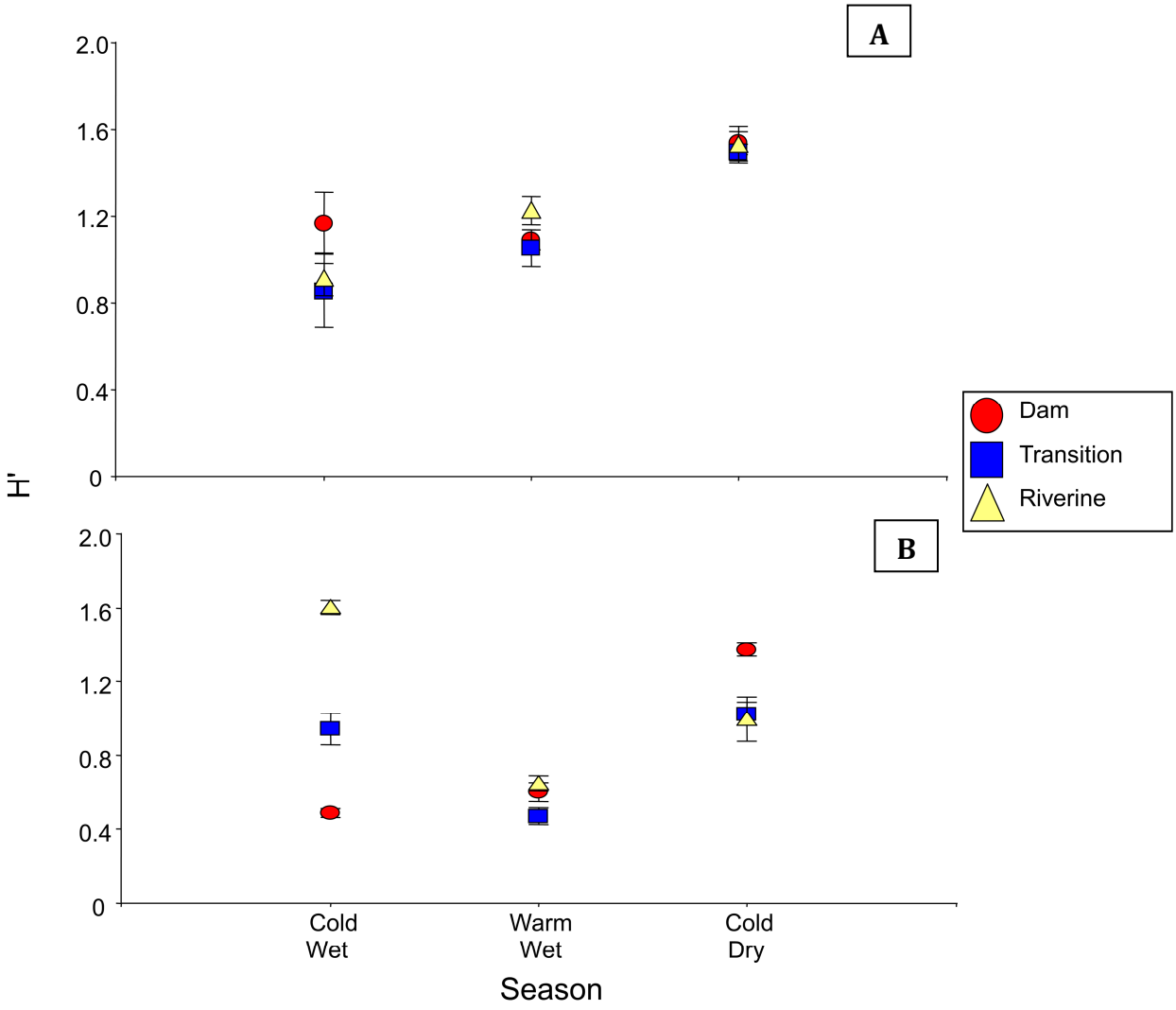

Figure 2.8. Mean H' calculated for each zone in (A) Guajataca and (B) La Plata. The bars associated with each point represent the standard error. If no bars are visible, it indicates a small standard error.

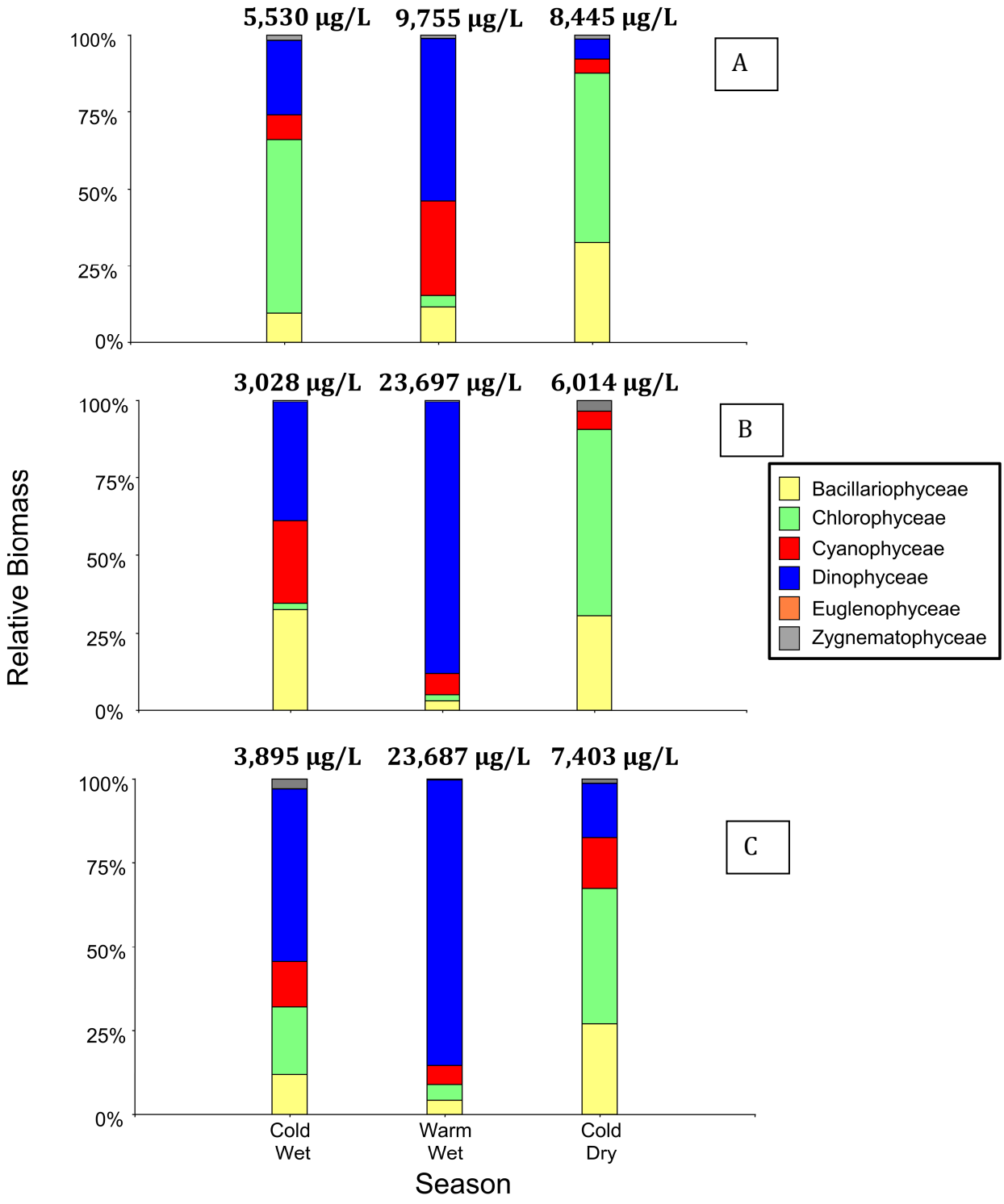

Figure 2.9. The relative phytoplankton biomass distributed by zone in the Guajataca reservoir. The phytoplankton were divided into classes (see text). Each class biomass is the sum of the 6 samples taken per zone. Graph A represents the dam zone, graph B represents the transition zone, and graph C represents the riverine zone. The biomass at the top of each bar represents the total biomass.

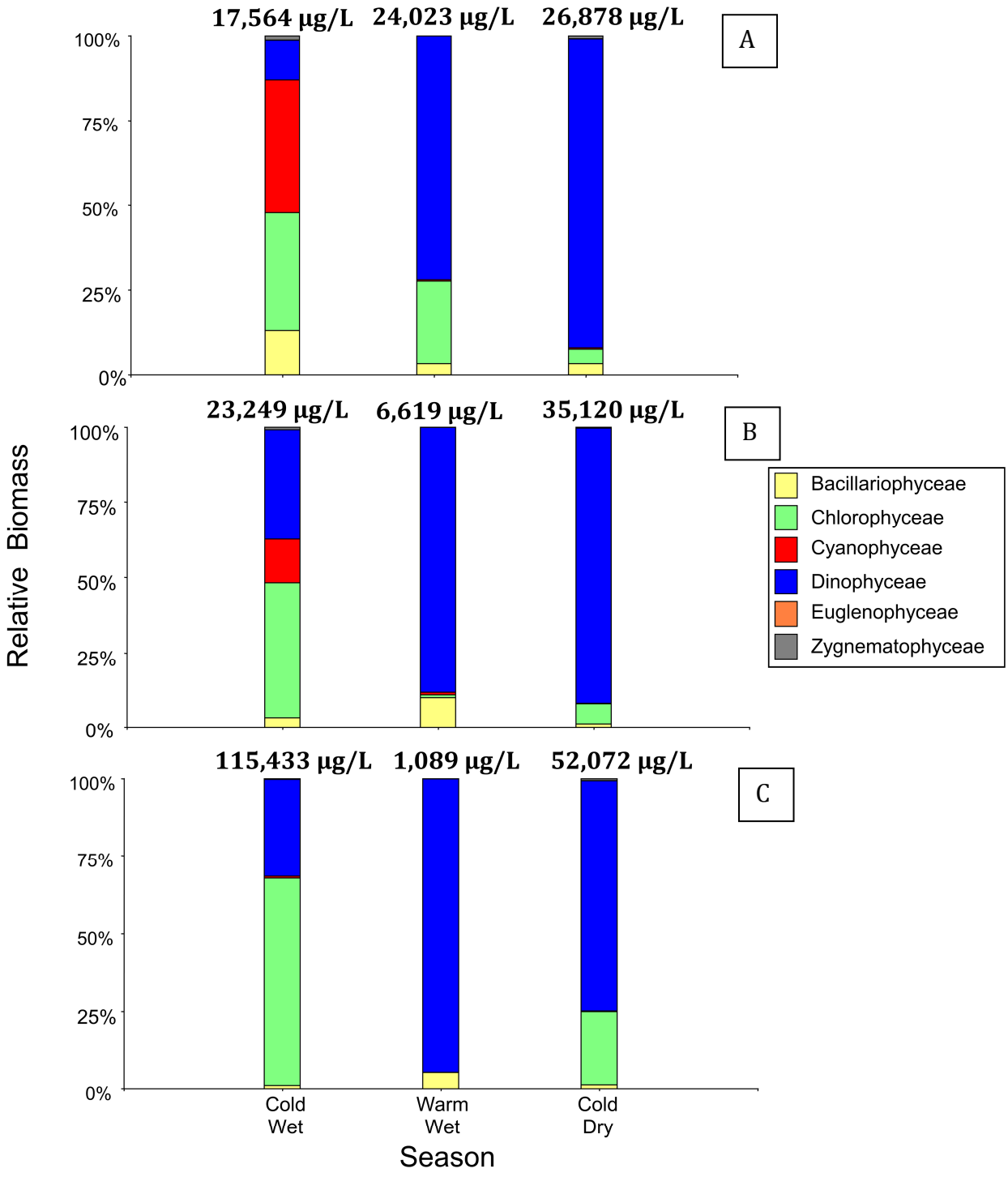

Figure 2.10. The relative phytoplankton biomass distributed by zone in the La Plata reservoir. The phytoplankton were divided into classes (see text). Each class biomass is the sum of the 6 samples taken per zone. Graph A represents the dam zone, graph B represents the transition zone, and graph C represents the riverine zone. The biomass at the top of each bar represents the total biomass.

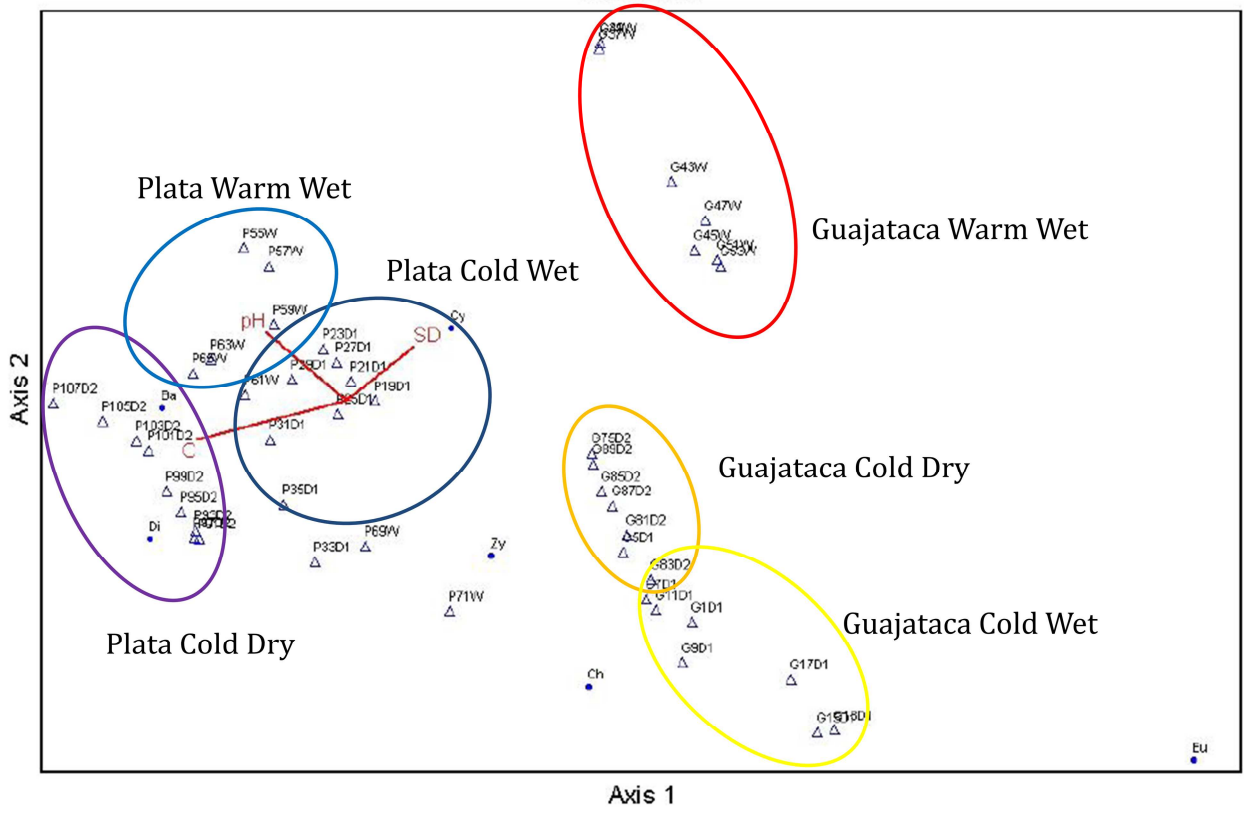

Figure 2.11. Canonical correspondence analysis ordination diagram showing the relationship between phytoplankton classes and the following environmental variables: pH, C (specific conductance), and SD (secchi disc depth). Note the season and reservoir sampled appear to play a strong role in determining where each point is located. Classes included are: Euglenophyceae, Chlorophyceae, Bacillariophyceae, Dinophyceae, Zygnemaphyceae, and Cyanophyceae.

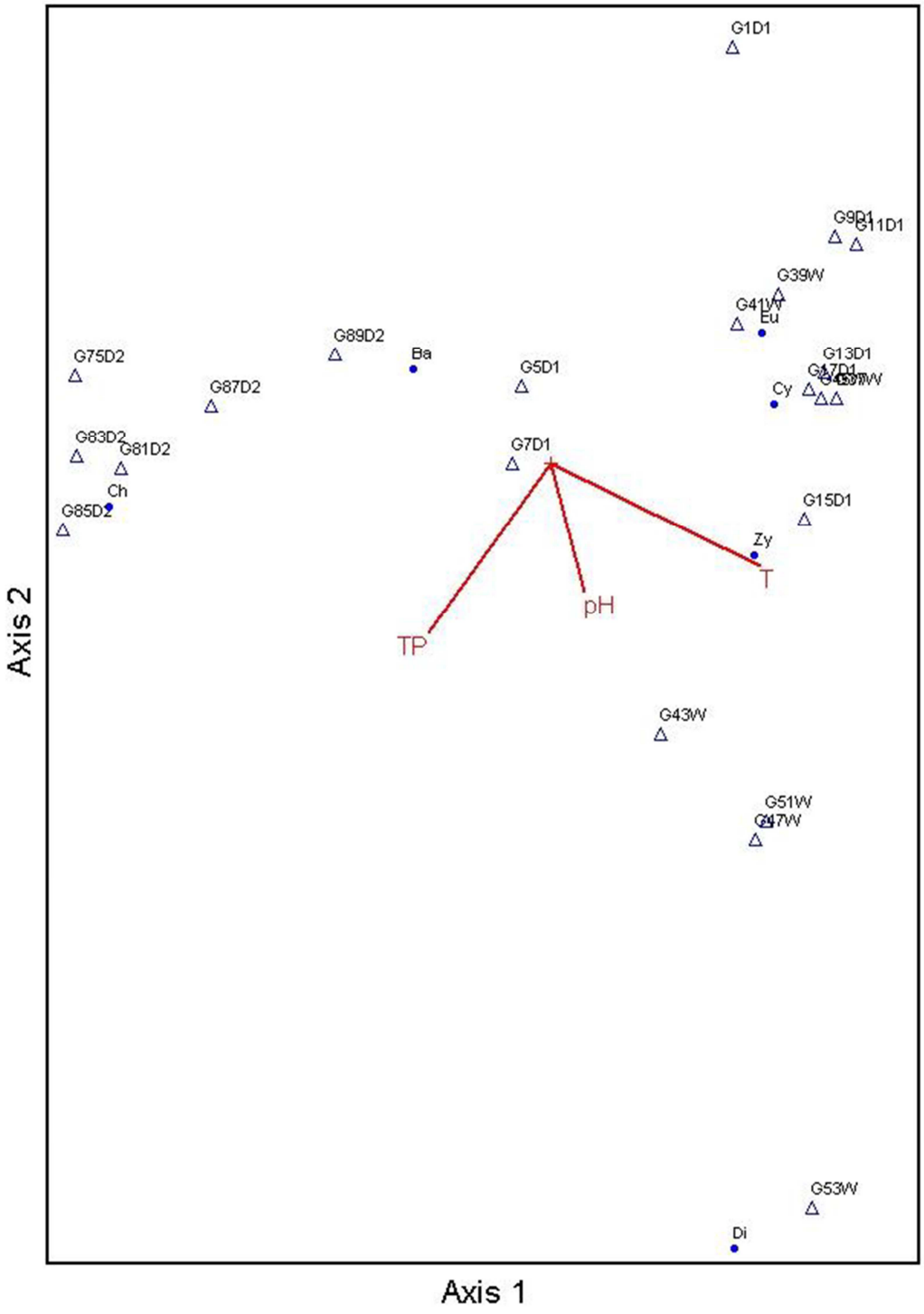

Figure 2.12. CCA ordination diagram of the Guajataca reservoir showing the relationship between phytoplankton classes and the following environmental variables: pH, TP, and T (temperature) in the Guajataca reservoir. Classes included are: Euglenophyceae, Chlorophyceae, Bacillariophyceae, Dinophyceae, Zygnemaphyceae, and Cyanophyceae.

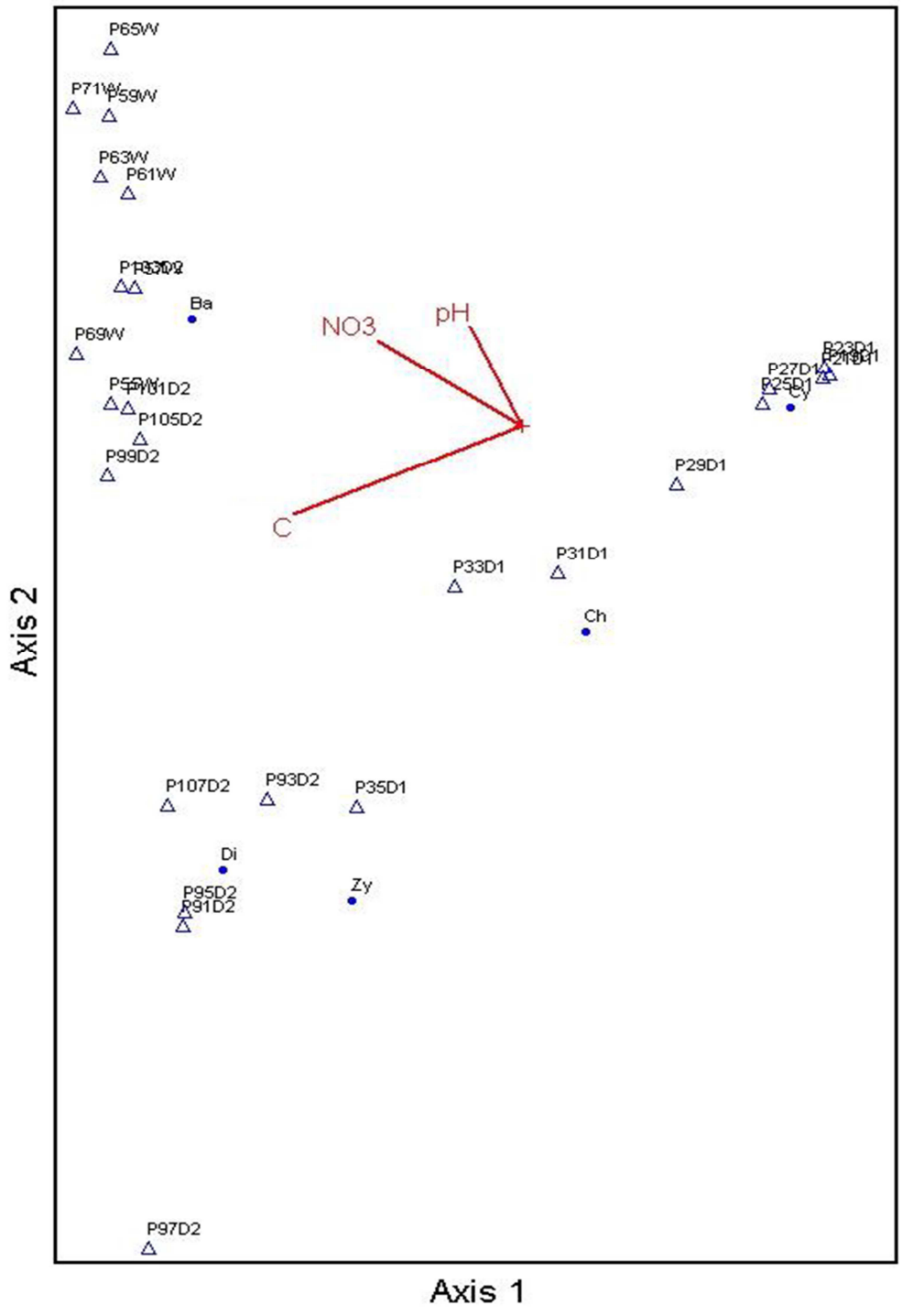

Figure 2.13. CCA ordination diagram of the La Plata reservoir showing the relationship between phytoplankton classes and the following environmental variables: pH, NO_3, and C (specific conductance). Classes included are: Euglenophyceae, Chlorophyceae, Bacillariophyceae, Dinophyceae, Zygnemaphyceae, and Cyanophyceae.

Chapter 3

Investigating the Impact of the Asian Clam on Phytoplankton Community Structure and Nutrient Concentrations

Introduction

Bivalves are known to impact aquatic systems (Strayer et.al. 1999; Vaughn and Hakenkamp 2001). Such impacts include removing organic matter from the water column, increasing the depth of light penetration, and increasing nutrient availability (Strayer et al. 1999; De Stasio et al. 2008). Additionally, bivalves allow infaunal macroinvertebrates increased access to food sources, enable nutrient mobilization from the pelagic to the benthic zone, and influence the phytoplankton community structure (Strayer et al. 1999). While these impacts may be beneficial in a bivalve's native environment, they can become problematic if the bivalve has invaded a new community. Invasive burrowing bivalves create additional conditions in their environment, such as increased habitat on which benthic organisms can attach, increased water clarity due to filter feeding, and decreased abundance of benthic organisms (Sousa et al. 2009; Hakenkamp et al. 2001). A burrowing bivalve that is becoming increasingly invasive in freshwater systems is *Corbicula fluminea*, commonly known as the Asian clam.

One of the main reasons *C. fluminea* is such an effective invader is due to its ability to survive in a variety of environments and its fast reproductive strategy (McMahon 2002). Once the species is established, it can influence decreases in native bivalve abundance and diversity due to increased competition and decreases in oxygen due to large population die offs (Sousa et al. 2008). These factors make *C. fluminea* a potential detriment to any environment it invades.

The Asian clam exists naturally in southern Asia, Africa, and Australia but is an invasive species in the United States, South America, Europe, and many reservoirs across Puerto Rico (Beasely et al. 2003; McMahon 2002; Williams et al. 2001). The first documented discovery of *C. fluminea* in Puerto Rico was in 1998 in the Cayey River. Since its discovery, *C. fluminea* has been found in three different river systems and it is believed that its spread will continue throughout fresh and brackish waters across Puerto Rico, potentially driving the few native bivalves that currently exist to extinction (Williams et al. 2001). The impact of the Asian clam on the phytoplankton community in Puerto Rican reservoirs needs to be understood before the Asian clam becomes established throughout the island.

There are a variety of ways in which *C. fluminea* can change its environment, making it adept at impacting the entire ecosystem. They have the ability to increase turbidity and the organic matter within the sediment in addition to impacting the water column through filter feeding (Vaughn and Hakenkamp 2001); however, the impact *C. fluminea* has on the water column represents a more pressing concern. Although studies have shown *C. fluminea* is capable of impacting the general phytoplankton community (Cohen et al. 1984), no study has evaluated its impact on specific phytoplankton components. Experiments examining how an invasive bivalve alters specific members of the phytoplankton community have been conducted using the zebra mussel, *Dreissena polymorpha*.

The zebra mussel, is extremely invasive in freshwater systems throughout North America and has been intensively studied due to its large economic impact (Silverman et al. 1995; Bastiviken et al. 1998; Vanderploeg et al. 2001; Dionision-Pires et al. 2005; Sarnelle et al. 2005; De Stasio et al. 2008; Knoll et al. 2008). Zebra mussel invasions have been found to be associated with changes in the phytoplankton community, specifically with increases in the cyanobacteria population (Raikow et al. 2004). Most studies conclude zebra mussels are able to impact the phytoplankton community through two mechanisms: selective filter feeding or increased nutrient cycling (Bastviken et al. 1998; De Stasio et al. 2008). Although *D. polymorpha* and *C. fluminea* are different in many ways including habitat preference and reproduction method, both are invasive freshwater bivalves which utilize filter feeding to acquire food (McMahon 2002). Thus, it is assumed the Asian clam may impact the phytoplankton community through similar mechanisms.

Although *C. fluminea* does obtain food through filter feeding it is also able to pedal feed. Pedal feeding is a form of deposit feeding that involves the cilia on the foot uncovering and collecting organic matter (Sousa et al. 2009; Way et al. 1990). Pedal feeding has been found to play an important role in the diet of Asian clams, as clams that are allowed to pedal and filter feed grow at a faster rate than clams that are only permitted to filter feed (Hakenkamp and Palmer 1999). Additionally, one study found Asian clams cause a decrease in the bacteria and flagellate abundance when able to pedal feed, proving pedal feeding is used consistently enough to cause an impact (Hakenkamp et al. 2001).

Another way the Asian clam can affect phytoplankton community structure besides direct consumption is by altering the nutrient concentrations of the system. Several studies using

various bivalve species have examined a similar question with differing conclusions. Some studies have found bivalves play a large role in increasing the amount of phosphate available by accelerating the rate of the phosphorous cycling (Arnott and Vanni 1996). However, other studies demonstrate bivalves are able to decrease nutrient concentrations in the water column through filter feeding (Nakamura and Kerciku 2000; Cha et al. 2011). These studies show bivalves are able to remove nutrients from the water column and sequester them in the sediment through the process of filter feeding and feces production. One experiment specifically examined the ammonia and phosphate excretion rate of *C. fluminea* and found their nutrient excretions could serve as an important nutrient source for phytoplankton (Lauritsen and Mozley 1989).

The objective for this study was to determine whether the Asian clam is able to impact the nutrient concentration and phytoplankton community within two reservoirs in Puerto Rico. As the Asian clam is able to feed in two distinct ways, the two feeding methods were also examined to determine whether the Asian clam has a larger impact with filter feeding or a combination of filter and pedal feeding. Mesocosm experiments were used to address these objectives. Additionally, the two reservoirs selected differed in trophic level in order to study whether the initial nutrient concentration influences the impacts of *C. fluminea*.

This study will be the first to examine the impacts of *C. fluminea* in the tropics. Additionally, although studies have examined the impact of *C. fluminea* on phytoplankton abundance, no one has tried to determine their impact on specific genera within the community.

Methods

Mesocosm experiments were used to examine the impact of *C. fluminea* on nutrient concentrations and the phytoplankton community. Creating mesocosms allowed the impacts of filter and pedal feeding to be separated, as well as to limit the number of uncontrollable variables. Two separate mesocosm studies were conducted using water from two reservoirs: the mesotrophic Guajataca reservoir, and the eutrophic La Plata reservoir. The studies were carried out identically, with only the sediment and water source changing depending on which reservoir was being examined. For the site description of both reservoirs, see the methods section of chapter 2.

In order to separate the two feeding mechanisms of the Asian clam, four different treatments were utilized. One treatment included clams, reservoir water, and reservoir sediment

(C+W+S). A second treatment had no clams, reservoir water, and reservoir sediment (W+S). The third treatment had clams and reservoir water (C+W). And the fourth mesocosm was a control of the C+W treatment, with only reservoir water and no clams (W). Each treatment had four replicates, for a total of 16 mesocosms per study. The mesocosms were established at the Finca Alzamora at the University of Puerto Rico, Mayaguez (18° 12' 39.920" N, 67° 8' 38.306" W) (Figure 1).

The Asian clams used in this study were obtained from a pond located at the Agriculture Experimental Station in Lajas, Puerto Rico (18° 2' 11.652" N, 67° 3' 55.008" W) (Figure 2.1). The clams were collected three days before the experiment, and were kept in deionized water that was replaced daily in order to starve the clams. This 72 hour starvation block ensured no pseudofeces were released into the water column of the mesocosm experiment (Silverman et al. 1995), preventing contamination of the mesocosm study by phytoplankton from the clam's initial habitat.

As noted previously, the water and sediment came from two different reservoirs: Guajataca and La Plata. The sediment and water were collected one day prior to beginning the mesocosm study. Both the sediment and water were transported from the reservoirs to the site of the mesocosm (approximately 2 hours total). The water from each reservoir was collected at the boat ramp using plastic buckets. The sediment of each reservoir was collected from the littoral zone. The location of the sediment sample was selected in an attempt to maintain similar sediment composition between reservoirs.

Upon return from the field, all containers were aerated using a system of bubblers over night before being distributed to the mesocosms the following day. Plastic buckets (20L) were used to conduct the mesocosm experiments. Each bucket, which represented a mesocosm, received 11 L of reservoir water, and the treatments with sediment received 1 L of sediment that was evenly distributed in the bottom of the bucket. An air bubbling system was set up to maintain oxygen levels near saturation in all mesocosms throughout the experiment, allowing both the Asian clams and the phytoplankton to receive optimal oxygen concentrations. Evaporation occurred throughout each experiment, but there was no significant difference between treatments in water volume ($p=0.11$). The treatments that contained no sediment (C+W and W) were stirred 15 times daily to re-suspend phytoplankton by hand.

The Asian clam treatments (C+W and C+W+S) received 10 clams per bucket. This number was derived from the maximum density (172 clams per m^2) found by Karatayev et al. (2003) in Lake Nacogdoches, East Texas, and scaling the density to the size of the mesocosm. This density was employed as no ambient Asian clam density values existed for Puerto Rico at the time of this study. Biomass of the Asian clam was also consistent across treatments for each mesocosm study. However, the biomass of *C. fluminea* used in the Guajataca study was significantly smaller than in the La Plata study ($p<0.001$). The Asian clams ranged from 7.76g to18.89 g for the Guajataca reservoir experiment, while that of the clams used for the La Plata reservoir experiment ranged from 10.19g to 22.31g.

The mesocosms were arranged into 2 rows of 8 on top of a metal table underneath a clear plastic covering (Figure 3.1). This allowed ambient light to reach the mesocosms but it shielded them from rainfall. Each mesocosm experiment lasted 14 days. Temperature, dissolved oxygen, specific conductance, and pH were measured in each mesocosm every other day using a handheld YSI Pro Plus multisensor.

Water samples were taken on days 0, 1, 7 and 14 to determine nitrate (NO_3^-), ammonium (NH_4^+), dissolved phosphate (dissolved P), total phosphate, (TP), chlorophyll *a* levels, and the phytoplankton community structure. The day 0 sample was taken before the water collected from the reservoirs was added to the mesocosms in order to have an accurate initial survey for all parameters tracked through the study. Treatments without sediment (C+W and W) were stirred first in order to take a homogenous water column sample. Treatments with sediment (C+W+S and W+S) could not be stirred, so a large glass pipette was fashioned to allow water samples to be taken from the bottom, middle, and surface of the mesocosms to achieve the same homogenous effect. The ammonium and calcium concentrations were determined by the Institute of Tropical Forestry in San Juan, Puerto Rico. Nitrate and phosphate analyses were conducted by the Soil and Water Quality Laboratory located at the Rio Piedras Agricultural Experimental Station in Rio Piedras, Puerto Rico.

The chlorophyll *a*, phytoplankton community, biomass, and Shannon Index (H') of each bucket was determined using the same method that is described in the methods section of chapter 2. H' takes into account species richness and evenness within the sample. The phytoplankton community structure was examined for all the days water samples were taken in treatments without sediment. The phytoplankton community in treatments with sediment could not be

determined until Day 14 due to difficulties identifying phytoplankton cells among sediment suspended in the sample.

The chlorophyll *a* concentration, the physical parameters, phytoplankton biomass, and H' were compared using a repeated-measure ANOVA in SAS (version 9.1, © 2002-2003). Several models were tested, but the best model was selected based on the lowest AIC value observed. The analysis of each reservoir was done separately. Comparisons were focused on examining changes in all parameters both within and between treatments.

Results

The temperature in the Guajataca mesocosms ranged from 18.7 to 29.2°C during the 14 day period, while temperatures in the La Plata mesocosms ranged from 23.9 to 32.2°C. This large temperature fluctuation was seen in all mesocosms, regardless of treatment (Appendix E), and is not unexpected due to the small size of the mesocosms. In both mesocosm studies, there was no significant difference between the temperature, pH, or dissolved oxygen of the four treatments. Due to bubbling, dissolved oxygen concentrations remained high (7.82 mg/L for Guajataca, and 7.14 mg/L for La Plata), however the dissolved oxygen decreased about 10% in each study across all treatments from day 13 to day 14. The pH levels measured in the mesocosms were within the pH range typically seen in the reservoirs. The Guajataca mesocosms averaged 8.19 ± 0.12 in all treatments, while La Plata averaged 8.33 ± 0.09, except for the C+W+S treatment which was 8.25 ± 0.13 ($p<0.001$).

The specific conductance in the Guajataca mesocosms was lower on day 1 than in the water taken from the reservoir ($p<0.001$) (Figure 3.2A). On day 1, there were clear differences between mesocosms with sediment and those without ($p<0.001$). The specific conductance varied through time in treatments with Asian clams, and on day 14 the C+W treatment was found to have the lowest value at 190.8 µS/cm, with the C+W+S treatment having the second lowest specific conductance at 217.3 µS/cm. The specific conductance within the W+S treatment was the highest (254.8 µS/cm) and it did not change during the experiment, while the specific conductance within the W treatment decreased by less than 15% from day 0, with a final value of 230.3 µS/cm..

In the La Plata mesocosms, the specific conductance was lower on day 1 in the treatments with sediment than in the treatments without ($p=0.007$) (Figure 3.2B). However, this relationship

was not constant throughout the experiment. The specific conductance in all treatments excluding C+W+S increased from day 1 to day 5 at the same rate. After day 5, the specific conductance continued to increase in treatments without clams at a slower rate (W+S and W) than the first 5 days. After day 5, the specific conductance remained constant in the treatments with clams (C+W+S and C+W). The specific conductance was the lowest in the C+W+S treatment on day 14 (335.7 µS/cm). The W+S and C+W treatments were not significantly different on day 14, with an average specific conductance of 378.2 µS/cm, which was an increase of less than 10% from day 0. The W treatment had the highest specific conductance of 405.1 µS/cm, a 16% increase from day 0 (Figure 3.2B).

In the Guajataca mesocosms, the chlorophyll *a* level was lower in the C+W treatment than in the W treatment on day 1 (9.0 µg/L and 35.13 µg/L, respectively) ($p<0.001$). This represented an 80% decrease in the chlorophyll *a* of the C+W treatment in 24 hours. On day 14, the chlorophyll *a* values from all treatments, including those with sediment, were compared. The C+W treatment had the highest chlorophyll *a* at 8.06 ± 0.95 µg/L, which is over 30% higher than all the other treatments (mean 4.87 ± 1.41 µg/L) ($p=0.019$) (Appendix E). In the La Plata mesocosm, chlorophyll *a* was found to be higher in the C+W treatment than in the W treatment ($p<0.0001$). However, the chlorophyll *a* in the C+W treatment decreased by 70% each week from day 1 ($p<0.001$) while the chlorophyll *a* in the W treatment decreased by over 90% from day 1 to day 7. On day 14, there was no difference in chlorophyll *a* between the four treatments.

In the Guajataca reservoir, the ammonium concentrations in the C+W and W treatment were not significantly altered during the experiment. However, the ammonium concentration initially doubled in treatments with sediment within 24 hours (Figure 3.3A), and continued to increase to 1.2 mg/L on day 7 in the C+W+S treatment. These high levels had decreased by day 14. The ammonium concentration in the W+S treatment, however, decreased from day 1 to day 7 by 95% (Figure 3.3A). On day 1, the ammonium level in the La Plata mesocosm was highest in the sediment treatments (C+W+S and W+S), with an average of 2.28 ± 0.72 mg/L, while the treatments without sediment (C+W and W) had an average concentration of 0.07 ± 05 mg/L ($p<0.001$) (Figure 3.4A). As observed in the Guajataca mesocosm, there was no significant change in the ammonium concentration in the C+W or W treatment throughout the experiment. All treatments, regardless of experiment, had similar ammonium concentrations on day 14.

The nitrate concentration in the Guajataca mesocosm with and without sediment (C+W+S, W+S and C+W, W, respectively) did not differ due to the presence of clams throughout the experiment ($p=0.66$ and $p=0.89$, respectively). The nitrate concentration began to increase in the C+W+S and W+S treatments in the middle of the experiment and by day 14 had final concentrations of 5 mg/L and 3.06 mg/L, respectively (Figure 3.3B). The nitrate within the non sediment treatments (C+W and W) increased slightly to 1mg/L on day 14. Nitrate concentrations also increased in the C+W+S and W+S treatments by the middle of the experiment for La Plata, with the highest average nitrate concentration in the C+W+S treatment on day 7 (2.27 mg/L) ($p<0.001$). However, there was over a 95% decrease in the nitrate level of both sediment treatments (C+W+S and W+S) from the middle to the end of the experiment (Figure 3.4B). The nitrate level in both the C+W and W treatments were lower than the other treatments and did not change over the 14 day period.

There was an immediate increase in the P in treatments with sediment in both mesocosm experiments, which then decreased during the experiment (Figures 3.3C and 3.4C). Additionally, the P concentration did not vary in the non sediment treatments of both experiments. In the Guajataca mesocosms, the DP levels peaked on day 1 at 4.5 μg/L and then decreased by over 60% to day 14, where the DP levels were not different between treatments ($p=0.49$). The highest TP level was 1.59 ± 0.63 mg/L in the sediment treatments in La Plata. The TP pattern is the same as in Guajataca, with over a 70% decrease from day 1 to day 7 until day 14 where there are no differences in TP between treatments ($p=0.09$).

Species diversity, based on the Shannon Index (H'), decreased by at least 30% in all treatments by the end of both experiments (Table 3.1). The average H' in the La Plata mesocosms (0.65 ± 0.34) was lower than in the Guajataca mesocosms (1.10 ± 0.43) throughout the experiment ($p=0.02$). When examining the treatments without sediment in the Guajataca mesocosm, H' decreased twice as fast with clams (C+W) than water alone from day 1 to day 7 ($p=0.014$). In the La Plata mesocosms this decrease from day 1 to day 7 was 30% with clams, while there was no change in diversity with water alone. Only on day 14 could the treatments with sediment be compared. There were no differences in the Guajataca mesocosms on day 14, while the sediment treatments in La Plata had a lower H' than those without sediment (0.22 ± 0.30 and 0.70 ± 0.25, respectively). ($p=0.01$).

The biomass was also examined to determine whether there was an impact of *C. fluminea* on the phytoplankton community. The La Plata mesocosms clearly had a higher total phytoplankton biomass than the Guajataca mesocosms at the onset of the experiments with values of 29,783 µg/L and 1,473 µg/L, respectively. To determine whether the phytoplankton community shifted through time, the genera biomass within the C+W and W treatments were compared for each experiment. For a complete list of phytoplankton species identified in both mesocosm experiments, see Appendix A.

In the Guajataca mesocosms, there were differences in the succession of the phytoplankton genera over time, and these patterns varied between treatments with and without clams (C+W and W) (Figure 3.5). In the C+W treatment, the biomass of all genera decreased except that of *Tetraedron*, which disappeared by day 7. On day 7, the "other" group dominated until the end, which mostly consisted of green algae. Without clams, *Tetraedron* never disappeared and there is a shift in the dominance of the community by *Navicula* (35% of total biomass) at the beginning to *Synedra* (76% of total biomass) at the end (Figure 3.5B). Due to the large amount of variation, the *Synedra* biomass was not significantly higher in the W treatment than the C+W treatment.

On day 1 in the La Plata mesocosm, the biomass in the C+W treatment was higher than in the W treatment (38.01 mg/L and 28.24 mg/L respectively) ($p=0.034$). *Peridinium* dominated in both the C+W and W treatments in the beginning of the experiment ($p<0.001$), making up over 95% of the total biomass. However, the biomass of *Peridinium* in both treatments decreased by over 90% from the beginning to the middle of the experiment (Figure 3.6A, B), when Synedra began to appear. Although *Synedra* was the genus that tended to dominate at the end of the La Plata experiment in both the C+W (87% of total biomass) and W treatments (92% of total biomass), the *Synedra* biomass in the W treatment was nearly double that of the C+W treatment (8.06 mg/L and 4.51 mg/L, respectively) (Figure 3.7B). This is similar to the Guajataca mesocosms, where *Synedra* was also able to dominate the W treatment (Figure 3.7A). Additionally, although both non sediment treatments decreased in total biomass from day 0 to day 14, the C+W treatment experienced a 92% decrease, while the W treatment only decreased its biomass by 70%.

When looking across all treatments on day 14, there are some differences in the phytoplankton genera for each experiment (Figure 3.7A,B). In both mesocosm experiments,

treatments with sediment have a lower biomass than those without (p= 0.05 for Guajataca, p=0.021 for La Plata). Additionally, sediment treatments without clams (W+S) were dominated by the "other" group, making up 100% of the biomass in Guajataca and 74% in La Plata; however, the "other" group also dominated the C+W treatment in Guajataca comprising 67% of the total biomass. In the La Plata experiment, *Navicula* dominated the C+W+S treatment with 98% of the biomass, while in Guajataca *Navicula* only made up 25% of the biomass in the same treatment on day 14.

Discussion

C. fluminea do not have a large impact in the mesocosms in this study. There was no observable effect of *C. fluminea* on the nutrient concentration in either experiment. In fact, sediment presence altered nutrient concentrations more than clam presence (Figures 3.3 and 3.4). However, *C. fluminea* was able to keep the phytoplankton community from becoming dominated by *Synedra* which allowed for a more diverse community structure. As the effect of *C. fluminea* is not significant, this may suggest the Asian clam is not able to impact tropical systems as severely as temperate ones. However, the lack of a significant impact observed for *C. fluminea* in this newly invaded system is unusual and should be further investigated.

Environmental Variables and Nutrient Concentrations

The La Plata reservoir was found to have a higher ammonium and nitrate concentration as well as a higher initial concentration of chlorophyll *a* than the Guajataca reservoir. This was expected as the La Plata reservoir is eutrophic, while the Guajtaca reservoir is mesotrophic. Although the initial nutrient levels were different, *C. fluminea* was expected to increase the nutrient concentration in both systems as clams produce metabolic wastes that include ammonium and phosphate (Lauritsen and Mozley 1989). However, examining the ammonium, nitrate, and phosphate concentrations within both mesocosm experiments, it is apparent treatments with sediment (W+S and C+W+S) had a higher concentration of nutrients than treatments without sediment (C+W and W), regardless of the presence of *C. fluminea*. It was difficult to determine whether limiting the Asian clam to only filter feeding or allowing the clam to filter and pedal feed impacted nutrient concentrations differently as sediment presence

obscured these results. From these experiments it seems the Asian clam does not directly influence nutrient dynamics.

Examining the treatments with sediment (C+W+S and W+S) as a resuspension event, which commonly occurs in lakes (Wetzel 2001), the increase of ammonium may be better understood. As the sediment was suspended, the ammonium present in the sediment also experienced a flux into the water column (Reddy et al. 1996). This flux could explain why ammonium concentrations tended to be high in the C+W+S and W+S treatments on day 1 in both mesocosm experiments.

The increase in the nitrate concentration of all treatments in both mesocosm experiments after an initial spike in the ammonium concentration was most likely due to the process of nitrification (Wetzel 2001). The larger increase of nitrate observed in the treatments with sediment could be due to the larger concentration of active bacteria in the sediment than the water column (Jones 1979). Since the sediment was suspended in the water column, the bacteria were in aerobic conditions which favored nitrification. Although nitrification was also occurring in treatments without sediment in Guajataca, it is possible there were fewer bacteria due to the lack of sediment in the water column.

In addition to the sediment having an impact on the nitrogen dynamics, it also seems to have had an impact on the phosphate concentration. The phosphate level decreased over time in treatments with sediment, which inversely correlated with water transparency (personal observation). Thus, it is likely the increased phosphorous concentration is also due to the initial suspension of sediment. This result agrees with previous studies which documented an increase in phosphorous concentrations within the water column due to the resuspension of sediment through wind action (Kristensen et al. 1992; Reddy et al. 1996). The initial total phosphorous concentration of the Guajataca sediment was calculated as 9.35 mg/kg utilizing the Olsen method (Sims 2000), while 20.25 mg/kg was the level found for the La Plata sediment. These levels of phosphorous further suggest the sediment is the most likely source of the increased phosphorous concentrations observed.

There are several reasons which could explain why an increase in the nutrient concentration was not detected due to *C. fluminea*. One possibility could be food limitation though Hakenkamp et al. (2001) would disagree. It is possible the phytoplankton concentration was limited and thus *C. fluminea* could not produce and release nutrients within the mesocosms.

Foe and Knight (1985) found the Asian clam becomes food limited at chlorophyll *a* concentrations below 47.3 ug/L during the summer season. Although the initial chlorophyll *a* concentration for both experiments was high (50.0 µg/L in Guajataca, 336.4 µg/L in La Plata), it fell to well below the limiting level by the end of the experiment (5.7 µg/L Guajataca, 11.3 µg/L in La Plata). Additionally, *C. fluminea* is known to decrease its filter feeding rate as its food concentration decreases, not allowing it to compensate for lowered food levels with increased filter feeding (Vohmann et al. 2010). It is possible there was a low food concentration within the sediment as well; however abundance of food in the sediment was not quantified.

In addition to lowered food concentrations, a compounding problem could be high water temperatures. Although the highest temperature measured during the study was well within the biological limits of *C. fluminea*, 29°C, it lies toward the upper limit of its range (McMahon 2002). As an ectotherm's metabolism increases with elevated temperatures, *C. fluminea* would most likely need even more food than its normally high metabolic rate requires (Hakenkamp and Palmer 1999). If *C. fluminea* were starving due to a lowered food concentration and elevated temperature, we might expect to observe a decrease in the Asian clam's body mass as did Vohmann et al. (2010). The fact that the Asian clam biomass did not differ between the beginning and end of either study does not mean *C. fluminea* was not food limited. It could merely suggest the duration of the experiment was not long enough to observe a decrease, as the Vohmann study occurred over a 5 month period. Thus, it is possible *C. fluminea* was food limited, causing a decrease in its feeding activity and resulting in little to no nutrients being excreted. Although the Asian clams may have been food limited, the conditions of the mesocosm were replicating natural ones, suggesting food limitation could also be an issue for *C. fluminea* in the reservoirs of Puerto Rico.

If, however, *C. fluminea* was not food limited when able to filter and pedal feed, it is possible an increase in the nutrient concentration due to the Asian clam occurred but was undetected. Ammonium has been found to be the preferred source of nitrogen for plankton (Liao and Lean 1978), thus any ammonium produced by *C. fluminea* could have been immediately utilized by plankton within the system. Although a large increase in the phytoplankton community was not observed (Figures 3.5 and 3.6), there was an increase in the amount of periphyton in the mesocosms after day 10 (personal observation), which was not quantified. Although there was some periphyton growth in nearly all treatments, the most abundant growth

was observed in the C+W+S treatment. This could have been due to the treatment receiving nutrient inputs from both *C. fluminea* and the sediment, as there was less periphyton in the C+W treatment. However, *C. fluminea* had no quantifiable impact on nutrient concentrations, which contrasts with the results of previous studies (Lauritsen and Mozley 1989; Way et al. 1990; Hakenkamp et al. 2001). This surprising find could be due to the interaction of the suspended sediment within the water column. As the results from both mesocosms were similar, this demonstrates that *C. fluminea* is not able to alter the nutrient concentration in either a eutrophic or mesotrophic system in Puerto Rico.

Though *C. fluminea* does not seem to impact nutrients, it does have an effect on other ions within the system. The specific conductance was expected to increase throughout the experiment due to concentration via evaporation. This was observed in the La Plata mesocosms from day 1 to day 5, as the rate of increase in the specific conductance was similar in each treatment. However, only the W+S treatment maintained a high specific conductance in the Guajataca mesocosm (Figure 3.2A) while all others decreased. In both experiments, the treatments with the lowest specific conductance on day 14 contained Asian clams (Figure 3.2A, B), suggesting Asian clams have the ability to regulate specific conductance.

In order to determine which ions might be affected by *C. fluminea*, other ions were examined. Calcium was found to have a high positive correlation with specific conductance in the Guajataca treatments with clams (r^2= 0.92 for C+W and r^2= 0.98 for C+W+S, $p<0.001$). Calcification was most likely the reason a decrease in calcium ions was observed in the clam treatments, as bivalves use calcium in their shell synthesis (Chauvaud et al. 2003). The daily calcification rate for *C. fluminea*, as calculated from Miller and Payne (1994) (0.5-3 g $CaCO_3$ m^{-2} d^{-1}), suggests *C. fluminea* would have the ability to lower calcium concentrations over the 14 day experiment period as the highest calcium concentration measured was 0.041 g/L. However, a high positive correlation between calcium and specific conductance was only found in the La Plata C+W+S treatment (r^2= 0.85 for C+W+S, $p<0.001$ and r^2= 0.02 for C+W, $p=0.96$). This could be due to the higher concentration of other ions within the system, causing calcium to directly impact the specific conductance less even though Ca^{+2} is still decreasing within the system by the Asian clams to make $CaCO_3$.

Phytoplankton Community

Overall, the Guajataca mesocosm had higher diversity within the phytoplankton community than the La Plata mesocosm and this pattern was maintained throughout the study (Table 3.1). The La Plata mesocosm, however, had a phytoplankton biomass that was 20 times larger than the Guajataca mesocosm (Figures 3.5 and 3.6). These differences can be attributed to the different initial nutrient concentrations of each reservoir, as eutrophic systems are known to have a higher phytoplankton biomass and lower diversity (Wetzel 1983). Although this is opposite of the pattern observed in the reservoir sampling, an inverse relationship between phytoplankton diversity and biomass has been shown in other systems and is attributed to the resource-competition theory which states that diversity is directly proportional to the number of limiting resources within a system (Interlandi and Kilham 2001). Although the phytoplankton communities were different in the two mesocosms, *C. fluminea* did not allow *Synedra* to dominate in either experiment. Thus, it seems the initial nutrient concentration of the environment did not alter the Asian clam's ability to impact the phytoplankton community structure.

The diversity of the phytoplankton community was described by H', which accounts for both species richness and evenness. The lowest diversity value (0.04) found for the entire La Plata mesocosm study was on day 14 in the C+W+S treatment (Table 3.1). This correlates with a low chlorophyll *a* and indicates a general decrease in the phytoplankton biomass and not a shift in the community. It must be taken into consideration that much of the phytoplankton community may have settled out of the water column as the treatment was not stirred daily to prevent sediment resuspension. Additionally, a decreased diversity in both the C+W and W treatments on day 14 can also be attributed to decreases in the overall phytoplankton abundance as the chlorophyll *a* level also decreased (Appendix E).

The same trends are true for the phytoplankton diversity in the Guajataca mesocosm, although the diversity never differed significantly between treatments. In both mesocosm experiments *C. fluminea* did not seem to impact phytoplankton diversity; however, it is difficult to determine exact trends in the phytoplankton examining only H', as a lower H' was typically the result of a decrease in overall phytoplankton abundance (Appendix F). Thus, the biomass of the phytoplankton genera was examined as well.

The total phytoplankton biomass was expected to decrease the most in the C+W treatment, as *C. fluminea* filter feeds on phytoplankton. Although changes in the biomass were observed over time (Figure 3.5 and 3.6), the total phytoplankton biomass was not significantly different between the C+W and W treatments in either mesocosm study on day 14 (Figure 3.7). There is no reason to believe a difference in the phytoplankton biomass was not observed due to the lack of the Asian clam filter feeding, as phytoplankton was their only food source in the C+W treatments. Additionally, it is not probable the duration of the experiment was too short to allow the Asian clam access to the entire water column within the mesocosm. Utilizing filtration rates found by other studies examining *C. fluminea* (Way et al. 1990), it was determined 10 Asian clams could filter all the water within the 11L mesocosm within 1-5 days, depending on which filtration rate is assumed. Another potential reason which could explain why no significant difference in the biomass between the C+W and W treatments was found could be the high variability within each treatment.

Although there was no effect of *C. fluminea* on the total phytoplankton biomass, it did seem to alter the biomass of some phytoplankton groups. *C. fluminea* was not expected to favor any phytoplankton group as previous studies reported no preferential feeding (Way et al. 1990; Boltovskoy et al. 1995). However, the "other" group was able to dominate on day 7 in the C+W treatment in the Guajataca study due to the inclusion of several *Pediastrum*, a genus large in size and biomass. It is possible *Pediastrum* was able to increase in number due to its ability to escape predation, as it can be larger (up to 23 μm) than the upper particle size limit *C. fluminea* has been predicted to be able to process (20μm) (Way et al. 1990). Additionally, some species have been found to have a shape that is highly resistant to sinking which could have also assisted in escaping clam predation (Padisak et al. 2003). However, it seems *Pediastrum* was only able to elude *C. fluminea* for a short period until the individuals, who cannot swim, eventually sank to the bottom of the mesocosm and were consumed.

In the W treatment of both experiments, the genus *Synedra* was able to increase its population by the end of the experiment (Figure 3.7). One factor that may explain this increase is its high affinity for phosphorous (Makulla and Sommer 1993). *Synedra* is a long, needle-like pennate diatom, giving it a high surface area to volume ratio and making it more competitive for phosphorous uptake (Grover 1989). Thus, the increase in the *Synedra* population observed on day 14 could be due to the genus outcompeting other phytoplankton for phosphorous. However,

if this were the only reason *Synedra* was able to dominate it would be expected that an increase in the *Synedra* population would also occur in the C+W treatment. Since that was not observed, it is likely *C. fluminea* contributed to maintaining a lower *Synedra* population.

Predation is the probable explanation for why the population of *Synedra* was smaller in treatments with *C. fluminea* as a previous study found *Synedra* in the gut of the Asian clam in the Parana River, Argentina (Boltovskoy et al. 1995). Additionally, Boltovskoy et al. (1995) found *C. fluminea* lacks a feeding preference which supports the results of this experiment as the relative biomass of each genera in treatments with *C. fluminea* in both mesocosms indicate no genera was excluded from filtration (Figure 3.7). The Asian clam was thought to not have a feeding preference in Argentina due to food scarcity, which may also be true in Puerto Rico.

One big difference between the Guajataca and La Plata mesocosms is the initial dominance of the phytoplankton community by *Peridinium* in the La Plata mesocosm. This is most likely due to the time of year the experiment was conducted, as tropical lakes have been found to have a pattern of phytoplankton succession similar to that found in temperate lakes (Lewis 1978). The phytoplankton succession pattern predicts an increase in dinoflagellates once nutrients become limited due to a lengthy stratification (Lewis 1978). It is possible *Peridinium* did not dominate the phytoplankton community in the Guajataca experiment as it was completed two months prior to the La Plata experiment and may have been in a different stage of the phytoplankton succession pattern. However, *Peridinium* only dominated phytoplankton communities in Guajataca in August based on seasonal sampling (Chappell, chp. 2). As the phytoplankton community structure can shift from season to season it is likely that if this experiment had been conducted at a different time a distinct phytoplankton community would have been used for this study.

Although *Peridinium* initially dominated the phytoplankton community in La Plata, its biomass rapidly decreased within the first week of the experiment. It is unlikely this decrease is due to *C. fluminea*, as the *Peridinium* population decreased in both the C+W and W treatment. One explanation could be excess light exposure. *Peridinium* can migrate over 2 m to reach depths that are optimal for photosynthesis (Regel et al. 2004), which was not possible in the shallow mesocosms where the average depth was less than 0.5 m. Additionally, dinoflagellates have been shown to experience negative growth rates when above an optimal depth of approximately 0.6-0.73 m (Whittington et al. 2000; Regel et al. 2004). Irradiance measurements

were taken above the mesocosms to determine if there was excess light. All values were found to be higher than the irradiance level at which significant photoinhibition was observed for *Peridinium cinctum* (1100 μmol m^{-2} s^{-1}) (Kok 1956; Regel et al. 2004). It is thus highly plausible *Peridinium* within the mesocosms experienced photoinhibition and were unable to migrate to a more optimal depth, explaining the dramatic decline of *Peridinium* observed in the La Plata mesocosms.

Despite the fact the treatments with sediment were not disturbed after the beginning of the experiment, the phytoplankton community composition was examined on day 14. This was done to determine whether the Asian clam impacted the phytoplankton differently when able to both filter and pedal feed; however, the low phytoplankton biomass observed for all sediment treatments in both mesocosm experiments is a result of the phytoplankton genera sinking out of the water column due to a lack of disturbance (Figure 3.7). Thus, the impact of the Asian clam on the phytoplankton community structure when able to filter and pedal feed compared to only filter feeding could not be assessed, as overall phytoplankton biomass decreased due to limitations in the experimental design.

Although there were significant differences between the two mesocosm experiments, such as phytoplankton composition and biomass, the final results of both mesocosms were similar. The only observable effect *C. fluminea* had in both studies was to keep *Synedra* from dominating the phytoplankton community through predation. *C. fluminea* was predicted to impact the phytoplankton community differently depending on the initial nutrient level as this has been found to be true for other invasive bivalves (Raikow et al. 2004; Sarnelle et al. 2005; Knoll et al. 2008). However, it appears different initial nutrient concentrations do not affect the impact of *C. fluminea* as the Asian clam does not cause large changes in the phytoplankton community.

Potential Impact of C. fluminea on Cyanobacteria

It was hypothesized *C. fluminea* could increase the concentration of cyanobacteria, specifically *Microcystis aeruginosa*, through creating conditions conducive for cyanobacteria blooms as zebra mussels, another freshwater invasive bivalve, have been found to do so in various systems (Bykova et al. 2006; Knoll et al. 2008; De Stasio et al. 2008). If a relationship between *C. fluminea* and *M. aeruginosa* existed, it could have major implications for Puerto Rico

as *C. fluminea* is spreading throughout the island (Williams et al. 2001), and *M. aeruginosa* has been identified in a majority of the reservoirs (Martinez et al. 2005). Additionally, 70% of the potable water in Puerto Rico originates from its reservoirs (Ortíz-Zayas et al. 2004), which means a bloom of *M. aeruginosa* could become a public health issue as it has been found to contain toxins which can cause cancer in humans (Araoz et al. 2009).

As *M. aeruginosa* was not present within the water column when this experiment was conducted, the impact of *C. fluminea* on the cyanobacteria could not be examined directly. Instead, whether *C. fluminea* altered the N:P ratio was used as a proxy, as cyanobacteria has been found to favor low N:P ratios (Jacoby et al. 2000; Bykova et al. 2006). In this study, high N:P ratios tended to be found in the C+W+S treatment in both the Guajataca and La Plata experiments (Appendix E). The high ratio is due to a combination of two factors: increasing nitrogen and decreasing phosphate concentrations. The nitrogen increased due to a flux from the sediment, while the initial phosphorous level decreased due to sediment settling out of the water column. It must be noted a higher increase in the N:P ratio was observed in treatments with sediment (C+W+S and W+S), especially in Guajataca. Thus sediment dynamics are more likely responsible for the increase in the N:P ratio than *C. fluminea*.

A previous study found *M. aeruginosa* blooms increased as the *C. fluminea* population decreased in the Potomac River (Phelps 1994). It suggested *C. fluminea* discouraged *M. aeruginosa* growth by filtering phosphate out of the water column, which supports the theory of *C. fluminea* creating a high N:P ratio. However, the results of this study did not find *C. fluminea* to be more efficient at removing phosphate than the sediment naturally settling out of the water column. Although it is possible *C. fluminea* increased the N:P ratio in the C+W+S treatment, the results do not clearly support this idea and thus the potential impact of *C. fluminea* on *M. aeruginosa* cannot be determined. However, predation of *Synedra* by *C. fluminea* may help avoid an increase in *M. aeruginosa* as a bloom of *Synedra* can create low N:P ratios (Rocha et al. 2002). By keeping the *Synedra* population low, *C. fluminea* is helping to avoid environmental conditions which induce cyanobacteria blooms.

Constraints and Conclusion

One constraint of the experiment is the reservoir water used in this study was taken from sites with a known *C. fluminea* population. This could explain why a larger shift in the

phytoplankton community and nutrient concentrations was not observed: *C. fluminea* has already altered the system. This seems unlikely, however, as the natural *C. fluminea* population in both reservoirs appears to be smaller than in other invaded freshwater systems (Cohen et al. 1984; Karatayev et al. 2003; Caffery et al. 2011) and the mesocosms were stocked at high densities typical of temperate systems. Although the filtration rate of *C. fluminea* has the ability to be high under certain conditions (Way et al. 1990), its confinement to sediment (Sousa et al. 2008) limits which part of the water column it can filter. Additionally, as reservoirs typically have a higher renewal rate than traditional lakes, it is unlikely a bivalve with a low population and limited spatial distribution could have impacted both reservoirs so thoroughly.

An additional constraint is the size of the mesocosms. The small size of the mesocosms utilized may have created changes within the system, specifically in the phytoplankton community, which may not have been observed in larger mesocosms. A small size was necessary, however, in order to deliver sufficient oxygen to the entire system and to manage treatment replication. Regardless of its size, this represents the first experimental attempt to examine the impact of *C. fluminea* on a natural phytoplankton community within a controlled setting. Other experiments have studied the effect of *C. fluminea* on the phytoplankton community in the field (Cohen et al. 1984; Phelps 1984; Boltovskoy et al. 1995), but no one has attempted to replicate a natural system and monitor the impact of *C. fluminea* on specific phytoplankton genera.

Previous studies have shown *C. fluminea* to be capable of creating large changes within an ecosystem (Cohen et al. 1984; Sousa et al. 2008); however, this was not the case for Puerto Rico based on this mesocosm study. *C. fluminea* was able to prevent the phytoplankton community from becoming dominated by *Synedra* in both studies; however it did not seem to impact the nutrient concentration. This could be due to food limitation or increased nutrients being immediately utilized. In fact, sediment within the mesocosm was able to alter the nutrient concentration more than the Asian clam. Thus it is possible the impact of *C. fluminea* could not be detected in the mesocosm experiment due to limitations in the experimental design, such as the length of the experiment, size of the mesocosms, or timing of sampling. Additionally, other studies showing a large impact of *C. fluminea* on the phytoplankton have examined these impacts in the natural environment and not within a mesocosm experiment (Cohen et al. 1984; Phelps 1984). As this was the first study to examine the impact of *C. fluminea* in a tropical

system, it is possible *C. fluminea* is not able to impact tropical systems as severely as temperate ones potentially based on its apparent low natural abundance. Further studies need to be conducted in order to validate these conclusions.

Table 3.1. The H' calculated for each phytoplankton sample from both mesocosms. The standard error is given for each index. H' was not calculated for the treatments with sediment until day 14 because the quantity of suspended sediment made the phytoplankton difficult to identify.

Treatment	Day Sampled	Guajataca Reservoir H'	La Plata Reservoir H'
Collection Container	0	1.50 ± 0.04	1.10 ± 0.06
Clams, Water (C+W)	1	1.35 ± 0.26	0.89 ± 0.13
	7	0.83 ± 0.30	0.59 ± 0.27
	14	0.99 ± 0.36	0.73 ± 0.33
Clams, Water, Sediment (C+W+S)	1	-	-
	7	-	-
	14	0.72 ± 0.18	0.04 ± 0.08
Water, Sediment (W+S)	1	-	-
	7	-	-
	14	0.64 ± 0.0	0.40 ± 0.35
Water (W)	1	1.61 ± 0.34	0.76 ± 0.13
	7	1.37 ± 0.11	0.83 ± 0.11
	14	0.61 0.42	0.66 ± 0.19

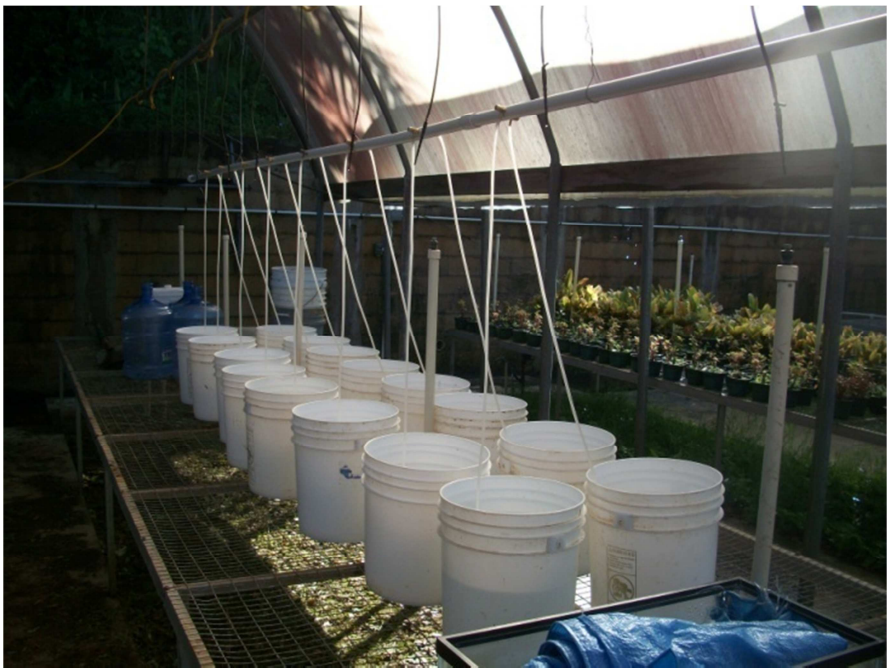

Figure 3.1. The experimental set-up. Each bucket represents a mesocosm. The transparent tubes represent the aeration system. At the top of the picture, the clear covering is visible which allows sunlight to penetrate but not rain.

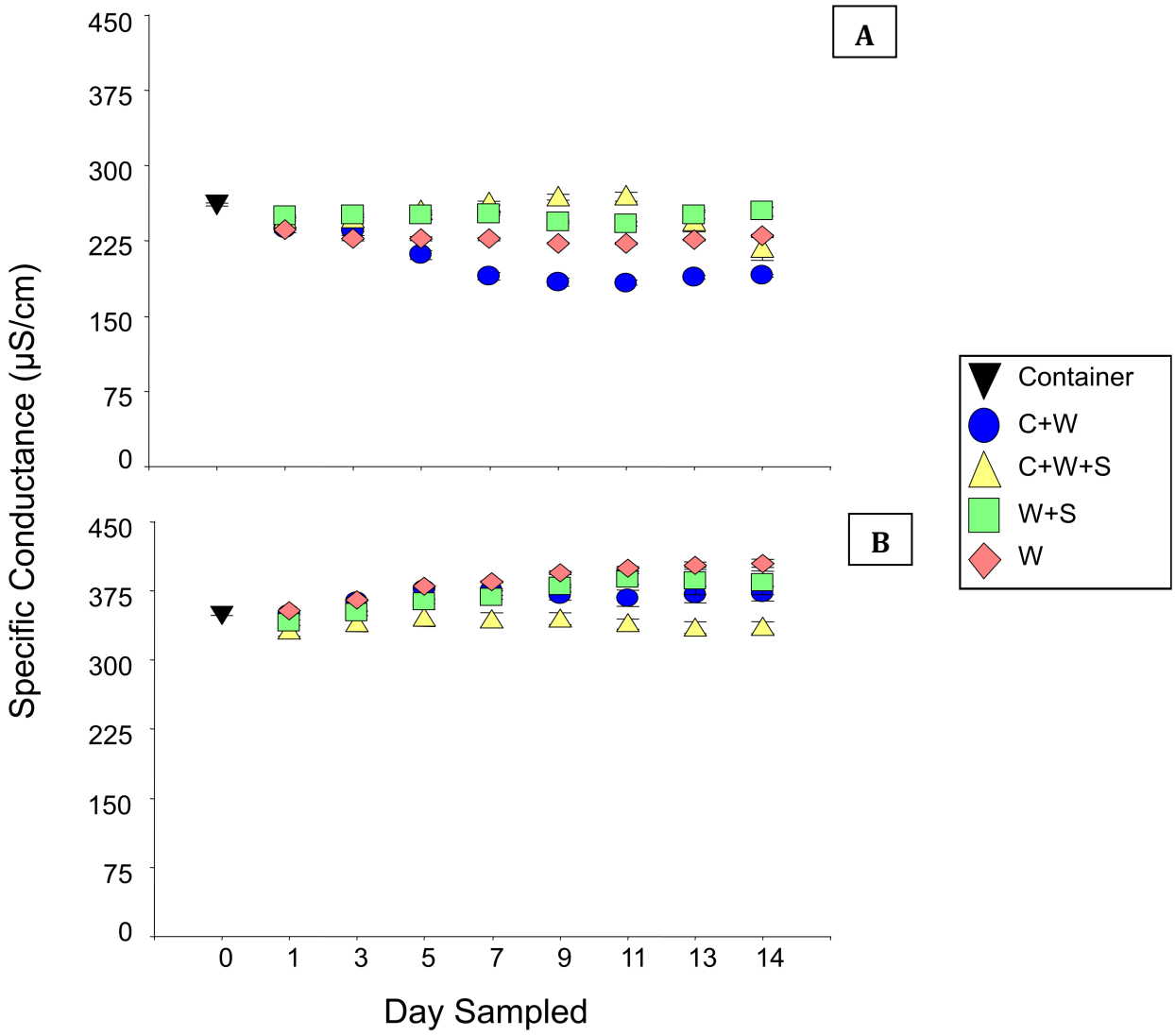

Figure 3.2. The mean specific conductance measured in (A) Guajataca and (B) La Plata mesocosm experiment. Day 0 is the value measured in the containers, before the water was distributed. The bars associated with each point represent the standard error. If no bars are visible, it indicates a small error.

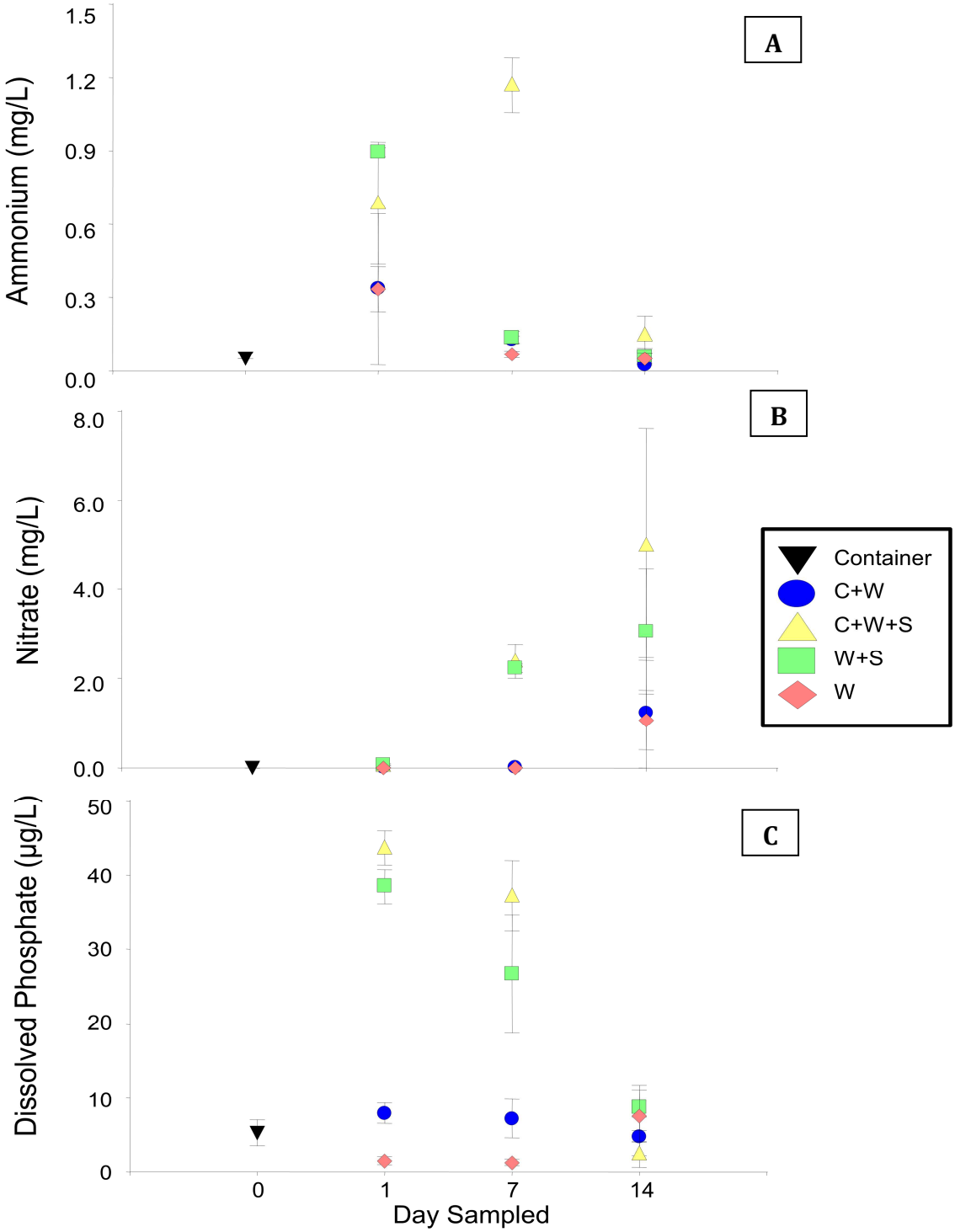

Figure 3.3. Mean concentrations measured in the Guajataca mesocosm experiment for (A) ammonium, (B) nitrate, and (C) dissolved phosphate. Day 0 is the concentration measured in the containers, before the water was distributed. The bars associated with each point represent the standard error. If no bars are visible, it indicates a small error. Note that the scales and units are not the same.

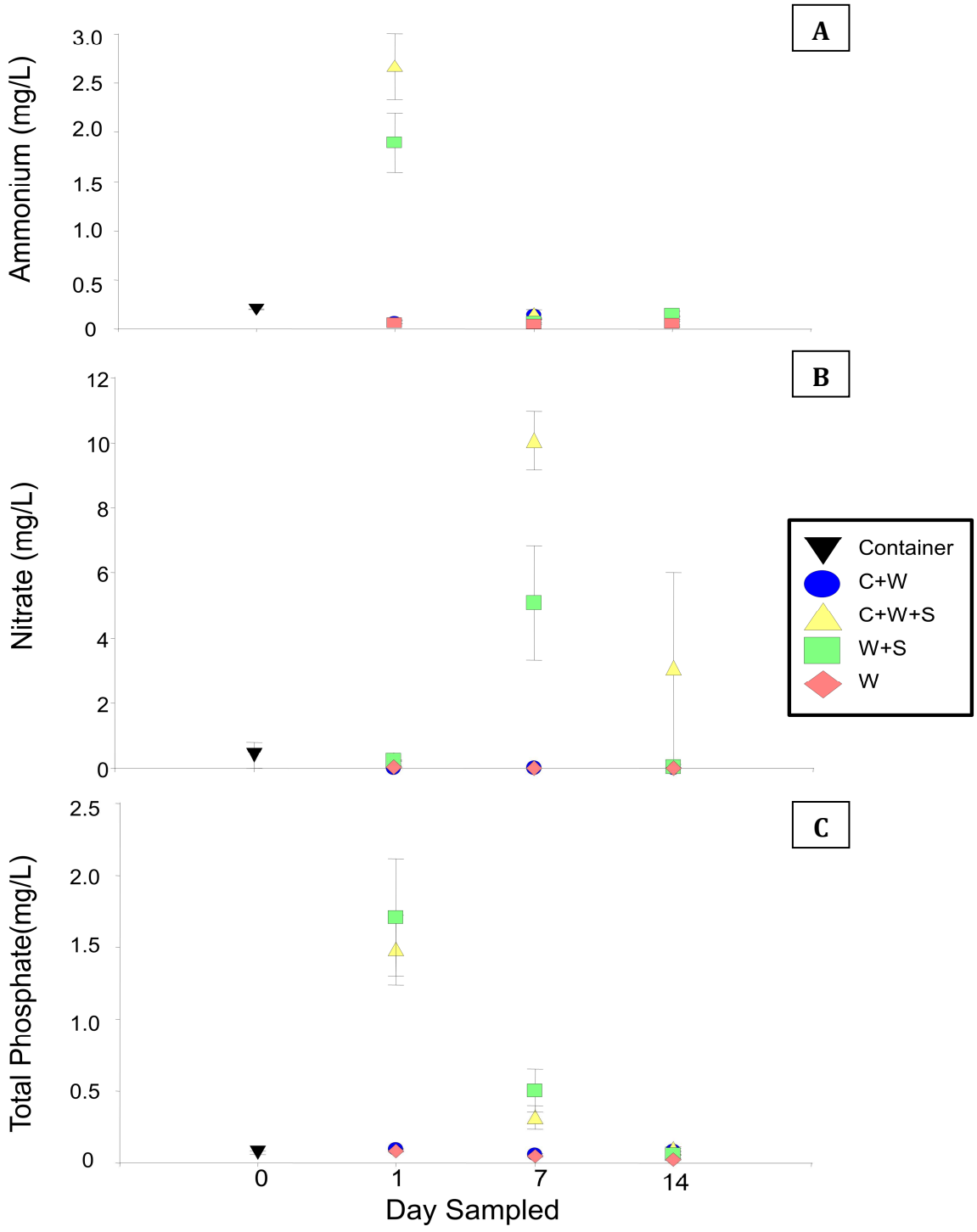

Figure 3.4. Mean concentrations measured in the La Plata mesocosm experiment for (A) ammonium, (B) nitrate, and (C) total phosphate. Day 0 is the concentration measured in the containers, before the water was distributed. The bars associated with each point represent the standard error. If no bars are visible, it indicates a small error. Note that the scales are not the same.

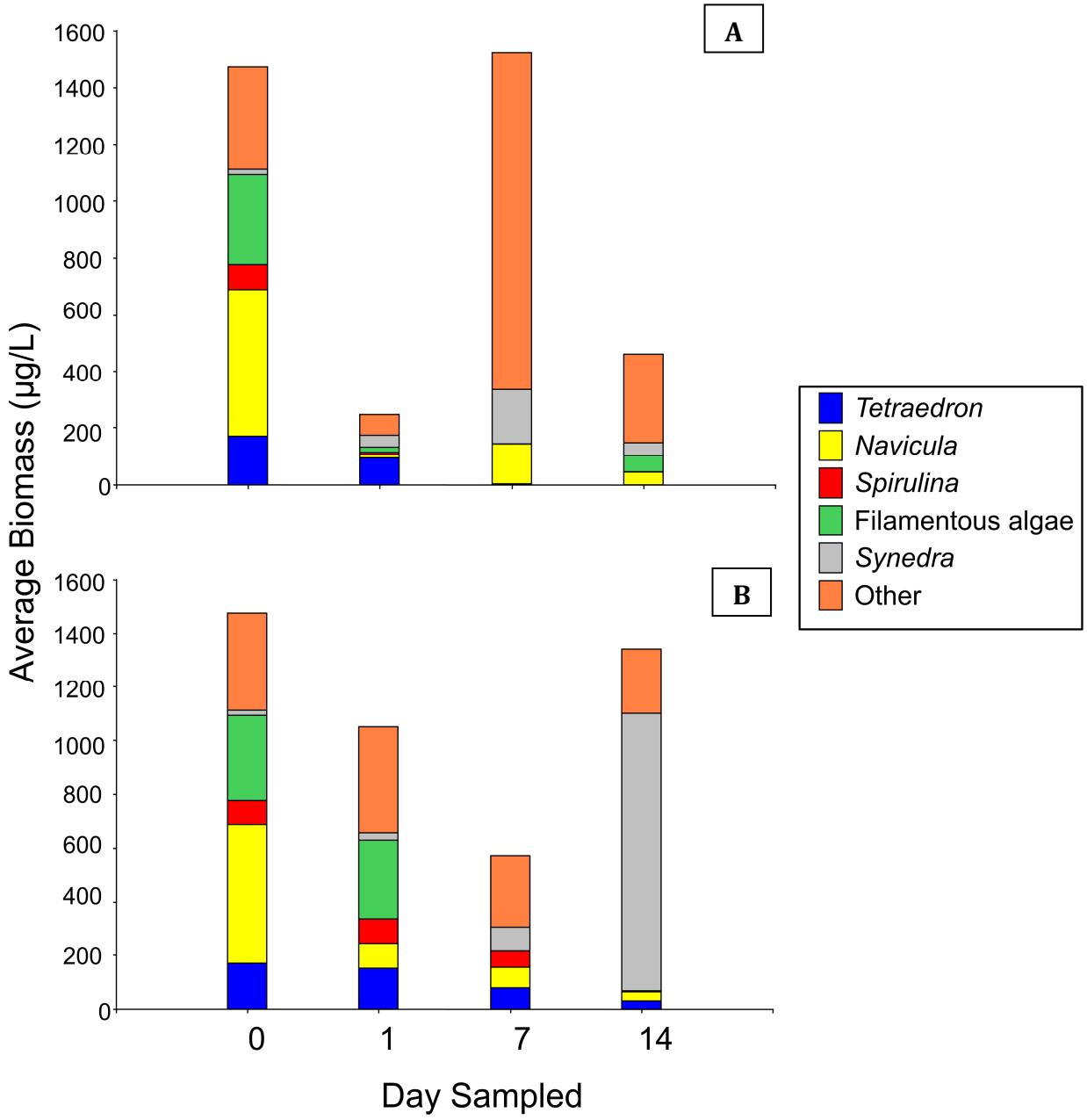

Figure 3.5. The mean biomass from the Guajataca reservoir mesocosms separated into genera for (A) the C+W treatment and (B) the W treatment. The genera which was determined to make up a lower percentage of the biomass were added together to form the category "other". These minor genera include: *Peridinium*, *Pandorina*, *Starastrum, Crucigenia*, *Merismopedia*, *Treubaria*, *Scenedesmus*, *Pediastrum*, centric diatoms, desmid, and an unidentified genera.

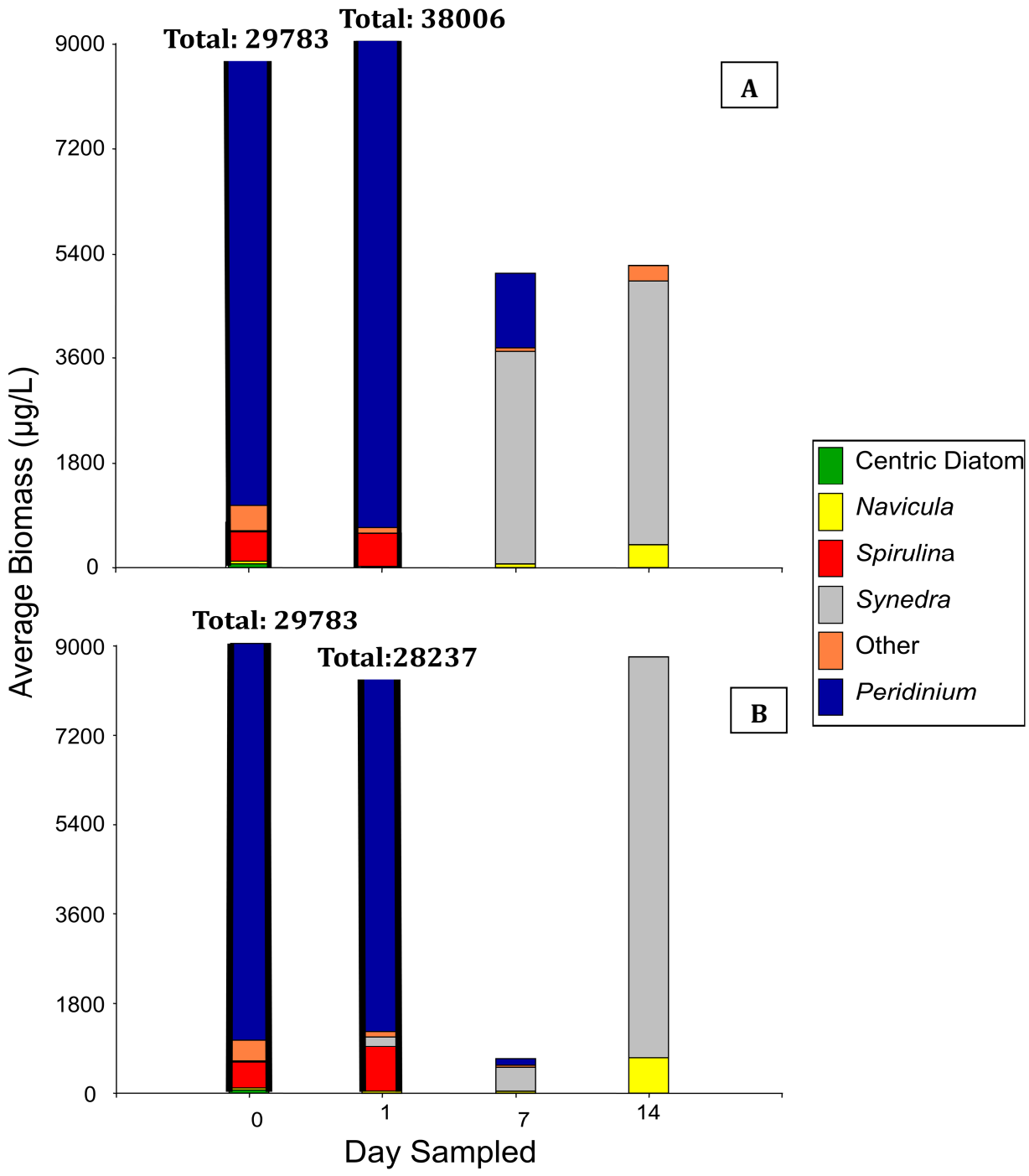

Figure 3.6. The mean biomass from the La Plata reservoir mesocosms separated into genera for (A) the C+W treatment and (B) the W treatment. The genera which was determined to make up a lower percentage of the biomass were added together to form the category "other". These minor genera include: *Starastrum*, *Eudorina*, *Scenedesmus*, *Merismopedia*, *Crucigenia*, *Anabaena*, *Pandorina*, *Coelastrum*, *Tetraedron*, filamentous algae, and desmid.

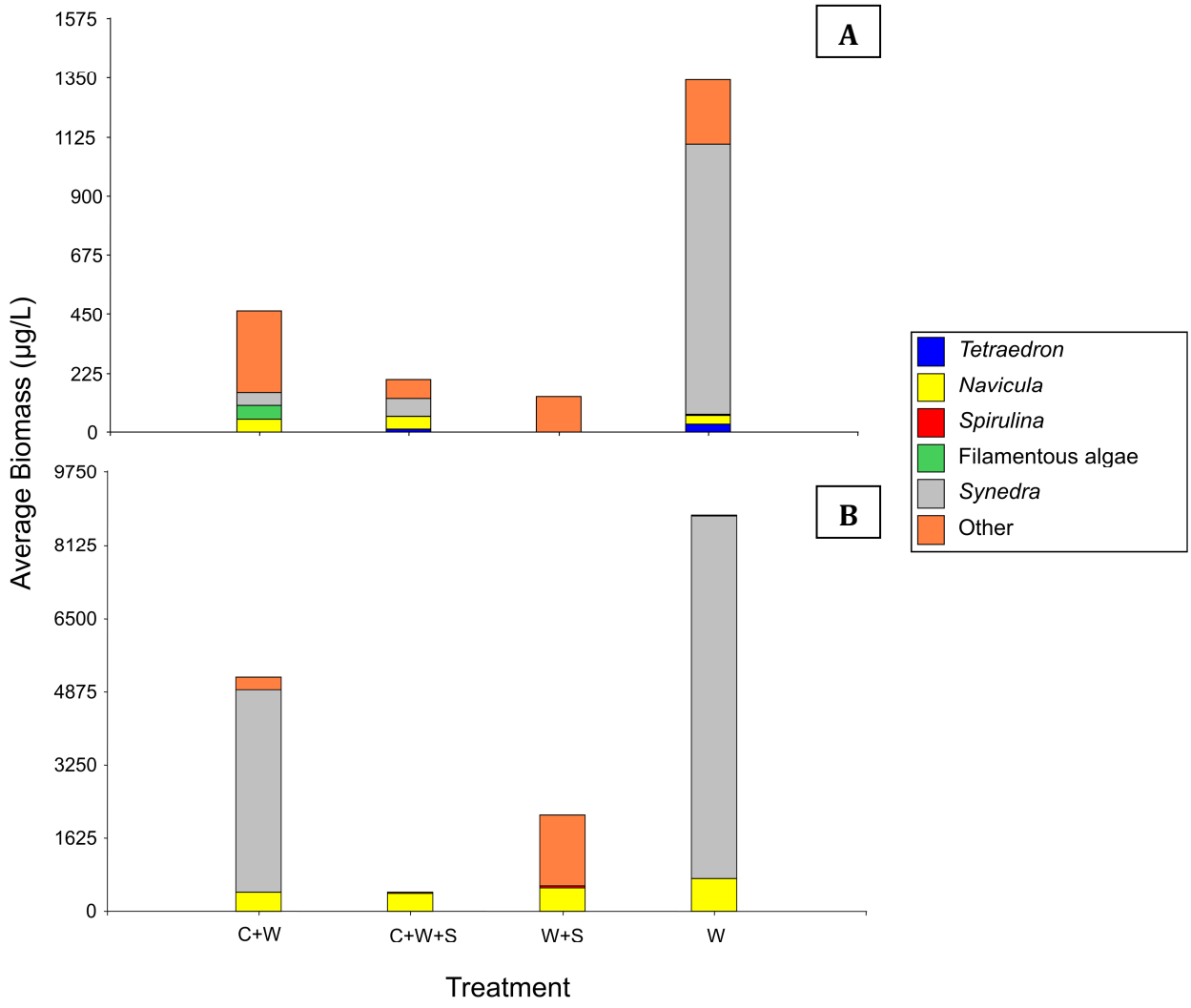

Figure 3.7. The mean biomass separated into genera on day 14 for (A) the Guajataca mesocosm experiment and (B) the La Plata mesocosm experiment. The "other" group in Graph A contains the same genera as listed for Figure 16. The "other" group in Graph B contains the same genera as listed for Figure 17. Note the scales are not the same.

Chapter 4

Conclusions

Good water quality within the reservoirs in Puerto Rico is important to many people, as they represent a major source of drinking water. The health of the phytoplankton community is a large factor in determining water quality, as some members contain toxins and have the ability to create large blooms (Jacoby et al. 2000). These blooms can be both dangerous to public health and discourage recreational activities (Paerl 1988; Araoz et al. 2009). Thus, the conditions which influence phytoplankton community members must be understood. Previous research has suggested nutrient concentrations are a major factor in determining phytoplankton abundance and diversity (Gallegos et al. 1992; Brett and Goldman 1997; Dodson et al. 2000), indicating nutrient concentrations also determine water quality. Although changes in nutrient concentrations are typically caused by human activity (Smith et al. 1999), some invasive bivalves have also been found to influence nutrient cycling (Heck et. al 2004). In addition to nutrients, environmental parameters such as temperature and pH have also been found to influence the phytoplankton community (Grover and Chrzanowski 2006; Dantas et al. 2008). Thus, the variables which impact the phytoplankton distribution the most within the reservoirs must be identified to avoid poor water quality.

In order to determine which parameters impact the phytoplankton community structure, field sampling and mesocosm experiments were conducted. Field sampling was completed in two reservoirs, one mesotrophic and one eutrophic, to examine whether the conditions which impact the phytoplankton community structure vary with trophic status. Additionally, temporal and spatial patterns in environmental variables and the phytoplankton community were evaluated within each reservoir. The mesocosm experiments were necessary to examine the direct impact of the invasive bivalve, *Corbicula fluminea*, on the phytoplankton community. In the following paragraphs a summary of the most important results found in this thesis, reservoir management suggestions, and potential ideas for future studies are presented.

Spatial and temporal differences were found in both reservoirs studied. Spatial differences were expected as a gradient has been proven to exist from the dam to the riverine zone by previous studies (Martinez et al. 2005; Pantoja-Agreda et al. 2009). Though chlorophyll *a* and H' differed spatially within the reservoirs, nutrient concentrations and total biomass varied

spatially only in the eutrophic reservoir. Although spatial differences existed, the temporal variation of environmental parameters seemed to have a larger impact on the phytoplankton community. Additionally, the environmental factors which influence community structure seem to differ depending on the trophic status of the system. Canonical correspondence analysis (CCA) determined pH and TP were the most influential in the mesotrophic reservoir, while specific conductance was the most important parameter in the eutrophic reservoir. Thus, trophic status impacts the phytoplankton by determining which parameters heavily influence the community structure.

In addition to examining whether the environmental parameters impacting the phytoplankton community change with the trophic status, the impact of *C. fluminea* was also studied in both reservoirs. Though sediment from the reservoirs was found to increase nutrient concentrations, *C. fluminea* did not, which was unexpected as it is contrary to the findings of previous studies (Lauritsen and Mozley 1989; Hakenkamp et. al 2001). However, *C. fluminea* was able to maintain the phytoplankton diversity within the mesocosms, as treatments without *C. fluminea* experienced a dominance of *Synedra*, a pennate diatom, at the end of the experiment. A reduction in *Synedra* biomass most likely occurred due to predation. No feeding preference was demonstrated by *C. fluminea* as all phytoplankton genera declined, which supports previous studies (Boltovskoy et al. 1995). As the effects caused by *C. fluminea* were the same in both experiments, it appears their impacts are independent of trophic status.

These findings could assist reservoir managers in determining where to focus their efforts to continue to maintain high water quality standards. Although the results of this study do not suggest a toxic cyanobacteria bloom is imminent, previous surveys have identified a species of cyanobacteria in 19 reservoirs on the island (Martinez et al. 2005). Thus, precautions should be taken to avoid a bloom, such as reduced phosphorous loading. Small measures such as reducing the amount of nutrients generated and consumed in the watershed could have a large impact on the amount of phosphorous loading into the system (Carpenter et al. 1998). Additionally, managers should be aware of the spatial gradient within the reservoirs and should take this phenomenon into account when conducting future monitoring projects. Also, managers should recognize different factors impact the phytoplankton community depending on whether the system is eutrophic or mesotrophic, as changing environmental parameters will have different effects depending on the trophic status of the system. Additionally, although *C. fluminea* has

been found to create large problems within invaded systems (Cohen et al. 1984; Sousa et al. 2008), it does not seem to have the same impact in the reservoirs of Puerto Rico based on this study. Thus, despite its reputation as a successful invader (McMahon 2002), at this time it would be best to avoid wasting resources on eradication efforts and instead focus on limiting the spread of the species to other watersheds.

Although several revealing conclusions can be drawn as to what factors impact the phytoplankton community structure within the reservoirs of Puerto Rico, there is still much work to be done. In order to be more certain which processes impact the phytoplankton community, sampling needs to occur over a longer time period as the seasons are clearly highly variable year to year. Additionally, in order to reduce sampling effort, one could focus on the dam and riverine zones as they represented the highest range of parameters measured within the reservoirs. Also, sampling more than once within a season is recommended as parameters are most likely highly variable within seasons as well. Further studies also need to be carried out examining the impact of *C. fluminea* on both the phytoplankton community and nutrient concentrations. In future mesocosm studies, sediment should be avoided as its presence can complicate effects created by *C. fluminea*. Additionally, water samples should be taken more frequently to determine whether shifts are occurring from day to day. It is also recommended that a better system be examined in order to ensure constant suspension of phytoplankton, such as strong air bubblers on the bottom of the mesocosm. Although this thesis has increased the understanding of which variables impact the phytoplankton community structure within the reservoirs on the island, these conclusions need to be supported by continuing research.

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Appendix A. Total list of all genera found in both studies. GR= Guajataca Reservoir, LPR= La Plata Reservoir, GM= Guajataca Mesocosm, LPM= La Plata Mesocosm.

Genus	**Class**	**GR**	**LPR**	**GM**	**LPM**
Anabaena	Cyanophyceae		X		X
Centric Diatom	Bacillariophyceae	X	X	X	X
Coelastrum	Chlorophyceae	X	X		X
Crucigenia	Chlorophyceae	X	X	X	X
Desmid	Zygnemaphyceae	X		X	X
Eudorina	Chlorophyceae		X		X
Filamentous Algae	Chlorophyceae	X	X	X	X
Gonium	Chlorophyceae	X			
Merismopedia	Cyanophyceae	X	X	X	X
Navicula	Bacillariophyceae	X	X	X	X
Oocystis	Chlorophyceae	X	X		
Pandorina	Chlorophyceae	X	X	X	X
Pediastrum	Chlorophyceae	X	X	X	
Peridinium	Dinophyceae	X	X	X	X
Scenedesmus	Chlorophyceae	X	X	X	X
Spirulina	Cyanophyceae	X	X	X	X
Starastrum	Zygnemaphyceae	X	X	X	X
Synedra	Bacillariophyceae	X	X	X	X
Tetraedron	Chlorophyceae	X	X	X	X
Trachelmona	Euglenophyceae	X	X		
Treubaria	Chlorophyceae	X	X	X	
Unknown Cyanobacteria	Cyanophyceae	X		X	

Appendix B. Determining phytoplankton biovolume.

The number of cells per mL was determined through:

$$\frac{\text{Number of Cells}}{\text{mL}} = \frac{\left\{\left[\frac{\text{Number of Cells Counted}}{50}\right] X \text{ Conversion Factor}\right\}}{\frac{210}{10}}$$

where

Number of Cells Counted= number of individual phytoplankton counted per genera

50= number of square fields counted under the microscope

Conversion factor= factor based on the magnification used to count the cells

210= volume present in the Palmer chamber

10= the concentration factor of the sample

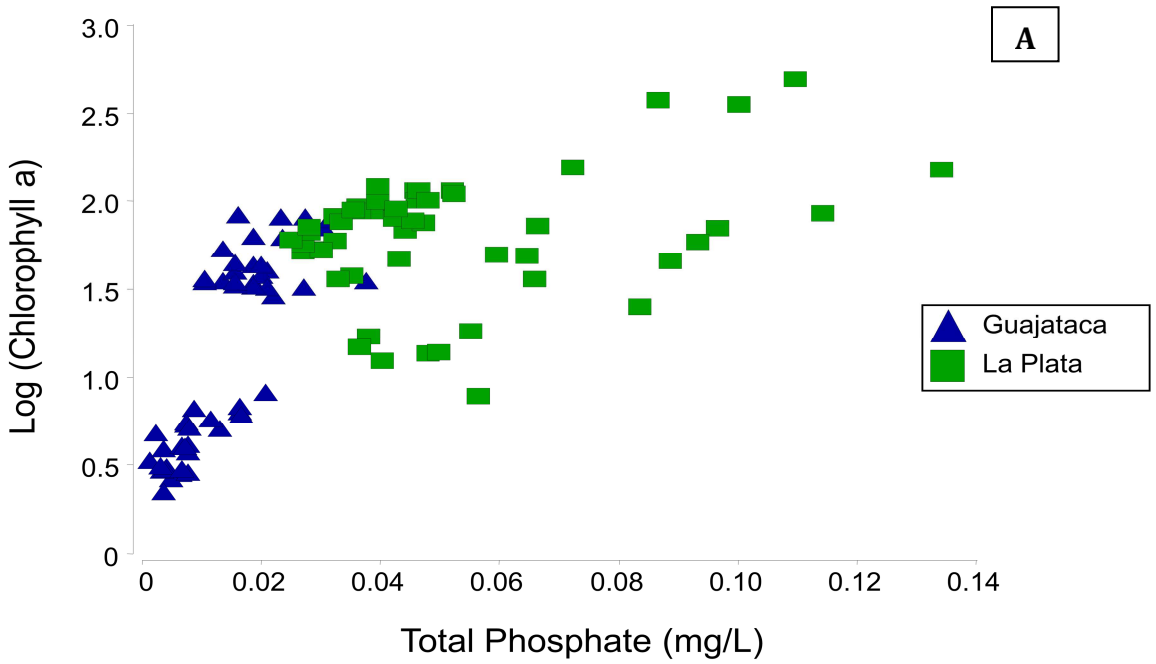
Table appendix B. The equations used to estimate biovolume for each genera found in the samples. The name of the equation is listed in each box, along with the specific formula used. The geometric equations are associated with genera that have a similar shape.

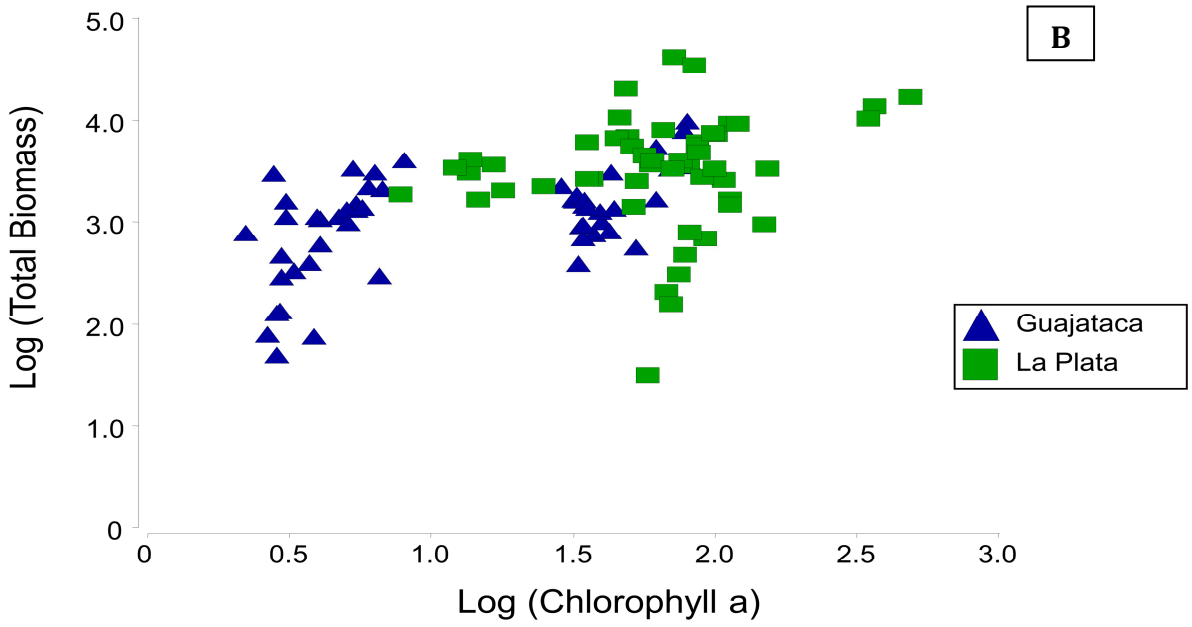
Geometric Equation (μm^3)	Genera
Ellipsoid $4 \times \left\{\frac{\text{length}}{2}\right\} \left\{\frac{\text{width}}{2}\right\}^2$	*Peridinium*, *Navicula*, *Starastrum*, *Crucugenia*, *Scenedesmus*, *Treubaria*, *Pediastrum*, *Pandorina*
Cylinder $3.14 \times \text{length} \times \text{width}^2$	*Spirulina*, *Synedra*, centric diatom
Sphere $4 \left\{\frac{\text{diameter}}{2}\right\}^3$	*Coelastrum*, *Eudorina*
Box $\text{length} * \text{width} * \text{depth}$	Desmid
Cube $(\text{length})^3$	*Tetraedron*, *Merismopedia*
Prolate spheroid $3.14 \times \text{length} \times \text{width}^2$	*Oocystis*

Appendix C. The latitude and longitude of all sample points in both the Guajataca and La Plata reservoir used in Chapter 2. The different numbers refer to transects within the specified zone.

	Guajataca Reservoir		La Plata Reservoir	
Point Location	**Latitude**	**Longitude**	**Latitude**	**Longitude**
Dam Littoral 1	18.39417833	-66.92082333	18.343313	-66.233575
Dam Middle 1	18.39415167	-66.92222278	18.341633	-66.234919
Dam Littoral 2	18.39275806	-66.92091583	18.338832	-66.234247
Dam Middle 2	18.39280194	-66.9220483	18.339728	-66.232343
Dam Littoral 3	18.39187722	-66923205	18.337039	-66.236151
Dam Middle 3	18.39192139	-66.92201472	18.335919	-66.235031
Transition Littoral 1	18.37967611	-66.91584722	18.32662	-66.235815
Transition Middle 1	18.37751278	-66.92080806	18.32774	-66.234583
Transition Littoral 2	18.37126694	-66.91810306	18.327516	-66.233238
Transition Middle 2	18.3761	-66.91557417	18.326956	-66.234135
Transition Littoral 3	18.37549222	-66.92607028	18.327628	-66.229429
Transition Middle 3	18.374825	-66.9250825	18.327068	-66.228421
Riverine Littoral 1	18.37173583	-66.91181694	18.333006	-66.2177777
Riverine Middle 1	18.37347667	-6690905139	18.333902	-66.217889
Riverine Littoral 2	18.37357444	-66.90715333	18.329533	-66.214752
Riverine Middle 2	18.37181917	-66.911188222	18.329084	-66.215312
Riverine Littoral 3	18.3726775	-66.90674972	18.323819	-66.213631
Riverine Middle 3	18.37155583	-66.90809278	18.324043	-66.212847

Appendix D. Correlations between several of the parameters measured in both reservoirs. Graph A represents the correlation between TP and the log of chlorophyll a. Graph B is the correlation between the log of chlorophyll a and the log of the total biovolume. For the correlation values, see Chapter 2.





Appendix E. The physical parameters sampled in each mesocosm from chapter 3. The values measured before the water was distributed to all treatments is represented in Day 0. For the four treatments, the initial and final values of each parameter are given, along with the standard deviation. The chlorophyll *a* of the sediment treatments was not able to be calculated on Day 1 due to the large amount of suspended sediment, which interfered with the reading. Table A represents the values recorded in the Guajataca mesocosms, and table B represents the values measured in the La Plata mesocosms. Note the P used to calculate the N/P ratio was DP in Guajataca and TP in La Plata.

A

	Container	C+W		C+W+S		W+S		W	
	0	1	14	1	14	1	14	1	14
Chl a (µg/L)	49.97± 1.78	8.96± 0.68	8.06± 0.95	-	4.82± 0.58	-	5.48± 2.28	35.13± 1.86	4.30± 0.93
N/P ratio	1.96± 1.47	3.25± 0.51	301.13± 587.20	1.60± 0.30	3297.61± 4099.45	2.65± 0.51	698.16± 680.90	7.72± 7.64	103.58± 103.40
DO (mg/L)	7.53± 0.17	7.90± 0.14	6.69± 0.17	8.13± 0.28	6.90 ± 0.21	8.04± 0.17	6.63± 0.33	7.86± 0.25	6.45± 0.12
Temp °C	26.7± 0.2	20.5± 0.2	27.9± 0.6	20.6± 0.2	28.3 ± 0.9	20.6± 0.2	28.1± 0.9	20.6± 0.2	28.0± 0.9
pH	8.37± 0.05	8.34± 0.02	8.22± 0.05	8.32± 0.04	8.16 ± 0.07	8.34± 0.02	8.22± 0.03	8.27± 0.17	8.16± 0.10

B

	Container	C+W		C+W+S		W+S		W	
	0	1	14	1	14	1	14	1	14
Chl a (µg/L)	336.45± 8.01	266.50± 33.77	18.53± 23.12	-	7.02± 2.92	-	8.48± 5.56	204.67± 31.18	11.06± 2.88
N/P ratio	10.28± 17.79	0.17± 0.19	0.01± 0.03	0.21± 0.06	41.04± 78.81	0.21± 0.15	0.73± 0.47	0.36± 0.38	0.56± 1.10
DO (mg/L)	7.41± 0.11	6.55± 0.23	6.03± 0.33	6.22± 0.14	6.07 ± 0.25	6.41± 0.33	6.39± 0.15	6.39± 0.29	6.11± 0.18
Temp °C	26.5± 0.1	29.5± 0.3	31.4± 0.3	29.7± 0.4	31.8± 0.4	29.5± 0.4	31.5± 0.5	29.3± 0.4	31.2± 0.4
pH	7.99± 0.13	8.42± 0.09	8.39± 0.07	8.22± 0.02	8.55 ± 0.11	8.21± 0.03	8.60± 0.08	8.38± 0.02	8.44± 0.03

Appendix F. Significant correlations between various parameters from chapter 3. Table A represents the Pearson correlations for Guajataca, while table B has the Pearson correlations for La Plata. The significant correlations are shown with an asterisk.

A

	H’	DP	Log Chla	Log total biomass
H’	1.00			
DP	-0.37*	1.00		
Log Chla	0.62*	0.08	1.00	
Log total biomass	0.96*	-0.40*	0.55*	1.00

B

	H’	TP	Log Chla	Log total biomass
H’	1.00			
TP	0.14	1.00		
Log Chla	0.64*	0.34	1.00	
Log total biomass	0.53*	0.45*	0.61*	1.00

Appendix G. The ANOVA results for Chapter 2. Results shown are: (A) TP, (B) TKN, (C) NO_3, (D) temperature, (E) specific conductance, (F) secchi disc depth, (G) chlorophyll *a* concentration, (H) H', and (I) total biomass. In each output, Lake 1= Guajataca; Lake 2= La Plata; Month 1= Cold Wet; Month 2= Warm Wet; Month 3= Cold Dry; Zone 1= Dam; Zone 2= Transition; Zone 3= Riverine.

A

Variable	N	R^2	R^2 Aj	CV
TP Value (mg/L)	104	0.79	0.74	37.66

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	0.05	17	3.2E-03	18.69	<0.0001
Lake	0.04	1	0.04	222.47	<0.0001
Zone	0.01	2	4.5E-03	26.17	<0.0001
Month	9.6E-04	2	4.8E-04	2.76	0.0686
Lake*Month	3.0E-03	2	1.5E-03	8.64	0.0004
Lake*Zone	0.01	2	2.9E-03	16.61	<0.0001
Month*Zone	8.8E-04	4	2.2E-04	1.27	0.2869
Lake*Month*Zone	3.6E-04	4	9.1E-05	0.53	0.7162
Error	0.01	86	1.7E-04		
Total	0.07	103			

Test:Bonferroni Alfa=0.05 DMS=0.01350

Error: 0.0002 gl: 86

Lake	Month	Medias	n	E.E.			
1.00	1.00	0.01	18	3.1E-03	A		
1.00	2.00	0.02	16	3.3E-03	A		
1.00	3.00	0.02	18	3.1E-03	A		
2.00	3.00	0.05	18	3.1E-03		B	
2.00	1.00	0.06	18	3.1E-03		B	C
2.00	2.00	0.06	16	3.3E-03			C

Medias con una letra común no son significativamente diferentes(p<= 0.05)

Test:Bonferroni Alfa=0.05 DMS=0.01350

Error: 0.0002 gl: 86

Lake	Zone	Medias	n	E.E.			
1.00	1.00	0.01	17	3.2E-03	A		
1.00	2.00	0.02	18	3.1E-03	A		
1.00	3.00	0.02	17	3.2E-03	A		
2.00	1.00	0.04	18	3.1E-03		B	
2.00	2.00	0.05	18	3.1E-03		B	
2.00	3.00	0.08	16	3.3E-03			C

Medias con una letra común no son significativamente diferentes(p<= 0.05)

B

Variable	N	R^2	R^2 Aj	CV
TKN (mg/L)	104	0.25	0.10	84.22

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	9.69	17	0.57	1.68	0.0624
Lake	1.02	1	1.02	3.01	0.0861
Month	1.56	2	0.78	2.30	0.1069
Zone	0.43	2	0.21	0.63	0.5351
Lake*Month	0.71	2	0.35	1.04	0.3584
Lake*Zone	2.34	2	1.17	3.44	0.0365
Month*Zone	1.79	4	0.45	1.32	0.2686
Lake*Month*Zone	1.48	4	0.37	1.09	0.3685
Error	29.20	86	0.34		
Total	38.90	103			

Test:Bonferroni Alfa=0.05 DMS=0.59793

Error: 0.3396 gl: 86

Lake	Zone	Medias	n	E.E.	
2.00	1.00	0.42	18	0.14	A
2.00	2.00	0.55	18	0.14	A
1.00	3.00	0.57	17	0.14	A
2.00	3.00	0.81	16	0.15	A
1.00	1.00	0.81	17	0.14	A
1.00	2.00	0.99	18	0.14	A

Medias con una letra común no son significativamente diferentes(p<= 0.05)

C

Variable	N	R^2	R^2 Aj	CV
NO3 (mg/L)	104	0.96	0.95	71.51

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	16.97	17	1.00	110.26	<0.0001
Lake	2.65	1	2.65	292.23	<0.0001
Month	5.12	2	2.56	282.93	<0.0001
Zone	1.53	2	0.76	84.46	<0.0001
Lake*Month	5.03	2	2.52	277.80	<0.0001
Lake*Zone	1.55	2	0.78	85.78	<0.0001
Month*Zone	2.81	4	0.70	77.57	<0.0001
Lake*Month*Zone	2.87	4	0.72	79.16	<0.0001
Error	0.78	86	0.01		
Total	17.75	103			

Test:Bonferroni Alfa=0.05 DMS=0.21013

Error: 0.0091 gl: 86

Lake	Month	Zone	Medias	n	E.E.				
1.00	1.00	1.00	0.00	6	0.04	A			
1.00	1.00	3.00	0.00	6	0.04	A			
2.00	3.00	2.00	0.00	6	0.04	A			
1.00	1.00	2.00	0.00	6	0.04	A			
2.00	1.00	2.00	1.8E-03	6	0.04	A			
2.00	3.00	3.00	3.2E-03	6	0.04	A			
2.00	3.00	1.00	3.3E-03	6	0.04	A			
1.00	2.00	2.00	4.1E-03	6	0.04	A			
1.00	2.00	3.00	4.2E-03	5	0.04	A			
1.00	3.00	2.00	0.01	6	0.04	A			
1.00	3.00	1.00	0.01	6	0.04	A			
1.00	3.00	3.00	0.01	6	0.04	A			
2.00	1.00	1.00	0.01	6	0.04	A			
2.00	1.00	3.00	0.01	6	0.04	A			
1.00	2.00	1.00	0.02	5	0.04	A			
2.00	2.00	1.00	0.31	6	0.04		B		
2.00	2.00	2.00	0.59	6	0.04			C	
2.00	2.00	3.00	2.01	4	0.05				D

Medias con una letra común no son significativamente diferentes(p<= 0.05)

D

Variable	N	R^2	R^2 Aj	CV
Temp (C)	103	0.97	0.96	1.18

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	299.49	17	17.62	161.97	<0.0001
Lake	0.13	1	0.13	1.21	0.2737
Month	269.76	2	134.88	1240.13	<0.0001
Zone	0.67	2	0.34	3.09	0.0507
Lake*Month	16.85	2	8.43	77.47	<0.0001
Lake*Zone	2.39	2	1.20	10.99	0.0001
Month*Zone	2.78	4	0.70	6.39	0.0002
Lake*Month*Zone	1.10	4	0.28	2.54	0.0457
Error	9.24	85	0.11		
Total	308.73	102			

Test:Bonferroni Alfa=0.05 DMS=0.73315

Error: 0.1088 gl: 85

Lake	Month	Zone	Medias	n	E.E.								
1.00	3.00	1.00	25.68	6	0.13	A							
2.00	3.00	1.00	25.92	6	0.13	A	B						
2.00	3.00	2.00	26.08	6	0.13	A	B	C					
2.00	3.00	3.00	26.15	6	0.13	A	B	C					
1.00	3.00	2.00	26.38	6	0.13	A	B	C	D				
1.00	3.00	3.00	26.42	6	0.13		B	C	D				
1.00	1.00	1.00	26.73	4	0.16			C	D	E			
1.00	1.00	3.00	26.92	6	0.13				D	E			
1.00	1.00	2.00	27.17	6	0.13					E			
2.00	1.00	3.00	27.50	6	0.13					E	F		
2.00	1.00	2.00	28.10	6	0.13						F	G	
2.00	1.00	1.00	28.70	6	0.13							G	
2.00	2.00	3.00	29.58	4	0.16								H
2.00	2.00	2.00	29.65	6	0.13								H
2.00	2.00	1.00	29.68	6	0.13								H
1.00	2.00	1.00	30.37	6	0.13								H I
1.00	2.00	3.00	30.40	5	0.15								H I
1.00	2.00	2.00	30.65	6	0.13								I

Medias con una letra común no son significativamente diferentes(p<= 0.05)

E

Variable	N	R^2	R^2 Aj	CV
SPC	103	1.00	0.99	1.10

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	203428.49	17	11966.38	1093.18	<0.0001
Lake	147795.33	1	147795.33	13501.70	<0.0001
Month	33127.13	2	16563.57	1513.15	<0.0001
Zone	267.18	2	133.59	12.20	<0.0001
Lake*Month	11771.89	2	5885.94	537.70	<0.0001
Lake*Zone	1189.47	2	594.73	54.33	<0.0001
Month*Zone	474.98	4	118.75	10.85	<0.0001
Lake*Month*Zone	2426.54	4	606.63	55.42	<0.0001
Error	930.45	85	10.95		
Total	204358.93	102			

Test:Bonferroni Alfa=0.05 DMS=7.35501

Error: 10.9464 gl: 85

Lake	Month	Zone	Medias	n	E.E.							
1.00	2.00	3.00	228.16	5	1.48	A						
1.00	2.00	2.00	229.37	6	1.35	A						
1.00	2.00	1.00	229.68	6	1.35	A						
1.00	1.00	3.00	264.68	6	1.35		B					
1.00	3.00	3.00	277.45	6	1.35			C				
1.00	3.00	2.00	278.30	6	1.35			C				
1.00	3.00	1.00	278.56	6	1.35			C				
1.00	1.00	1.00	282.60	4	1.65			C				
1.00	1.00	2.00	283.43	6	1.35			C				
2.00	1.00	1.00	313.02	6	1.35				D			
2.00	2.00	1.00	317.28	6	1.35				D	E		
2.00	1.00	2.00	318.80	6	1.35				D	E		
2.00	2.00	3.00	322.03	4	1.65					E		
2.00	2.00	2.00	337.22	6	1.35						F	
2.00	1.00	3.00	337.40	6	1.35						F	
2.00	3.00	2.00	362.58	6	1.35							G
2.00	3.00	1.00	365.10	6	1.35							G
2.00	3.00	3.00	366.52	6	1.35							G

Medias con una letra común no son significativamente diferentes(p<= 0.05)

F

Variable	N	R^2	R^2 Aj	CV
Secchi (m)	100	0.90	0.88	15.34

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	35.80	17	2.11	43.59	<0.0001
Lake	11.62	1	11.62	240.46	<0.0001
Month	1.79	2	0.89	18.52	<0.0001
Zone	10.24	2	5.12	105.94	<0.0001
Lake*Month	8.31	2	4.15	85.99	<0.0001
Lake*Zone	0.16	2	0.08	1.64	0.2002
Month*Zone	1.42	4	0.35	7.34	<0.0001
Lake*Month*Zone	0.47	4	0.12	2.44	0.0532
Error	3.96	82	0.05		
Total	39.76	99			

Test:Bonferroni Alfa=0.05 DMS=0.23048

Error: 0.0483 gl: 82

Lake	Month	Medias	n	E.E.				
2.00	2.00	0.80	16	0.06	A			
2.00	1.00	1.05	18	0.05		B		
2.00	3.00	1.39	16	0.06			C	
1.00	1.00	1.44	18	0.05			C	
1.00	3.00	1.54	17	0.05			C	
1.00	2.00	2.34	15	0.06				D

Medias con una letra común no son significativamente diferentes(p<= 0.05)

Test:Bonferroni Alfa=0.05 DMS=0.31097

Error: 0.0483 gl: 82

Month	Zone	Medias	n	E.E.					
1.00	3.00	0.92	12	0.06	A				
2.00	3.00	0.94	7	0.08	A				
3.00	3.00	1.19	12	0.06	A	B			
1.00	2.00	1.32	12	0.06		B	C		
3.00	2.00	1.37	12	0.06		B	C		
1.00	1.00	1.50	12	0.06			C		
2.00	2.00	1.61	12	0.06			C	D	
3.00	1.00	1.85	9	0.07				D	E
2.00	1.00	2.15	12	0.06					E

Medias con una letra común no son significativamente diferentes(p<= 0.05)

G

Variable	N	R^2	R^2 Aj	CV
Chl A Calculated (ug/L)	108	0.60	0.53	89.96

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	323272.51	17	19016.03	7.99	<0.0001
Lake	97891.22	1	97891.22	41.13	<0.0001
Month	71789.49	2	35894.75	15.08	<0.0001
Zone	33575.15	2	16787.58	7.05	0.0014
Lake*Month	16284.98	2	8142.49	3.42	0.0370
Lake*Zone	11346.72	2	5673.36	2.38	0.0980
Month*Zone	38460.39	4	9615.10	4.04	0.0047
Lake*Month*Zone	53924.55	4	13481.14	5.66	0.0004
Error	214221.13	90	2380.23		
Total	537493.64	107			

Test:Bonferroni Alfa=0.05 DMS=105.25818

Error: 2380.2348 gl: 90

Lake	Month	Zone	Medias	n	E.E.		
1.00	1.00	1.00	2.75	6	19.92	A	
1.00	1.00	2.00	3.47	6	19.92	A	
1.00	1.00	3.00	4.22	6	19.92	A	
1.00	2.00	1.00	5.34	6	19.92	A	
2.00	1.00	1.00	13.17	6	19.92	A	
1.00	3.00	1.00	34.75	6	19.92	A	
1.00	3.00	2.00	35.43	6	19.92	A	
1.00	2.00	2.00	37.08	6	19.92	A	
1.00	3.00	3.00	44.10	6	19.92	A	
1.00	2.00	3.00	49.98	6	19.92	A	
2.00	1.00	2.00	52.87	6	19.92	A	
2.00	1.00	3.00	56.15	6	19.92	A	
2.00	3.00	1.00	57.72	6	19.92	A	
2.00	2.00	3.00	59.33	6	19.92	A	
2.00	2.00	1.00	87.40	6	19.92	A	
2.00	2.00	2.00	93.05	6	19.92	A	
2.00	3.00	2.00	93.45	6	19.92	A	
2.00	3.00	3.00	245.90	6	19.92		B

Medias con una letra común no son significativamente diferentes(p<= 0.05)

H

Variable	N	R^2	R^2 Aj	CV
H'	104	0.79	0.75	18.58

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	12.75	17	0.75	19.14	<0.0001
Lake	2.31	1	2.31	59.00	<0.0001
Zone	0.53	2	0.27	6.77	0.0019
Month	4.05	2	2.03	51.70	<0.0001
Lake*Zone	0.38	2	0.19	4.87	0.0099
Lake*Month	1.54	2	0.77	19.63	<0.0001
Zone*Month	1.17	4	0.29	7.44	<0.0001
Lake*Zone*Month	2.63	4	0.66	16.76	<0.0001
Error	3.37	86	0.04		
Total	16.12	103			

Test:Bonferroni Alfa=0.05 DMS=0.43848

Error: 0.0392 gl: 86

Lake	Zone	Month	Medias	n	E.E.								
2.00	2.00	2.00	0.47	6	0.08	A							
2.00	1.00	1.00	0.49	6	0.08	A	B						
2.00	1.00	2.00	0.60	6	0.08	A	B	C					
2.00	3.00	2.00	0.65	3	0.11	A	B	C	D				
1.00	2.00	1.00	0.85	6	0.08	A	B	C	D	E			
1.00	3.00	1.00	0.91	6	0.08		B	C	D	E			
2.00	2.00	1.00	0.94	6	0.08			C	D	E			
2.00	3.00	3.00	1.00	6	0.08			C	D	E	F		
2.00	2.00	3.00	1.02	6	0.08			C	D	E	F		
1.00	2.00	2.00	1.05	6	0.08				D	E	F		
1.00	1.00	2.00	1.09	6	0.08				D	E	F	G	
1.00	1.00	1.00	1.17	6	0.08				D	E	F	G	H
1.00	3.00	2.00	1.23	5	0.09					E	F	G	H I
2.00	1.00	3.00	1.37	6	0.08						F	G	H I
1.00	2.00	3.00	1.50	6	0.08							G	H I
1.00	3.00	3.00	1.53	6	0.08								H I
1.00	1.00	3.00	1.54	6	0.08								H I
2.00	3.00	1.00	1.60	6	0.08								I

Medias con una letra común no son significativamente diferentes(p<= 0.05)

I

Variable	N	R^2	R^2 Aj	CV
*Biomass	104	0.55	0.47	114.68

Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo	2013159127.87	17	118421125.17	6.28	<0.0001
Lake	371305011.28	1	371305011.28	19.70	<0.0001
Zone	215374401.40	2	107687200.70	5.71	0.0047
Month	68690297.75	2	34345148.88	1.82	0.1678
Lake*Zone	138495438.95	2	69247719.47	3.67	0.0294
Lake*Month	390930969.95	2	195465484.97	10.37	0.0001
Zone*Month	253799611.77	4	63449902.94	3.37	0.0131
Lake*Zone*Month	402563424.23	4	100640856.06	5.34	0.0007
Error	1620861407.70	86	18847225.67		
Total	3634020535.57	103			

Test:Bonferroni Alfa=0.05 DMS=9614.71273

Error: 18847225.6709 gl: 86

Lake	Zone	Month	Medias	n	E.E.		
2.00	3.00	2.00	373.28	3	2506.47	A	
1.00	2.00	1.00	504.61	6	1772.34	A	
1.00	3.00	1.00	649.10	6	1772.34	A	
1.00	1.00	1.00	921.66	6	1772.34	A	
1.00	2.00	3.00	1002.34	6	1772.34	A	
2.00	2.00	2.00	1103.10	6	1772.34	A	
1.00	3.00	3.00	1233.85	6	1772.34	A	
1.00	1.00	3.00	1407.45	6	1772.34	A	
1.00	1.00	2.00	1625.79	6	1772.34	A	
2.00	1.00	1.00	2927.42	6	1772.34	A	
2.00	2.00	1.00	3874.80	6	1772.34	A	
1.00	2.00	2.00	3949.46	6	1772.34	A	
2.00	1.00	2.00	4003.87	6	1772.34	A	
2.00	1.00	3.00	4479.71	6	1772.34	A	
1.00	3.00	2.00	4768.78	5	1941.51	A	
2.00	2.00	3.00	5853.27	6	1772.34	A	
2.00	3.00	3.00	8678.74	6	1772.34	A	
2.00	3.00	1.00	19238.80	6	1772.34		B

Medias con una letra común no son significativamente diferentes(p<= 0.05)

Appendix H. Mean concentration of nitrate found in each zone for (A) Guajataca and (B) La Plata in Chapter 2. The bars associated with each point represent the standard error. If no bars are visible, it indicates a small standard error.