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Nutrient Discharges from Mayagüez Bay Watershed

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EXECUTIVE SUMMARY

The identification of non-point sources of pollution within tropical watersheds and quantification of the relative nutrient, bacterial, and sediment contribution of these sources to surface waters are important for development of mitigation strategies and for total maximum daily load (TMDL) development and implementation. Water-quality problems such as elevated bacterial indicator concentrations and sediment concentrations are prevalent throughout Puerto Rico and often exceed water quality standards established by the PR Environmental Quality Board (JCA, 1990, Vachier, 1994). The fact that nutrients are yet to be considered a major pollution cause in Puerto Rico may be due to the lack of adequate standards, which has prevented the identification of nutrient impaired waters (JCA, 2002). We hypothesize that in-lake reservoir nutrient (nitrogen and phosphorus) concentrations are high enough to promote conditions leading to water-quality degradation, throughout Puerto Rico, and that these are continuously fed by nutrient enriched stream waters. In the case of the Rio Grande de Añasco River, it serves to drain nearly 50,000 ha of the Rio Grande de Añasco (RGA) watershed which is an important socio-economic natural resource, is an important scenic attraction, and eventually reaches an important ecological area which is the Mayagüez Bay watershed.

To address this issue, hydrologic discharges, nutrient concentrations, sediment, biological indicators and other water-quality parameters were evaluated at approximately 15-day intervals in five rural sub watersheds (Miraflores, Cerro Gordo, Cerrote, Chamorro, and Guaba), without known point sources of pollution within the Rio Grande de Añasco (RGA) watershed in western Puerto Rico from May 2002 to December 2003. A detailed geographical information system that described land use, soils, and hydrology was developed. The agricultural land areas ranged from 2.9 to 20% and urban/suburban areas ranged from 0.6 to 11.5 %. Secondary forest covered the majority of the land-areas studied. Geometric means of total coliform counts, *E. coli* and *Enterococcus spp*. bacteria within sub watersheds ranged from 34,135 to 85,921, 110 to 531 and 728 and 1842 cfu/100 mL, respectively. Total coliform bacterial concentrations increased from the watershed outlet to upstream areas. Bacterial transport was strongly associated with suspended sediments and weakly with hydrologic flow and nutrients in these sub watersheds. Resuspension of bottom sediments during runoff events, may serve as a mechanism for coliform and *Enterococcus* bacteria transport to the water column. The *Enterococcus* species detected indicate that the most probable origin of contamination are humans, animals, herbivores, and poultry.

Streamwater chlorophyll-a values were positively correlated to TKN (r=0.180), and negatively correlated to DP (r=-0.351), and DIN (r = -0.377). The stronger relationship of chlorophyll-a with dissolved nutrient constituents suggests that these nutrient fractions are readily available and are being actively utilized by primary producers within the streamwater column. There were no clear temporal patterns with regards to nutrient or sediment concentrations within sub watersheds. The sub watershed with the greatest agricultural land area also was associated with greater sediment concentrations and loads but not necessarily with nutrient concentrations nor loads.

Quantitative associations among land uses within the watersheds and nutrient concentrations and chlorophyll values could not be ascertained, because of the multiple land-uses interacting within the watershed. Empirical multiple regression models were developed that allowed prediction of in-stream concentrations based on changes in land-use proportions and allowed for evaluation of possible land-use scenarios on stream water quality. Conversion of 10% of the agricultural land area to suburban use presented the largest change (nearly 60 % increase) in TP and DP concentrations.

Water samples corresponding to high runoff events (storm) were collected with automated storm event samplers from two sub watersheds (Miraflores and Cerro Gordo) and analyzed for nutrients. Annual synthetic runoff hydrographs were generated using the HEC-HMS (Hydrologic Simulation Program), and the Curve Number Method of USDA-NRCS. The annual flow data for all sub-watersheds were separated into base-flow and runoff. Correlation analysis related mean daily volume and nutrient loads. Annual nutrient loads were calculated based on a daily time-series integration. Annual TP yields (kg/ha) were 0.57, 0.29, 0.37, 0.51, and 0.16 for Miraflores, Cerro Gordo, Cerrote, Chamorro, and Guaba, respectively.   Annual TKN yields (kg/ha) were 7.03, 4.0, 0.56, 0.30, and 0.70 for Miraflores, Cerro Gordo, Cerrote, Chamorro, and Guaba, respectively. These values may be underestimated for sub watersheds in which storm events were not characterized (Cerrote, Chamorro, and Guaba).

The in-stream nutrient concentrations observed in this study, suggest that rural sub watersheds may be experiencing significant anthropogenic influence associated with unsewered communities, and sediment movement associated with agricultural land preparation and land construction. A reference state is not available to classify the degree of nutrient impact in the streams associate with these rural sub watersheds, except preliminary nutrient criteria for lakes that have been suggested by Martínez, 2003 and Martínez et al., 2004). The TKN and TP concentrations quantified in the rural sub watersheds were in the high range of suggested values (15 to 50 µg TP/L), and should serve as an indicator that steps should be taken towards reduction in nutrient source loadings. The nutrient impact to the RGA main tributary, may be reduced due to high sediment concentrations that have been documented in the river which can serve as nutrient sinks. Further work should be directed towards ascertaining quantitative nutrient loadings from specific land uses and to assess the nutrient dynamics in the water-column associated with base-flow and runoff events within the RGA main tributary.

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1.0 GENERAL INTRODUCTION

Nutrient over-enrichment of surface waters is a major source of water pollution in the United States, where approximately half of the waters reported by the States to be impaired are attributed to excess nutrients and related to biological growth (Parry, 1998; Daniel et al., 1998). Similar problems occur in Puerto Rico although less quantitative data is available to adequately assess the problem. Vachier (1994) suggested that as of 1993, sediments and the presence of high fecal coliform and streptococcal bacteria were the main surface water quality impairments. A recent analysis of water quality in the Río Cibuco watershed by Horsley and Witten, Inc. (2002), found that fecal coliform bacterial counts exceed the water quality standard limit of 2,000 CFU/100mL in 43% of the water samples taken in the mainstem of the river during the years 1990 to 2000. Sotomayor et al. (2001) summarized trends in total phosphorus (TP) concentrations over an eight year period for major rivers in Puerto Rico and found that TP concentrations in the 25 to 75 interquartile ranged from 0.04 to 0.29 mg P/L, with mean and median values of 0.30 and 0.09 mg P/L, respectively. In this study, there were strong correlations between TP and the presence of fecal coliform bacteria, fecal streptococcal bacteria, and total Kjeldahl nitrogen. Ramos-Ginés (1997) quantified mean TP concentrations greater than 0.1 mg P/L during both low and high flow events entering an eutrophic Lake in Central Puerto Rico which were due to a combination of point and non-point sources.

The Puerto Rico Environmental Quality Board (EQB) has included 60 water-bodies (all but one corresponding to rivers) in the list of impaired waters for Puerto Rico (303(d) list) (JCA, 2002). The reduction contrasts with the 199 water bodies reported in 1998 by USEPA (USEPA, 2002) because inclusion then was based solely on the suspected impact of point-sources of pollution. In the 2002 303(d) list, 66% of the river miles evaluated were negatively impacted with regards to one or more water quality parameters. Of the 102 watersheds in Puerto Rico, the Rio Grande de Añasco (RGA) watershed is of prime importance because it is a potable water source and has a strong influence on the water quality of the Mayagüez Bay. The RGA watershed also called the Mayagüez Bay watershed is one of the largest in Puerto Rico and lies within the municipalities of Adjuntas, Lares, San Sebastian, Maricao, Las Marías, Mayagüez and Añasco with a catchment area of about 48,130 ha (USGS, 1998). The Añasco River is born at elevation 1,204 m (3,950 ft) near Monte Guilarte and flows westward for 74 km to discharge into the Añasco/Mayagüez Bay. The major tributaries of this river are: Río Daguey, Río Humatas, Río Canas, Río Casei, Río Arenas, Río Mayagüecillo, Río Guaba, Río Prieto, and Río Blanco. Possible major sources of pollution include land disposal of wastewater systems, industrial point sources, and agricultural activities (JCA, 2002).

Our research group has conducted preliminary assessment of the water-quality status of lakes in Puerto Rico (Martínez, 2002), and has described potential contaminant sources, nutrient concentration dynamics and nutrient export in sub watersheds of the Rio Grande de Arecibo watershed (Sotomayor et al., 2004). Results suggest that current water quality standard for phosphorus (P) in Puerto Rico of 1000 μg/L (JCA, 1990), greatly underestimates the effects of nutrients on aquatic biomass growth and does not relate to numeric nutrient criteria developed in other ecoregions of the continental U.S. Phosphorus in the water column appears to be a limiting factor for primary productivity in many surface waters of Puerto Rico (Martínez, 2003). A value of 19.3 μg/L of total dissolved P could be used as an upper limit to assign pristine status to lakes in Puerto Rico, and a range of total dissolved P of 40 to 60 μg/L could be indicative of lake eutrophication. Historical total P concentrations of the Añasco River exceed these P concentrations in approximately 30% of sampling occasions (Sotomayor et al., unpublished information).

The transport of N and P to surface waters can occur via surface runoff and erosion from urban, rural, and agricultural lands. Seepage from septic tanks, and direct discharges from waste-water treatment facilities also contribute to nutrient loadings. The different processes governing P availability in soil and water (Frossard et al., 2000) make the identification of source areas difficult, yet studies done at a small enough scale with well defined land uses can serve to better identify non-point sources of pollution. Correlations between nutrient concentrations and bacterial indicators suggests biological sources of contamination to the water column.

In this research study we have identified the land-use distribution in five rural sub watersheds within the Rio Grande de Añasco watershed. Within the streams draining the sub watersheds we have evaluated the dynamics of bacterial, sediment, and nutrient concentrations and have quantified nutrient and sediment loads. To identify probable factors influencing nutrient concentrations and to determine the relative magnitude of point and non-point sources, trends in water-quality parameters were related to land-use, soil test P information, hydrologic discharge, and known point inputs. An empirical model that describes the risk of off-field transport from agricultural land areas was applied to assess the relative importance of agricultural activities and other land-uses as potential contributors of P to surface waters in this area. Finally, systematic and detailed evaluations relating hydrologic discharge and nutrient and bacterial concentrations during base-flow and storm events were performed.

2.0 OVERALL PROJECT OBJECTIVES

The objectives of this project were to:

- Quantify seasonal and annual nutrient, sediment, and bacterial concentrations and loads to sub watershed outlets during base-flow and storm events
- Characterize land-use factors influencing nutrient loads
- Assess the relative non-point source nutrient contribution to surface waters

3.0 MICROBIOLOGICAL SURFACE-WATER QUALITY OF THE RIO GRANDE DE AÑASCO WATERSHED IN WESTERN PUERTO RICO

3.1. SPECIFIC OBJECTIVES

- Quantify trends and concentrations of contaminant bacterial indicators in surface waters draining from sub watersheds of the Río Grande de Añasco
- Isolate and identify Coliform and Enterococcus groups

- Identify possible sources of origin of contaminant bacterial indicators

3.2. METHODOLOGY

Site description and geographical information system (GIS)

Five sub-watersheds within the RGA watershed were selected on the basis of: (i) 1977 land-use map (ii) accessibility to roadways, (iii) land use homogeneity (iv) aerial photography and (v) personal visits. The names, locations and type of water sample collected are shown in Table 3.1. Watershed and sub-watershed boundaries of the Rio Grande de Añasco watershed were delineated using the Watershed Modeling System (WMS ver. 6.1, EMS-I) (EMS, Utah) software from USGS "Digital Elevation Model Maps" (DEM) (1:20,000 scale). Boundaries for the corresponding points of interest were drawn using the TOPAZ subroutine within WMS.

Updated land use classification within the sub watersheds was performed by digitizing 1997 digital orthoquadrangle (DOQ) photographs for each of the sub watersheds. Using WMS 6.1, classification polygons with their respective attributes were drawn using the USGS classification system (Anderson et al. 1976 and http://landcover.usgs.gov/classes.html). Major land use groups included agricultural cropland (Agriculture), secondary forest (Forest), urban/sub-urban (Urban), herbaceous rangeland (Rangeland), and fertilized pasture (Pasture), the latter which included pasture-land used for cattle raising and meat production. This classification differs from that assigned to the one in the Rio Grande de Arecibo basin in which "Pasture land-use" was improved pasture used for dairy production which was amended with sludge from anaerobic waste-holding lagoons or fertilized with inorganic fertilizers. In the watersheds studied there is no official nor anecdotal evidence of pasture-land sprayed with organic waste so that the pasture-land was that which was used for grazing animals and which received minimal fertilizer input.

The boundaries of each catchment and polygon attributes were exported to ArcView (Environmental Systems Research Institute, 1994) to process along with other coverages of the GIS. The total area occupying urban or suburban was estimated. Buffer areas corresponding to"riparian zones with potential as a source of contamination from household waste-water discharges" (Rodríguez-Martínez, et al., 2001), were identified because the can increase the vulnerability of surface water contamination. The setback distance was established as a 100 m distance from the stream area. A database was generated that delineated sub watersheds, identified agricultural activities and their aerial extent, identified farms, land use, housing units, soils, and geology.

Sampling procedures

Sampling consisted of grab samples collected from representative portions of the stream channel at depths of aproximately 6 to 10 inches below the surface, following procedures delineated by USGS (Wilde et al., 1998) and others (Haygarth and Edwards, 2000). Samples were collected using a Whirl-Pak sampling pole and placed in sterile 500-mL Whirl-Pak® polyethylene bags. Sample bags were closed, placed on ice in a closed cooler, and transported to the laboratory for processing within 24 hours of collection. The temperature was monitored during processing and arrival to the laboratory using a 500ml water bottle within the ice chest. Five separate bags were collected, one of which was used for bacterial enumeration. Samples were collected at approximately bi-weekly intervals from 8 May 2003 to 18 December 2004. Samples were usually analyzed and processed within 24-h after field collection, and only a few of the samples (<10 %) collected were analyzed within 6-h of after collection.

Bacterial enumeration:
Bacterial counts included total coliform, *E. coli*, and *Enterococcus* spp. For coliform analyses, a 0.1- to 5-ml aliquot from each water sample was used to inoculate bottles filled with Coliscan Easygel® medium (Micrology, Laboratories LLC, IN, USA). Bottles with the inoculum-water mixture were swirled evenly to ensure complete mixing and poured onto sterile petri dishes. Care was taken to ensure that the medium-inoculum mixture covered the entire dish. Duplicated plates were incubated at 35°C for 48 hours. This procedure enabled quantification of *Escherichia coli* and coliform bacteria (*Enterobacter* spp*., Klebsiella* spp*., and Citrobacter* spp*.*). All purple-blue colonies on the Coliscan plates were counted and reported as *E. coli.* All pink colonies were counted and reported as coliforms. Total coliforms bacterial counts were quantified as the sum of *E. coli* colonies plus coliform colonies on a per 100 mL of water sample.

For *Enterococci* bacterial enumeration, a membrane filter procedure on which direct bacterial counts on the surface of the membrane filter was used. Water samples (10 to 100 mL) were filtered using sterile membrane filter units (filter base, funnel and a 0.45µm membrane). Membrane filters were removed from the funnels and placed over the surface of the m-Enterococcus agar (DIFCO-0746-17-0) plates. Plates were incubated at 35°C for 48 hours. All rose-burgundy colonies growing over the membrane filter were counted as enterococci spp. Both coliform and *Enterococci* colonies were counted using the Quantity One® Quantitation Software and Gel Doc hardware by BIO-RAD® (ver. 4.2, 2000) using visual correction procedure to account for false positives and negatives.

Quality control procedures
Changes in fecal coliform bacterial population as a function of time were analyzed in quadruplicate field samples at 2, 6, and 24 hours after collection on two occasions (sampling dates). Results demonstrate that there was no significant effect of time on bacterial numbers (data not shown). Quality control procedures included running laboratory blanks and field blanks within selected samplings.

Sources of bacteria to the water column
Studies by Jolley (2003), have shown that aquatic and marine bottom sediments act as reservoirs for both fecal coliform and *E. coli* bacteria. We compared total coliform and *E. coli* levels in surface water and bottom sediments within the Cerro Gordo watershed. The surface water sample consisted of a grab sample and was placed on ice for transportation to the laboratory as described above. A bottom sediment sample was taken directly below the surface water. Surface sediment material (0-10 cm) was gently

scraped and added to sterile-glass sampling bottles. The bottles were transported on ice to the laboratory. Each surface water sample was analyzed for indicator bacteria as described previously. Bottom sediment samples were shaken (by hand) for one minute and the supernatant sampled immediately for indicator bacteria. A preliminary experiment revealed that extraction efficiency was similar using sodium pyrophosphate (5%) and distilled water (data not shown). The sediment volume was measured by allowing the sediment to settle for 24 hrs in a graduated cylinder. Sediment volumes include the sediment and its interstitial water. Bacterial numbers were calculated from the resuspendable sediment.

Sampling transects were established in the Miraflores sub watershed to perform empirical source tracking for nutrients and fecal coliform bacterial counts. Sampling was performed on 5 March 2003, 15 June 2003, and 20 April 2004. Sampling was initiated at the point of interest of the sub watershed and at pre-selected points within the sub watershed. Sampling points were selected based land-use information of the watershed. Grab samples were collected, transported on ice to the laboratory and analyzed for nutrients and bacterial counts as specified previously.

Bacterial identification

Bacterial colonies from Coliscan Easygel plates and filtration membranes from m-Enterococcus agar, were selected at random. Colonies were isolated and inoculated on Tryptic Soy Agar (TSA) and incubated at 26°C for 24 hours. Colonies were tested for gram stains to confirm purity. Pure bacteria isolations were inoculated in Biolog Universal Growth Medium (BUG) + sheep blood and incubated at 26°C for 24 h. Bacterial suspensions were performed in the inoculation fluid and inoculated in 96 wells microplates, especially formulated with 95 biochemical tests for gram positive or negative bacteria. Microplates were incubated at 26°C for 24 h. Readings were performed 4h and 24 h after inoculation with a microplate reader complemented with a MicroLog (Release 4.2) software and Database (Release 6.01) for final identification (BIOLOG, 2001).

Other analysis

Water samples collected were also analyzed for other water-quality parameters such as suspended solids (residue, non-filterable) (EPA method 160.2), pH (EPA method 150.1), electrical conductivity (EPA method 120.1), total kjeldhal nitrogen (EPA method 351.2) and chlorophyll content (EPA method 445.0), dissolved reactive P and total reactive P (EPA method 365.2) (USEPA, 1999). *In situ* measurements included pH, electrical conductivity using a YSI water-quality meter and instantaneous stream-flow (See section 4.0).

Statistical analysis

An analysis of variance was used to compare means among sub-watersheds considering dates as a blocking effect. Means separation was performed using Tukeys least significant difference test. All bacterial indicator parameters were $\log_{10}$ transformed prior to analysis. Frequency distributions were performed using PROC

Univariate of SAS. Data were analyzed using SAS (Statistical Analysis System Corp.). Correlation analysis was performed using Pearson's correlation procedure.

3.3. RESULTS AND DISCUSSION

Land use description

Sub watershed areas ranged from 224 to 1320 ha, with Miraflores having the smallest area and Guaba having the largest area (Table 3.2). The combined total area of the sub watersheds was 2,949 ha and accounts for 5.6 % of the total land area of the Rio Grande de Añasco watershed. The sub watershed with the largest proportion of land area under suburban (which includes unsewered households and structures) development was Miraflores and the others had land areas ranging from 1.1 to 2.9%. Housing units ranged from 435 to 975 dwellings per sub watershed. Cerro Gordo had the largest land area under agricultural production (Banana, Coffee, Citrus, Plantain, Yam, and other commodities). None of the sub watersheds had pasture that received organic or intensive use of fertilizers (Pasture 1). The Cerro Gordo sub watershed had the largest land area in which pasture was grown for beef-cattle feed which apparently received minor inorganic fertilizer and/or organic waste from grazing animals. Secondary forests and herbaceous rangeland occupied the largest proportional land area of all the sub watersheds. The number of farms within each sub watershed were 25, 24, 32, 16, and 20 for the Miraflores, Cerro Gordo, Cerrote, Chamorro, and Guaba sub watersheds, respectively (Sotomayor et al., unpublished data).

Bacterial indicator patterns

Frequency distribution of total Coliform bacteria, *E. coli*, and *Enterococcus sp*. counts are presented in Figure 3.2. Geometric means of total coliform counts within sub watersheds ranged from 34,135 to 85,921. Sub watersheds Miraflores and Cerro Gordo presented significantly higher values than the other three sub watersheds. Geometric means of *E. Coli* and *Enterococcus spp*. bacteria ranged from 110 to 531 and 728 and 1842 cfu/100 mL. *Enterococcus* values for the Miraflores sub watershed was greater than Miraflores, Cerrote and Guaba but was similar as compared to Cerro Gordo. A similar pattern occurred for *E. Coli* numbers.

The sanitary quality standard for total Coliform bacteria concentrations is based on a geometric mean of 10,000 cfu/100 mL for five sequential samples (PREQB, 1990). Methodology differences preclude making statistical comparisons with PREQB limits, because of bacterial growth preferences among media types and other conditions of the microbiological assay. Nevertheless, the values demonstrate the consistent presence of total coliforms, *E. coli* and *Enterococcus* bacteria throughout the 18-month study period. Although *E. Coli* and *Enterococcus spp*. bacteria are not pathogenic, these have been correlated to the presence of waterborne, infectious disease-causing organisms present in wastes from warm-blooded animals including humans. According the local EQB these type of waters cannot contain any pathogenic organism as found in these waters.

Relationship with other studies

Various sources of information point to the consistent presence of indicator bacteria in tropical and subtropical waters. Colón-Guzman and Norát-Ramírez (2000), quantified total coliforms and *Enterococcus* in the Mayagüez Bay and Rio Grande de Añasco Bay during 1995 to 1996. Total coliforms ranged from 9,009 to 12,508 and Enterococcus bacteria ranged from 485 to 2,686 cfu/100 mL. Concentration of these and other indicator bacteria were found to be higher in areas that were influenced by waste-water treatment plants. In Rio Mameyes watershed (El Yunque), Hazen and Aranda (1981) quantified monthly fecal coliform bacteria counts that ranged from 100 to 10,000 cfu/100 mL. The authors suggested that human waste probably contributed to the higher densities of bacteria found in the lower parts of the watershed. In a follow-up study, Carrillo et al., 1985) found higher densities of bacterial indicators than would be found in comparable oligotrophic temperate streams, suggesting that fecal coliforms and E. coli were un-reliable as indicators of contamination in tropical environments. Rodriguez-Martínez et al., (2001) found that only 52% of stream segments analyzed which drained into Lago La Plata within municipality of Comerio met PREQB standards with regards to fecal coliform counts. They observed decreases in bacterial numbers with increases in household waste-water discharges from commercial and residential areas, where detergents and other chemicals may render bacteria incapable of survival and growth.

Relationship with water-quality parameters

All bacterial indicators were positively correlated to each other (Table 3.3 and Figure 3.3). The correlation coefficient values among bacterial indicators are similar as that found by Sotomayor et al, (2004) in five rural sub watersheds in north-central, Puerto Rico, and suggests that bacterial inputs, sources and survival were similar at these sub watersheds. All bacterial indicators were negatively correlated to electrical conductivity and pH. All bacterial indicators were positively correlated to the presence of suspended sediments and weakly to hydrologic flow. The relationship among indicator bacteria and nutrients was mixed. For example, all three indicator bacteria were positively correlated to dissolved P concentrations, but only *Enterococcus* were correlated to total P. Total coliforms and *Enterococcus* were correlated to TKN. The weak correlation with chlorophyll a should be viewed with caution as a few high values in chlorophyll a concentrations may have positively influenced the regression. The results demonstrate that bacterial transport is strongly associated with suspended sediments and weakly with hydrologic flow and nutrients in these sub watersheds.

Colón-Guzman and Norát-Ramírez (2000) found a significant positive relationship between total coliforms and Enterococcus and sediment concentrations and hydrologic flow at various monitoring stations of Mayagüez bay. Densities of fecal coliform bacteria were positively correlated with temperature, nitrates, phosphates, and chlorophyll a concentration (Hazen and Aranda, 1981). They concluded that nutrient supplying phytoplankton are the most important factor controlling the abundance of bacteria in a tropical watershed in Puerto Rico.

Sources of bacteria to the water column

Fecal contamination from urban areas may include illegal discharge of sewage to storm-water drains, malfunction of sanitary sewer ejectors, clogged and leaking sewage pipes. Potential contaminant sources from unsewered rural communities may include gray water discharge (which includes all waste water from household uses except sanitary wastes), septic tank leakage, unfenced livestock, runoff from livestock pens, and seepage from pits containing animal wastes (Rodríguez-Martínez, et al., 2001). The relative contribution from these sources increase in areas near the stream courses and has been designated as “riparian zones with potential as a source of contamination from household waste-water discharges” (Rodríguez-Martínez, et al., 2001), and can be used as an initial estimate to delimit potential sources of contamination to streams from unsewered communities.

The sampling transect used to evaluate sources of bacteria to Miraflores sub watershed is shown in Figures 3.5 and 3.6. In general, total coliform bacteria concentrations increased upstream from the point of interest. Except for the 5 March 2003 sampling concentrations peaked to the highest values at approximately between 500 and 600 meters from the point of interest coincident with the urban area (high and low density residential). An extremely low hydrologic flow was observed near the last sampling point on 5 March 2003, which suggests that groundwater contains contaminant bacterial indicators. Groundwater recharge during base-flow and low-flow events may possibly be contributing bacteria to the stream channel with dilution accounting for the observed reduction in concentrations.

Contamination sources that affect surface water-quality during base-flow conditions are distinct for urban and suburban areas. It is generalized that point sources provide significant proportion of total loads during base-flow and the inverse occurs for non-point sources (Horseley and Witten, Inc., 2002). Also, water-quality data collected in Puerto Rico shows that the best sanitary quality conditions occur at or near base-flow conditions when the water in stream courses is primarily derived from ground-water discharge (Rodriguez-Martínez, et al., 2001). Although, a small percentage of our samples were not at calculated base-flow (see section 5) most of the values were within conditions in which a specific runoff event was not immediately occurring.

The agricultural enterprises most likely to impact the sanitary quality of streams are poultry and broiler facilities, pig raising and breeding, and dairy and beef cattle production. It is estimated that the total head of dairy and beef cattle in the five sub watersheds is not substantial, because of the steep geomorphologic conditions of the sites. Through interviews with USDA-NRCS personnel (L. Nieves, Personal communication) and University of Puerto Rico Extension Agents (Añasco and Las Marías municipalities) we have no reports of formal or informal operations of pig raising-breeding, and poultry and broiler production within the sub watersheds.

Total coliform numbers were significantly higher in stream sediment as compared to the water column (Figure 3.7). Total coliforms were 2 $\log_{10}$ units and *Enterococcus* bacterial numbers were 1 $\log_{10}$ unit higher in sediment than in the water column. The results are in agreement with those found by Jolley (2003) and substantiates the hypothesis that aquatic bottom sediments act as reservoirs for coliform bacteria. Free-living bacteria in the water column have slow settling rates except when they are adsorbed to sediment particles. Sediment interstitial water may provide an environment were bacteria can survive for longer periods due to a physical protection from environmental stresses and predators, surface sorption, and nutrient rich environment. Resuspension of bottom sediments during runoff events, may serve as a mechanism for coliform and *Enterococcus* bacteria transport to the water column. Other studies have documented that Coliforms and *E. coli* are persistent in tropical and subtropical environments (Desmarais et al., 2002; Scott et al., 2002). This mechanism may partly explain the ubiquity of fecal coliforms and *E. coli* in otherwise uncontaminated environments (Hazen and Aranda, 1981; Carrillo et al., 1985).

Identification and origin of enterococcus and coliforms

A total of thirty-two isolations of bacteria corresponding to *Enterococcus* were isolated (Table 3.4). Of these, *E. faecalis*, and *E. gallinarum* were the most prevalent. The species detected indicate that the most probable origin of contamination are humans, animals, herbivores, and poultry (Suarez-Pita, 2002). A total of twenty-seven isolations of total coliforms were performed throughout the study period corresponding to six genera and eleven species (Table 3.5). The bacteria *E. coli*, *E. vulnevis* and *Klebsiella pnemoniae* ss *pnemoniae* were the species with the greatest incidence among the coliforms. The observed ranking in genera was in the order: *Escherichia* (26%), *Enterobacter* (26%), *Klebsiella* (22%), *Raoutella* (11), *Citrobacter* (7%), *Pectobacterium* (7%).

Table 3.1. Sub watershed name, location and type of water sample collected within the Rio Grande de Añasco watershed study area from May 2002 to December 2003. Storm events were collected from August 2003 to December 2004.

Name of sub-watershed	Type of sample collected	Location
1. Miraflores	Grab / Storm event	18°11’2’’ N - 66°57’29’’ W
2. Cerro Gordo	Grab / Storm event	18°17’9’’ N - 67°04’9’’ W
3. Cerrote	Grab	18°13’42’’ N - 66°55’41’’ W
4. Chamorro	Grab	18°13’53’’ N - 66°55’9’’ W
5. Guaba	Grab	18°16’48’’ N - 67°05’27’’ W

NA denotes not applicable

Table 3.2. Sub watershed areas, land-use areas and proportion of each land use to total area within the Rio Grande de Añasco watershed.

	Miraflores		**Cerro Gordo**		**Cerrote**		**Chamorro**		**Guaba**	
Land use Type	Area (ha)	(%)	Area (ha)	(%)	Area (ha)	(%)	Area (ha)	(%)	Area (ha)	(%)
Urban	25.8	11.5	8.0	1.1	8.6	2.9	2.4	0.6	17.6	1.3
Agricultural	8.6	3.9	144.6	20.2	25.9	8.8	39.8	10.0	153.6	11.6
Rangeland	51.7	23.1	96.3	13.5	34.5	11.8	23.4	5.9	141.1	10.7
Forest	137.8	61.5	393.5	55.1	224.3	76.5	331.7	83.5	1007.7	76.3
Pasture	0.0	0.0	72.3	10.1	0.0	0.0	0.0	0.0	0.0	0.0
Total	224		714		293		397		1320	
Housing units (units.)	560		776		435		433		975	

Table 3.3 Pearson correlation coefficients among physical and chemical parameters and bacterial indicators in five sub watersheds of the RGA watershed. Only coefficients with *P*<0.05 are included.

Parameter	Total coliforms	*E. coli*	*Enterococcus*
Suspended sediments	0.631 (0.001)	0.538 (0.001)	0.559 (0.001)
Hydrologic flow	0.259 (0.001)	0.235 (0.003)	0.176 (0.02)
Dissolved P	0.205 (0.01)	0.156 (0.05)	0.252 (0.001)
Total P	NS	NS	0.209 (0.01)
TKN	0.217 (0.001)	NS	0.316 (0.002)
Clorophyll$_a$	0.193 (0.05)	0.247 (0.01)	NS
pH	-0.449 (0.001)	-0.302 (0.001)	-0.389 (0.001)
Electrical conductivity	-0.483 (0.001)	-0.382 (0.001)	-0.464 (0.001)
Temperature	NS	NS	NS
E. coli	0.641 (0.001)	-	-
Enterococcus spp.	0.720 (0.001)	0.609 (0.001)	-

Table 3.4 Identification from bacterial isolates and possible source of origin of two genera and eight species of Enterococcus

Enterococcus / 32 isolations	% of total	% / genera	Origin [1]
Enterococcus faecalis	41		humans, poultry
Enterococcus gallinarum	13		poultry
Enterococcus casseliflavus	9		humans, herbivores
Enterococcus flavescens	9		unknown
Enterococcus mundtii	9		herbívores
Enterococcus faecium	3		humans, herbivores poultry
E. sulfureus	3		unknown
Enterococcus spp.	3		unknown
		90	
Lactococcus gaevieae (=*Enterococcus*)	6		unknown
L. lactis ss. lactis (=*Enterococcus*)	3		unknown
		10	

[1] Suarez-Pita, M. (2002)

Table 3.5. Identification from bacterial isolates of six genera and eleven species of coliform bacteria.

Coliforms / 27 isolations	%	% / genera
Citrobacter freundii	3.7	
C. koseri	3.7	
		7
Enterobacter cloacae	11	
E. aerogenes	3.7	
E. amnigenus	3.7	
E. cancerogenes	3.7	
Enterobacter sp	3.7	
		26
Escherichia coli	18.5	
Escherichia vulnevis	7	
		26
Klebsiella pnemoniae ss *pnemoniae*	18.5	
K. pnemoniae	3.7	
		22
Raoutella terrigena (=*Klebsiella*)	11	11
Pectobacterium caratovorum (=*Enterobacter*)	7	7

Figure 3.1. The Rio Grande de Añasco (RGA) watershed and sub watersheds being studied.

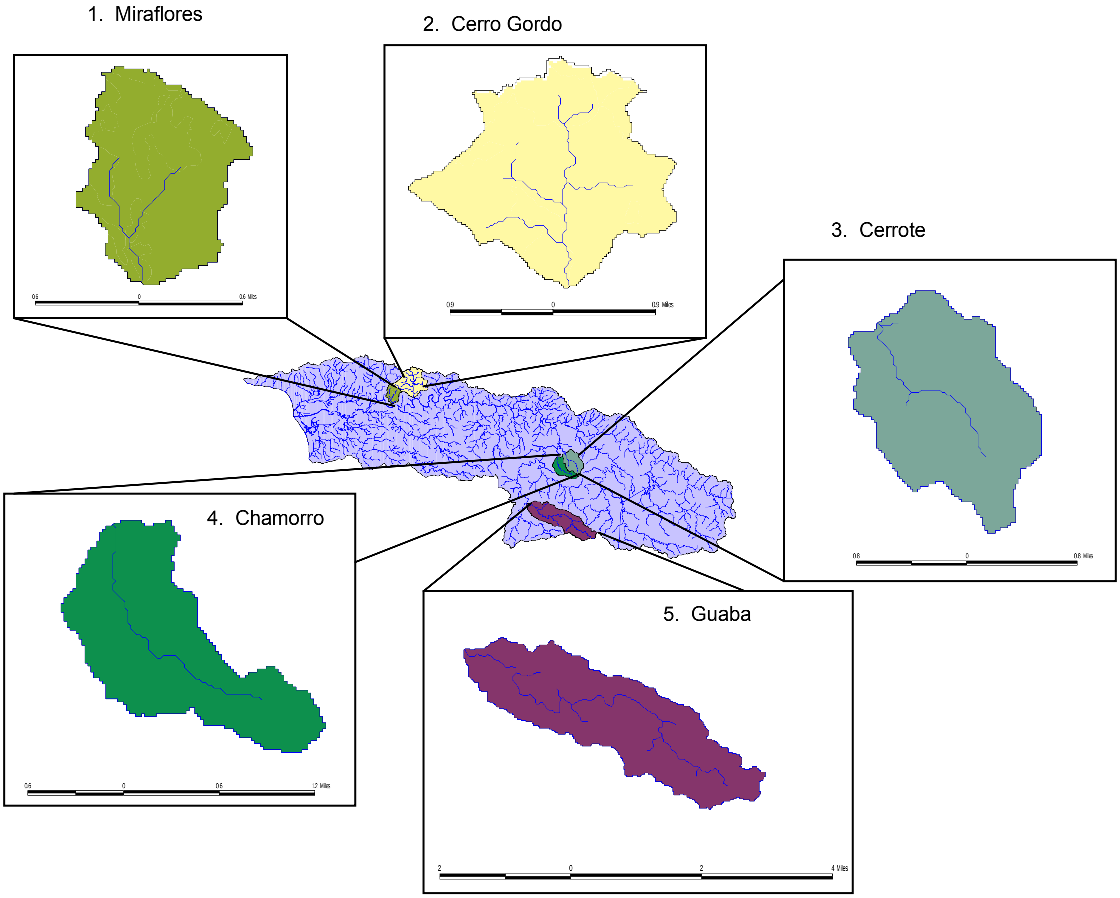


Figure 3.2. Frequency distribution, as described by box plots, of total coliform, E. coli, and Enterococcus bacterial indicator counts in five sub watersheds of the RGA watershed. Boxes with different letters are significantly different at p<0.01.

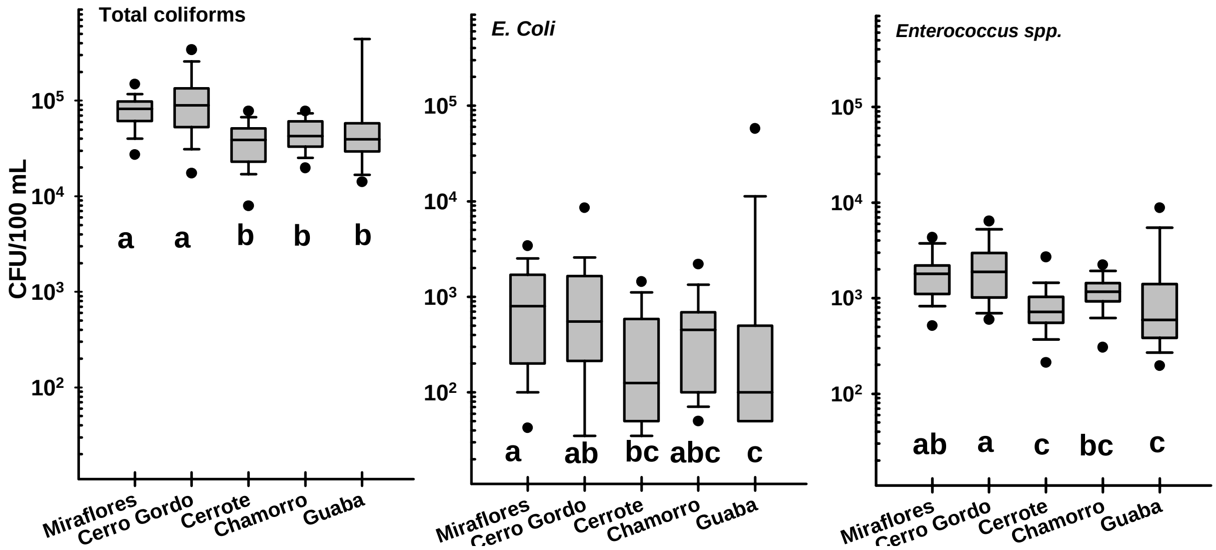


Figure 3.3. Relationships among bacterial indicator bacteria within sub watersheds of the RGA watershed.

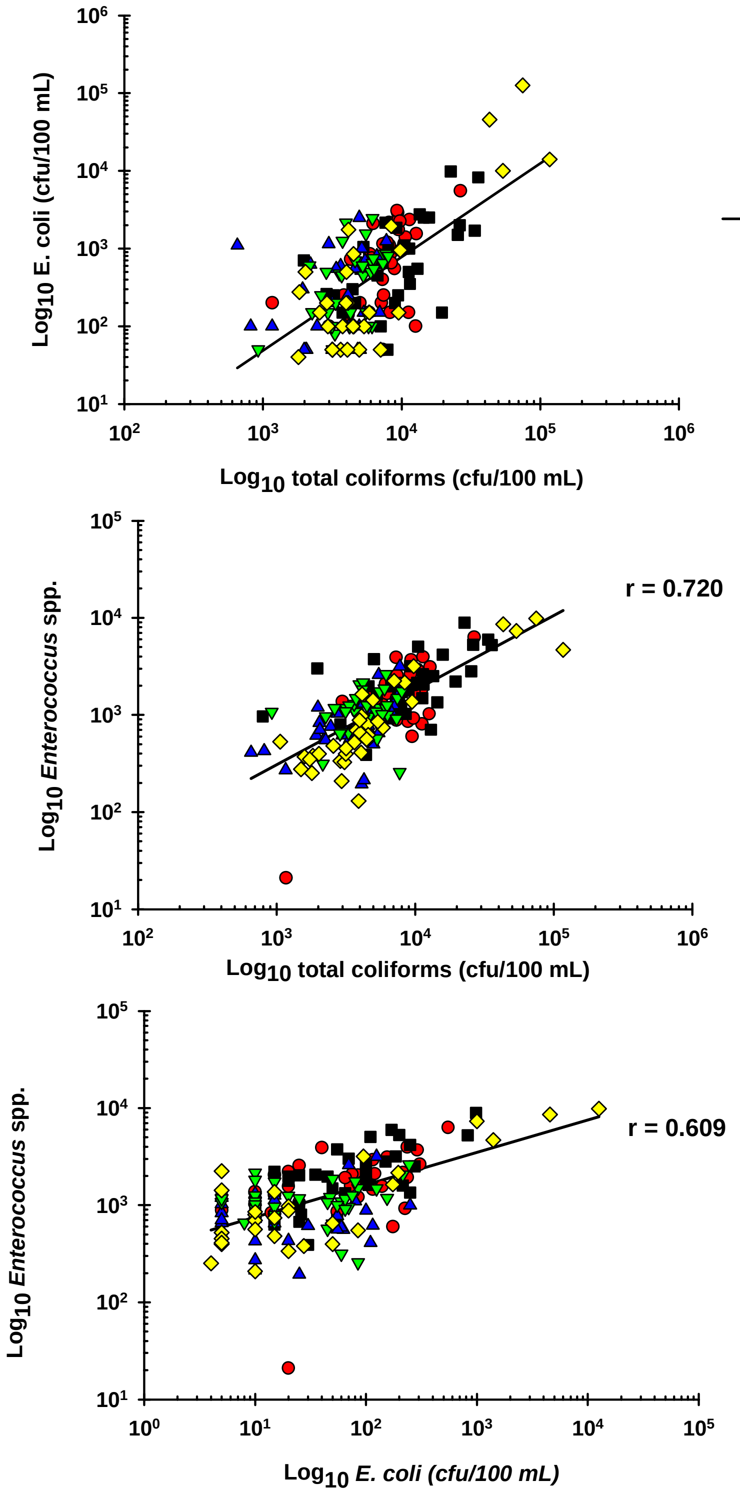


Figure 3.4. Relationships among bacterial indicator bacteria and suspended sediments within sub watersheds of the RGA watershed.

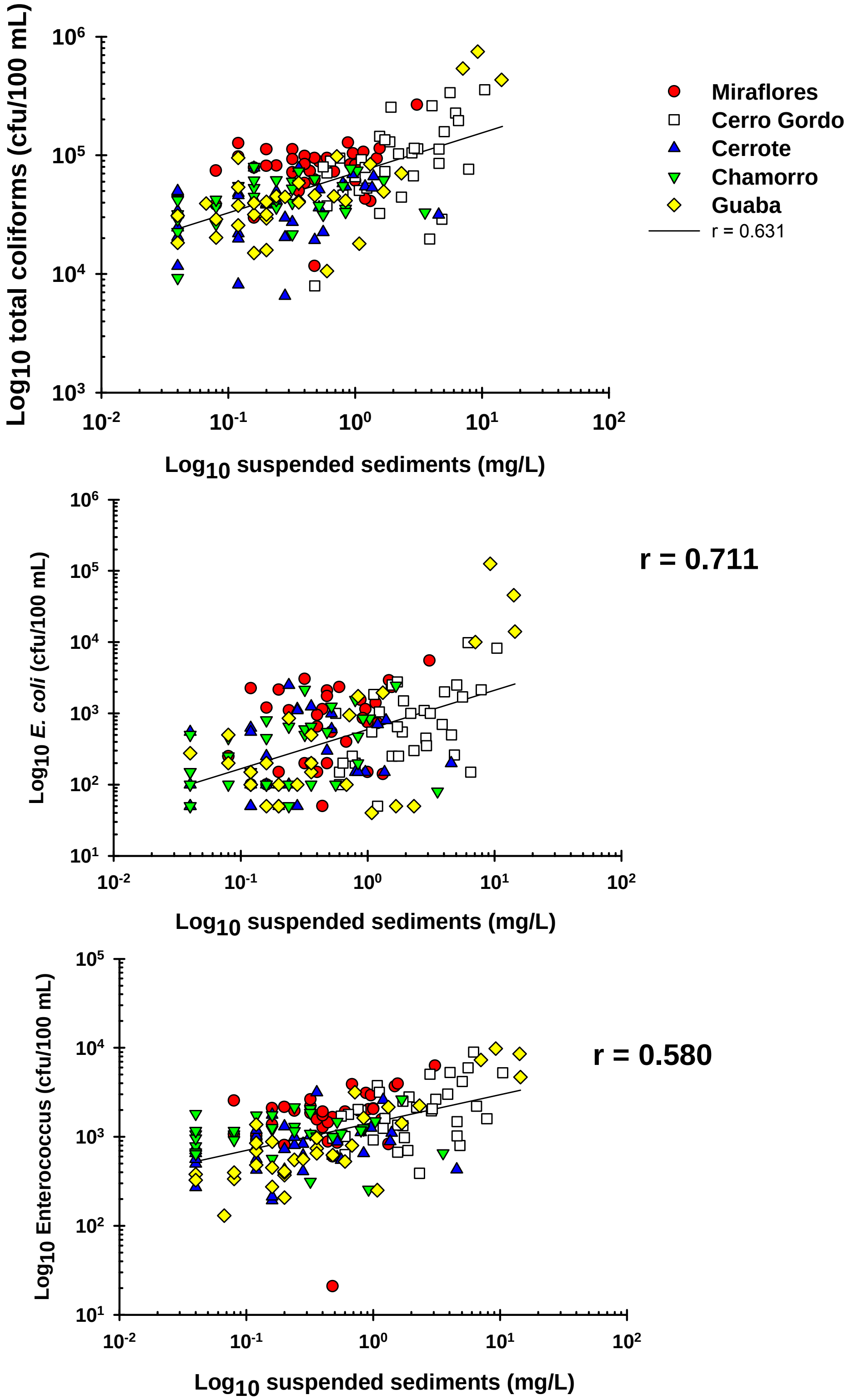


Figure 3.5. Land use distribution and location of sampling sites for bacterial analysis in Miraflores sub watershed.

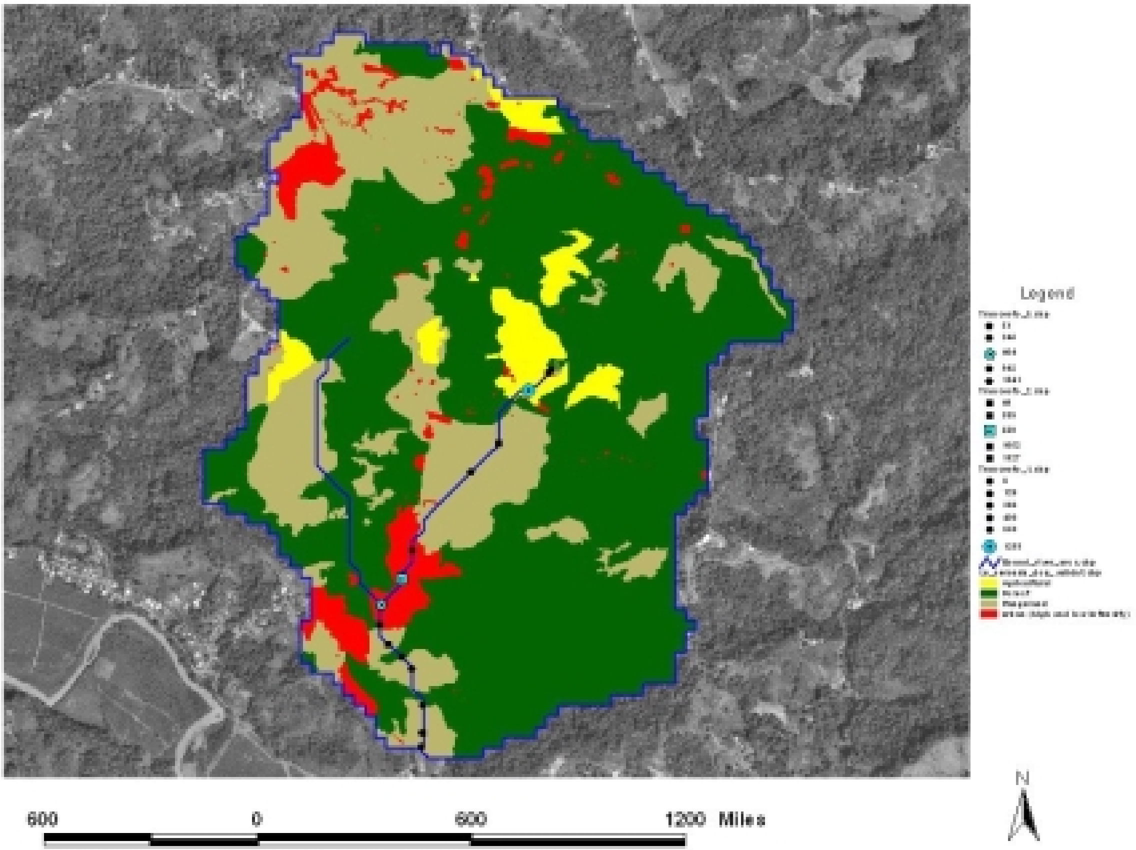


Figure 3.6. Total coliform counts as a function of distance from the point of interest in Miraflores sub watershed.

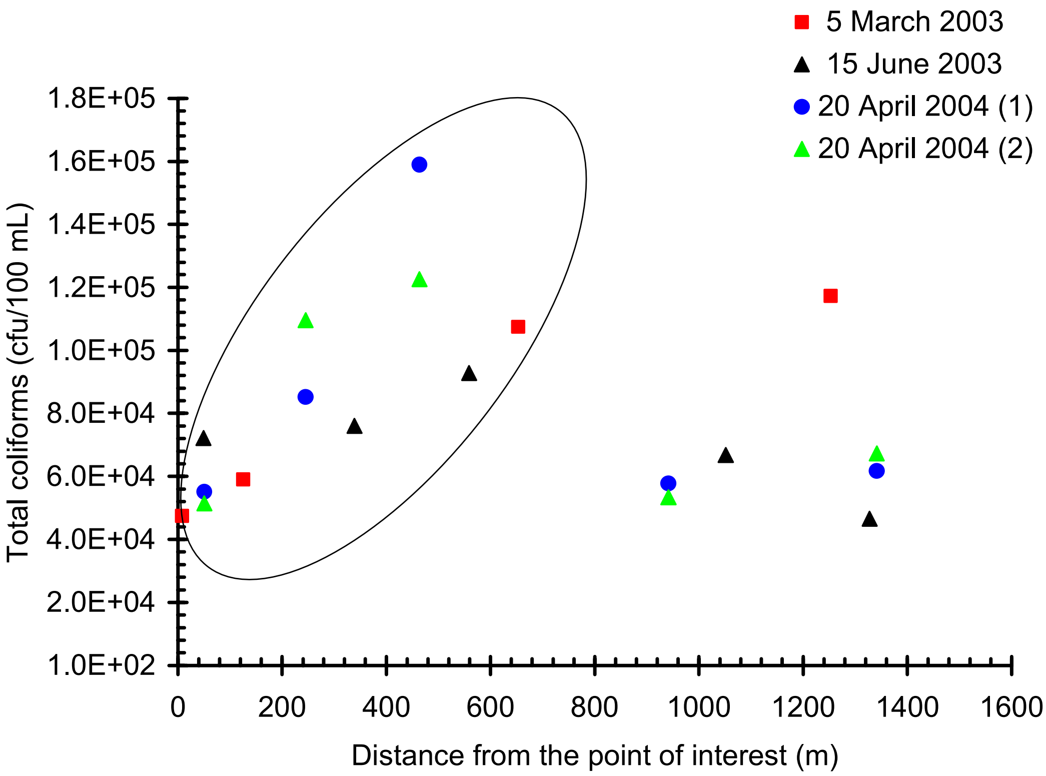
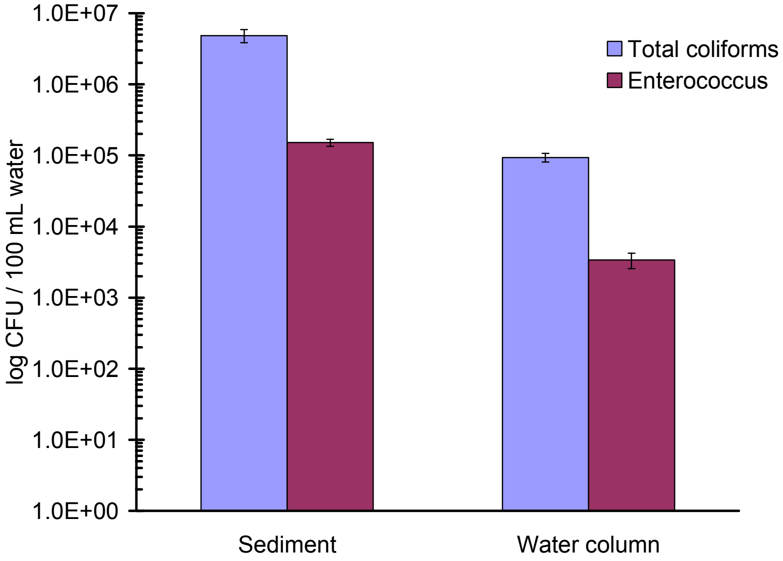


Figure 3.7. Total coliform and *Enterococcus spp*. bacterial concentrations in stream-water sediments and the water column.



4.0 NUTRIENT DYNAMICS AND EXPORTS WITHIN SUBWATERSHEDS OF THE RIO GRANDE DE AÑASCO WATERSHED

4.1. SPECIFIC OBJECTIVES

- Evaluate trends and quantify concentrations of causative (nitrogen and phosphorus) and response (chlorophyll-a) indicators in stream-waters draining from sub watersheds of the Río Grande de Añasco
- Describe the in-stream dynamics influencing causative and response indicators.
- Quantify nutrient loads and yields at sub watershed outlets

4.2. METHODOLOGY

Study area description

The Mayagüez Bay Watershed (also called Río Grande de Añasco [RGA]) watershed is one of the largest in Puerto Rico and lies within the municipalities of Adjuntas, Lares, San Sebastian, Maricao, Las Marías, Mayagüez and Añasco (USGS, 1998). Its east-west axis is aligned with the predominant weather patterns occurring in the Caribbean. The northern limit of the watershed is delineated by the Atalaya Mountains, where near the town of Añasco they merge with the dissected plateau remnants at slightly lower elevations. The Cordillera Central delineates eastward near the town of Adjuntas, and southeastern portions of the watershed rise south of the town of Maricao. Although elevations throughout the Cordillera Central range from 900 to 1200 m above mean sea level (mamsl) most of the peaks in the mountain ranges have elevations of between 300 to 340 mamsl (USDA-SCS, 1973).

All of the mountain masses have heavy precipitation on their north slopes as the trade winds from the northeast are forced to rise over them. Most of the upland areas exhibit rugged steep topography with frequent slopes ranging from 70 to 100 percent. On the south side of the watershed there are foothills with elevations ranging from 43 to 62 m above mean sea level which become progressively greater eastward with slopes varying from 25 to 45 percent.

The climate is characterized by warm, wet summers and warm but dry winters with progressively cooler temperatures occurring in the mountainous regions. The long term average annual rainfall at Mayagüez is approximately 220 cm; similar to the weighted average annual rainfall for the watershed of 236 cm (USDA-SCS, 1973). A wet season usually occurs from April through November and a drier season from December through March.

The Añasco River is born at elevation 1,204 m (3,950 ft) near Monte Guilarte and flows westward for 74 km to discharge into the Añasco/Mayagüez Bay. The major tributaries of this river are: Río Daguey, Río Humatas, Río Canas, Río Casei, Río Arenas, Río Mayagüecillo, Río Guaba, Río Prieto, and Río Blanco. Streams in the uplands carry a base flow at all times except during extreme drought. Some of the small streams in the Atalaya Mountains are classified as intermittent while those in the lowland are classified as perennial. Streams in the lowland are almost all modified channels (for agricultural purposes) or man-made ditches.

Of the total catchment area (52,278 ha), 48,130 ha are classified as mountainous and 4,148 ha are lowland (USDA-SCS, 1973). In 1973, more than 97% of the land area was either idle or planted to cropland that includes minor crops, coffee, and sugarcane (Table 1). Land use, specifically conversion from agricultural to urban land has changed significantly since then. As of 1973, approximately 50,000 people resided within the watershed (USDA-SCS, 1973). It is estimated that Río Grande de Añasco serves more than 150,000 people with drinking water (USDA-NRCS, 1998).

Soils in the uplands are mostly sub-lateritic, red, acid soils with a silty clay profiles. Soils in the bottomlands have developed from sediments of the upland soils. The bottomland soils have varying textures, but are mostly fine to moderately fine and vary in drainage properties from well to poorly drained (USDA-SCS, 1975).

Three main types of geologic groups predominate in the Río Grande de Añasco basin. Quarternary alluvium deposits predominate in the lower flood-plains and the river valleys. The northern part of the watershed including Atalaya mountains consist of Tertiary and Late Cretaceous volcanic and sedimentary rocks. In the eastern, central and southern parts of the watershed, Cretaceous sedimentary and volcanic rocks predominate (USDA-SCS, 1975).

Preparation of geographical information system (GIS)

Five sub-watersheds within the RGA watershed were selected on the basis of: (i) 1977 land-use map (ii) accessibility to roadways, (iii) land use homogeneity (iv) aerial photography and (v) personal visits. The names, locations and type of water sample collected are shown in Table 3.1. For each of the five sub watersheds, a database was created that identified geology, soil series distribution, location of farms, farm owners, crops grown, land-use distribution, housing units, K values of soils (erodibility potential), and P sorption capability distribution. Within the RGA watershed there is a daily rainfall and temperature record from stations at: Mayagüez Airport (Lat/Lon 18.15N 67.09W), San Sebastián (Lat/Lon 18.21 N 67.01W) and Maricao Fish Hatchery (Lat/Lon 18.10N 66.59W).

Digital copies of 1977 land-use maps, soil series, soil associations, and hydrologic soil grouping, were obtained from the Puerto Rico Planning Board. Later, a digital soils map was obtained from (http://ftw.nrcs.usda.gov/sssur_data.html). More specific and updated land use classification within the sub watersheds was performed by digitizing 1997 digital orthoquadrangle (DOQQ) photographs for each of the sub watersheds. Watershed and sub-watershed boundaries were delineated using the Watershed Modeling System (WMS ver. 6.1, EMS-I) (EMS, Utah) software from USGS "Digital Elevation Model Maps" (DEM) (1:20,000 scale). Boundaries for the corresponding points of interest were drawn using the TOPAZ subroutine within WMS.

Using WMS 6.1, classification polygons with their respective attributes were drawn using the USGS classification system (Anderson et al. 1976 and http://landcover.usgs.gov/classes.html). Major land use groups included agricultural cropland (Agriculture), secondary forest (Forest), urban/sub-urban (Urban), herbaceous rangeland (Rangeland), and fertilized pasture (Pasture). In these watersheds, we did not find official nor anecdotal evidence of pasture-land sprayed with organic waste so that the pasture-land was that which was used for grazing animals and which received minimal fertilizer input. Properties of each polygon were assigned after visual inspection of the area (ground truthing) and field data provided by local extension agents from UPRM, USDA-NRCS personnel and an undergraduate student (L. Págan). The boundaries of each catchment and polygon attributes were exported to ArcView (Environmental Systems Research Institute, 1994) to process along with other coverages of the GIS. Buffers of 30 m and 100 m around the stream channels in each sub watershed were created with ARCView, forming two spatial zones around each stream. The 30-m buffer includes land from 0 to 30 m from the stream and the 100-m buffer includes land from 0 to 100 m from the stream. Each land-use category was expressed as a proportion of the total land area at a given distance from the stream channel.

Land use description

Land use description of the areas are presented in Table 3.2. Polygons classified as agriculture included agricultural crops of human consumption, such as plantain (*Musa spp*.) coffee (*Coffea arabica*), and yam (*Dioscorea spp*.) and citrus (*Citrus spp*.) (Corvera-Gomringer, 2004). Polygons classified as secondary forest and herbaceous vegetation included pioneer species of low (up to 3 m height) medium (up to 10 m height) stands, resulting from the succession from agricultural land area. Polygons classified as pasture corresponded to nativized pastures that received minimum amounts of inorganic fertilizer and only organic waste directly excreted to the land area by grazing animals. We estimated that the number of grazing animals in the Cerro Gordo during the course of the study was < 80 animal units (1,200 lbs/animal unit). Polygons classified as urban corresponded to rural housing and their area of influence. This delimitation was based on direct observations from Digital Ortho Quad Quadrangle (DOQQ) photographs. These areas were characterized as not having a formal sewer system, so that their waste consisted of direct stream discharges or via septic systems.

Sampling procedures

Sampling consisted of grab samples collected from representative portions of the stream channel following procedures delineated by USGS (Wilde et al., 1998) and others (Haygarth and Edwards, 2000). Samples were collected using a Whirl-Pak sampling pole and placed in sterile 500-mL Whirl-Pak® polyethylene bags. Samples were closed and transported on ice in a closed cooler to the laboratory for processing within 24 hours of collection. The temperature was monitored during processing and arrival to the laboratory using a 500ml water bottle within the ice chest. Five separate bags were collected, one of which was used for bacterial enumeration (See section 3). Samples were collected at approximately bi-weekly intervals from 8 May 2003 to 18 December 2004.

Analysis performed

Temperature, pH, and electrical conductivity measurements were taken *in situ* at midchannel (at a depth of 15 cm from the water surface), with an YSI-63 Conductivity, Salinity and Temperature meter (Yellow Springs Instruments). Prior to sampling, the pre-calibrated instrument settings were corroborated using buffers and standard solutions.

Water velocity was measured with a Flow Probe Hand-held flowmeter (Forestry-Suppliers, Inc.), at each of the selected stream cross-sections. The stream at the sampling point was inspected from bank to bank, observing water velocity, width, depth distribution, accumulation of sediments and other debris. Channel depth (water column depth), and channel width was measured with a marking tape (tagline) at selected increments depending on observed discharge, and channel conditions. Homogeneity of the bottom channel was corroborated by measuring the water column depth at selected intervals within the channel. Usually three to five increments were selected. A diagram was drawn in the field data sheet that described the cross section. The flowmeter was inserted at middepth of each of the width increments of the water column and slowly moved vertically for a period of two minutes. An average reading corresponding to the time the instrument was left *in situ* was recorded. Stream flow for each of the increments was calculated from stream area (increment width x depth) and water velocity. Total stream flow (ft^3/s) was the weighted average of flow at each of the stream increments.

Samples were filtered within 24-hr of collection (usually on the same day). The filtrate was transferred to acid-washed 125 mL Nalgene bottles (Nalgene Company, Rochester, NY) and immediately stored frozen. Unfiltered samples were stored frozen in the same bags collected in the field.

Total suspended solids (hereafter referred to as suspended sediments) were filtered through glass fiber filter (Whatman GF/F) (EPA method 160.1. Total P was analyzed following EPA method 365.4. Phosphorus quantification was made using a Bran+Luebbe Autoanalyzer. Specific instrument specifications, reagent make-up, and additional details are outlined in Bran+Lubbe AutoAnalyzer Applications (Method no. 696A-82W). Standards encompassing the linear range were run before and after the run. Standards were prepared and run through the entire digestion process. Standard checks were run every 10 samples and had to conform to a relative standard deviation of 10%, or the set of samples were re-run. Duplicate blanks were run in every set of analyses by carrying the blanks through the entire digestion process. An analytical blank (0 ppm P) was included. Precision was estimated from 1 sample out of 20. Acceptance criteria is +/- 20%. Accuracy was determined from a spiked sample run for every 20 samples analyzed. Dissolved P was analyzed following EPA method 365.2. Phosphorus quantification was made manually using a UV-VIS spectrophotometer. Quality assurance procedures were similar to total P analysis. Total Kjeldahl nitrogen was analyzed using EPA method 351.2 and quantified using a Bran+Luebbe Autoanalyzer. Chlorophyll-a measurements were performed using EPA method 445.0 and quantified using a Turner model 10-AU Fluorometer (Application 998-9000). The instrument was calibrated using a standard chlorophyll values. Initial demonstration of performance was performed using lake waters and compared to values obtained using standard procedures with a UV-Vis spectrophotometer. At least one laboratory reagent blank was run with each sample batch.

Phosphorus load land-use relationships

Annual estimates of nutrient (TP, and DP) export at the point of interest of each sub watershed was performed using the following relationship:

$$\mathrm{Load}_{\mathrm{nutrient}} = \int_{\mathrm{Time}} Q_i C_i \, d\mathrm{t}$$

Where Q_i is the instantaneous hydrologic flow on a daily basis, or mean daily flow when available, C_i is the instantaneous nutrient concentration, *dt is* the time interval (approx. 15 days), integrated over a 365 day period. The export coefficient (EC) or yields (kg TP/ha) was obtained from:

EC (kg /ha/yr) = Load (kg) / sub watershed area (ha)

Nutrients are transported by base flow and storm events throughout the water year, and the methodology does not consider events occurring between samplings. Nevertheless, this approach has been used successfully in other areas because it homogenizes the complexity of land-use systems (Johnes, 1996; Hanrahan et al., 2001).

Statistical analyses

An analysis of variance was used to compare means among sub-watersheds considering dates as a blocking effect. Means separation was performed using Tukeys least significant difference test. Frequency distributions were performed using PROC Univariate of SAS. Data were analyzed using SAS (Statistical Analysis System Corp. Cary, NC).

Correlation analysis among TP, DP, TKN, DIN and other water-quality parameters were performed using Pearson's correlation procedure in SAS. Factors influencing nutrient concentrations were performed using multiple linear regressions using water quality parameters as independent variables and TP, DP, TKN, DIN, and Chl_a as dependent variables. Variable selection was performed using Stepwise method. The chosen model was verified that assumptions of normality, homogeneity of variance and lack of fit was met.

The proportions of land area corresponding to each of the land uses was utilized to examine the extent that these proportions influenced TP, DP, TKN annual loads (kg/watershed), yields and TP, DP, TKN and DIN mean annual concentrations. The land use proportions evaluated were Forest/Urban, Rangeland/Urban, Agriculture/Urban, Pasture/Urban, (Forest + Rangeland)/Urban, (Agriculture + Pasture)/Urban, Agriculture/Forest, (Agriculture + Pasture)/Forest, (Agriculture + Pasture)/(Forest + Rangeland). Similar ratios have been correlated to with nutrient concentrations in other studies (Castillo et al., 2000). A matrix of seventy correlations were calculated using all possible combinations. Significance was established when t_{alpha} was greater than calculated t_{crit} at P=0.1 and P=0.05.

Because individual land-use ratios may only predict a small proportion of the changes in the dependent variables, first-order least-squares multiple regression models were developed to relate land use to in-stream concentration of nutrients (TP, DP, TKN, and DIN) (Tufford et al., 1998) using SAS. We included data collected in a companion study which evaluated nutrient concentration changes and nutrient yields in five rural sub watersheds in north-central Puerto Rico (Sotomayor et al., 2004). The natural log of concentration data were regressed using the five land-use proportions in each sub watershed using the following model:

$$\hat{Y} = \beta_1X_1 + \beta_2X_2 + \beta_3X_3\ldots + \beta_nX_n$$

where X_i is the relative land use proportions of agriculture, forest, suburban, rangeland, pasture to the total sub watershed area; β_n are the estimated regression coefficients; and $\hat{Y}$ is the predicted nutrient concentration. Final values were expressed as the antilog values of predicted in-stream nutrient concentrations. The regression models for the ten sub watersheds were used to predict the impact of land-use change within all of the sub watesheds. The predicted concentrations were plotted as a function of the hypothetical scenario of changing 10% of the current land-use configuration as follows: forest to agricultural land use, rangeland to agricultural land use, agricultural to sub-urban land use, and forest to sub urban land use. The land use scenarios chosen were considered by our group as most likely to undergo land-use conversions in Puerto Rico. When the 10% land area of a particular land use was not available, we included in the regression model the maximum land area that was available.

4.3. RESULTS AND DISCUSSION

Water-quality concentrations and relationships among variables

Mean streamwater pH, electrical conductivity and temperature values ranged from 7.48 to 8.36, 153.3 to 225.9 dS/m and 23.4 to 24.4°C, respectively. Miraflores and Cerro Gordo sub watersheds had the lowest water pH mean values as compared to the other sub waterheds (Table 4.1). Cerro Gordo sub watershed had the lowest stream-water electrical conductivity mean values with no significant differences among the remainder sub watersheds. Although there were no large temperature differences among sub watersheds, there seems to be higher streamwater temperatures from June through October where more periphyton and phytoplankton are observed and higher chlorophyll-a concentrations occur. These general water-chemistry values are in accordance with other streamwater chemistry information being gathered in five other sub watersheds in the Rio Grande de Arecibo Puerto Rico (Sotomayor et al., 2004) and other published data for Puerto Rico (Díaz et al., 2002).

Hydrologic flow ranged from 0.015 to 6.3 m^3/s for all sub watersheds throughout the study period. Sub watersheds Cerro Gordo and Guaba had the highest flows (Table 4.2). For each sub watershed, hydrologic flow was at or near base-flow for only three of the thirty-six events sampled. The interquartile range (high and low values) of hydrologic flow for Miraflores, Cerro Gordo, Cerrote, Chamorro and Guaba was 0.030 to 0.081, 0.172 to 0.270, 0.098 to 0.188, 0.070 to 0.125, and 0.167 to 0.560 m^3/s, respectively. The sub watershed areas positively influenced mean annual (2002-2003) hydrologic flow, where the following equation was obtained:

$$\text{flow} = 2 \times 10^{-4} * \text{area} + 0.28; \; r^2 = 0.94$$

with the flow and area in m^3/s and hectares, respectively. A similar relationship was obtained for five sub watersheds within the Rio Grande de Arecibo watershed, and combining all ten sub watersheds yielded the relationship:

$$\log_{10}\text{flow} = 0.8852 * \log_{10}(\text{area}) - 3.0912; \; r^2 = 0.863$$

with the flow and area in m^3/s and hectares (from 152 to 9921 ha), respectively. Most of the instantaneous hydrologic flows measured were highly variable and exhibit extreme events that are the result of strong precipitation events during the bi-annual rainy season which occurs in Puerto Rico.

Suspended sediment concentrations ranged from undetectable (≤0.01 mg/L) to 145.2 mg/L. Cerro Gordo sub watershed is where the greatest land area dedicated to agricultural production occurs, and had greatest mean suspended sediment concentrations. It is expected that the same tendency, albeit at a greater magnitude occurs during runoff (storm) events (see Section 5.0 and Corvera-Gomringer, 2004). Considering all data, sediment transport was positively correlated to hydrologic flow (r=0.296) and TKN (r=0.303), and negatively correlated to pH (r = -0.293) and electrical conductivity (r = -0.380).

Detection limits for TP, DP, TKN, and DIN were 0.01, 0.01, 0.05, and 0.01 mg/L, respectively. Concentrations of TP, DP, TKN, and DIN ranged from 0.01 to 0.180, 0.01 to 0.161, 0.05 to 1.97, and 0.190 to 1.44 mg/L, respectively. There was no significant difference among sub watersheds with regards to TKN concentrations. Guaba sub watershed had the lowest TP and DP concentrations. The DP concentrations in Cerro Gordo and Miraflores where similar, Miraflores and Chamorro were similar, whereas Cerro Gordo and Cerrote were different. Concentrations of DIN were significantly higher for Guaba and Chamorro as compared to the other sub watersheds. There were no clear temporal patterns with regards to nutrient concentrations within sub watersheds.

Streamwater chlorophyll-a values ranged from 0.020 to 2.39 µg/L. Cerrote and Guaba sub waterheds had the greatest primary production (chlorophyll-a), yet these sub watersheds (specifically Guaba) had the lowest trends in nutrient (TP, DP, and TKN) concentrations. Streamwater chlorophyll-a values were positively correlated to TKN (r=0.180), and negatively correlated to DP (r=-0.351), and DIN (r = -0.377) (Table 4.3). The negative association and stronger relationship of chlorophyll-a with dissolved nutrient constituents suggests that these nutrient fractions are readily available and are being actively utilized by primary producers within the streamwater column. Regressions among chlorophyll-a and TP and TKN were positive and stronger when considering sub watersheds Miraflores, Cerro Gordo and Chamorro as a group (TP, r=0.253 and TKN, r=0.281) and Cerrote and Guaba (TP, r=0.247 and TKN, r=0.265) as another group.

There was a weak, though significant relationship between TP and DP concentrations. This is surprising considering that DP is a component of TP concentrations and that DP was nearly 90% of TP concentrations in streams of the Rio Grande de Arecibo watershed (Sotomayor et al., 2004). There was a significant negative correlation between hydrologic flow and DP and TP concentrations which suggests that dilution partially controlled the concentrations of these constituents in the water column, within the hydrologic flow ranges measured. This did not occur for DIN and suspended sediment concentrations as these relationships were positive.

The temporal trends in suspended sediment concentration exhibited sharp peaks at selected sampling intervals associated with increases in hydrologic discharge. The peaks were greatest in magnitude in Cerro Gordo and Guaba sub watersheds. Concentration peaks were lowest during the months of January through April.

Nutrient loads and export coefficient estimates

Monthly trends in suspended sediment loads (kg/time interval) did not demonstrate time periods of distinct months of greater or lower loads, because there was much variability across watersheds. This also occurred for nutrients.

Suspended sediment loads from the five sub watersheds for the 2003 year (January to December 2003) ranged from 12,177 to 1,496,602 kg/sub watershed. Lowest values were obtained for Chamorro sub watershed and highest values for the Guaba sub watershed. Suspended sediment yields ranged from 30.1 to 1133 kg/ha/yr, with highest values for the Guaba sub watershed. It is hypothesized that these estimates are in the lower proportion of actual values because sediment movement is primarily associated with runoff from precipitation events which probably did not coincide with our sampling events (see Section 5.0 and Corvera-Gomringer, 2004).

Rámos-Gines (1996) published total P export coefficients, and to our knowledge account for the only export coefficients for varying land-uses within tropical ecosystems. His data show that TP export coefficients for the land uses evaluated are higher than those for temperate-climate zones (See Ramós-Gines, 1996, Nearing et al., 1993, Hanrahan, et al., 2001, Johnes, 1996), and the values obtained for urban unsewered areas were 19 times higher than the coefficients obtained for secondary forests, agricultural/rural and urban sewered basis (Table 4.4).

DP and TP loads from the five sub watersheds for the 2003 year (January to December 2003) ranged from 77 to 424 and 93 to 745 kg/sub watershed, respectively (Table 4.5). Loads from the sub watersheds were in the order of: Guaba, Cerro Gordo, Cerrote, Chamorro, and Miraflores. Nutrient loads were converted to yields based on sub watershed areas. DP yields for the sub watersheds ranged from 0.308 to 0.436 kg/ha, and TP yields ranged from 0.374 to 0.713 kg/ha. Our experimentally determined values were from 4 to 65% different than those calculated by Rámos-Ginés (1996).

The annual TP load of the five sub watersheds evaluated in the Rio Grande de Añasco watershed can be described by the following relationship:

$\log_{10}(\text{TP load}) = 1.0757 * \log_{10}(\text{sub watershed area}) + 0.501$; $r^2 = 0.906$; $p<0.05$

If five other sub watersheds from the Rio Grande de Arecibo watershed (Sotomayor et al., 2003) are included, the following relationship is obtained:

$\log_{10}(\text{TP load}) = 0.963 * \log_{10}(\text{sub watershed area}) - 0.0596$; $r^2 = 0.880$; $p<0.05$

Hence, knowledge of the watershed area can be used to extrapolate TP loadings in the range of 152 to 9921 ha.

Nutrient concentrations land-use relationships

Multiple correlation models among water-quality parameters that predicted TP, DP, TKN, DIN, and chlorophyll concentrations in stream waters are shown in Table 4.6. TKN concentrations could not be predicted from any of the possible combinations tested. The sign and magnitude of the coefficients indicate the relationship of each associative parameter to predicted response (chlorophyll a) and causative variables (TP, DP, and DIN). For example, a positive sign of the independent variable is indicative of a positive influence on the dependent variable.

None of the different combinations of land-use ratios individually were significantly ($P>0.1$) correlated to TP and DP concentrations and TP and DP yields. There was a positive correlation between Agriculture/Urban and Agriculture + Pasture / Urban This is consistent with the use of fertilizers in agricultural and pasture land. There was a significant positive correlation between DIN and Forest/Urban, Forest + Rangeland/Urban and Vegetative/Urban land-use ratios (Vegetative land area included Forest, Rangeland, Agriculture and Pasture). This may be because of the slow but constant process of organic matter mineralization and subsequent transfer of inorganic N in runoff. Other ratios were not statistically significant.

Predicted in-stream nutrient concentrations after converting 10% of the current land use to other land uses are shown in Figures 4.1a, 4.1 b for P, and Figures 4.2a and 4.2b for N. An example of the equation obtained for TP is shown:

$\log_{10}\text{TP} = -0.775*(\text{Ag}) -1.373*(\text{For}) + 2.496*(\text{Urb}) -2.607*(\text{Ran}) -1.080*(\text{Pas})$; $r^2 = 0.96$

All of the models tested were statistically significant with models for DIN and TKN having lower predictive strengths (r^2 values). This difference probably reflects their differing pathways of chemical transport and transformation in the landscape. Nitrogen species are more mobile and have more pathways for transport, transformation and biotic uptake. Thus TP species and concentrations are more conservative and are a reflection of overall watershed characteristics, whereas nitrogen species may be better reflected by including land use closest to the stream channel. Tufford et al. (1998), observed that land close to the stream channel (<150 m) was a better predictor of nutrient concentrations than land away from the channel (>150 m).

For TP, conversion of 10% of the current land use from, forest to agriculture, rangeland to agriculture, agriculture to suburban, and forest to suburban, resulted in 15, 43, 60 and 16% increases in in-stream concentrations. For the same above stated land use conversions and DP concentrations, the changes were -3, 36, 62, and 18%. Positive increases in TKN concentrations were also observed, and much variation was observed for DIN concentrations.

Table 4.1. Summary of general water-quality parameters in five sub watersheds within the Rio Grande de Añasco watershed from May 2002 to December 2003.

	pH	EC	Temp
		dS/m	^{o}C
Miraflores	7.48 c	207.8 a	24.3 a
Cerro Gordo	7.84 c	153.3 b	23.4 b
Cerrote	8.22 ab	225.9 a	24.0 ab
Chamorro	8.16 b	207.0 a	23.5 b
Guaba	8.36 a	221.1 a	23.7 b

1 EC = electrical conductivity; Temp = water temperature

Table 4.2. Summary of physical and chemical parameters in five sub watersheds within the Rio Grande de Añasco watershed from May 2002 to December 2003.

	TP	DP	TKN	DIN	SS	Flow	Clor$_a$	N:P$_{(diss)}$
	------- µg/L------		----------mg/L-------------			m^3/s	µg/L	
Miraflores	55.9 a	48.7 ab	0.085 a	0.575 b	3.57 b	0.052 e	0.188 b	12.4 c
Cerro Gordo	54.8 a	40.3 bc	0.121 a	0.617 b	20.5 a	0.208 b	0.285 ab	16.5 bc
Cerrote	52.1 a	36.9 c	0.077 a	0.624 b	1.94 b	0.131 c	0.390 a	23.3 b
Chamorro	62.8 a	53.6 a	0.123 a	0.954 a	1.45 b	0.088 d	0.190 b	20.7 bc
Guaba	23.4 b	25.2 d	0.065 a	0.805 a	2.52 b	0.336 a	0.439 a	42.7 a

1 TP = Total P; DP = Dissolved P; TKN = Total Kjeldahl N; DIN = Dissolved inorganic N; SS = suspended sediments; Clor$_a$ = chlorophyll-a ; N:P$_{(diss)}$ = Proportion of DIN/DP.

Table 4.3. Pearson correlation coefficients among physical and chemical parameters in five sub watersheds of the RGA watershed. Only coefficients with $P<0.05$ are included.

Parameter	DP	TP	TKN	Clor$_a$	SS	DIN	N:P$_{(diss)}$
DP	-	-	-	-	-	-	-
TP	0.201 (0.01)	-	-	-	-	-	-
TKN	NS	0.192 (0.012)	-	-	-	-	-
Chla	-0.351 (0.001)	NS	0.180 (0.036)	-	-	-	-
SS	NS	NS	0.303 (0.002)	NS	-	-	-
DIN	0.267 (0.03)	NS	NS	-0.377 (0.003)	NS	-	-
N:P$_{(diss)}$	-0.604 (0.001)	-0.431 (0.001)	NS	NS	NS	NS	-
Flow	-0.185 (0.014)	-0.356 (0.001)	NS	NS	0.296 (0.001)	0.270 (0.03)	0.458 (0.001
Temp	NS	NS	NS	NS	NS	NS	-0.313 (0.01)
pH	-0.207 (0.019)	-0.246 (0.006)	-0.296 (0.001)	NS	-0.293 (0.001)	NS	0.394 (0.001)
EC	-0.241 (0.006)	NS	-0.188 (0.038)	NS	-0.380 (0.001)	NS	0.224 (0.07)

Table 4.4 Description of export coefficients from published literature.

Land Use	Ramos-Ginés, 1997 (Puerto Rico)	Rast and Lee 1978	Hanrahan, et al., 2001	Johnes,1996
Forest	0.37	0.1	0.02	0.02
Ag-rural (crops)	1.49	0.5	0.66	0.65
Ag-rural (Dairy)	1.98	0.5		1.4*
Urban-sewered	2.55	1.0	0.83	
Urban-unsewered	7.06		0.83	

Table 4.5. Annual TP and DP loads and yields at five sub watersheds of the Rio Grande de Añasco sub watershed from January to December 2003.

Sub watershed	TP	DP	TP	DP	TP(theoretical)[1]
	----kg/sub watershed-----		--------------------kg/ha---------------------------		
Miraflores	93	77	0.414	0.343	1.18
Cerro Gordo	367	242	0.514	0.339	0.834
Cerrote	209	128	0.713	0.436	0.665
Chamorro	149	122	0.374	0.308	0.523
Guaba	745	424	0.564	0.321	0.590

[1] Estimated using land-used export coefficients published by Rámos-Gines, 1997, and land-use information of this study.

Table 4.6. Multiple regression models between selected water-quality parameters and nutrient concentrations in five sub watersheds of the Rio Grande de Añasco watershed.

Dependent variable	Equation	Intercept	R^2
TP	-0.00307*(temp) – 0.02781*(flow) + 0.00569*(ecoli)	0.126	0.220
DP	-0.00858*(DIN) – 0.02781*(temp) + 0.00854*(ecoli) – 0.02728*(flow) – 0.0000842*(ecfld)	0.181	0.428
TKN	Non significant model		
DIN	-0.003*(temp) + 0.0053*(ecoli) + 0.157*(DP) – 0.0252*(flow)	0.120	0.223
Chla	0.00119*(EC) – 0.30417*(DIN) + 0.2552*(flow) + 5.718*(TP) – 0.545 *(DP)	0.027	0.616

Temp = temperature; flow = log10(hydrologic flow); ecoli = log10(Escherichia coli); DIN = dissolved inorganic nitrogen; TP = total phosphorus; DP = dissolved phosphorus; EC = electrical conductivity.

Figure 4.1. Predicted in-stream (A) total P and dissolved P (B) after converting 10% of the current land area.

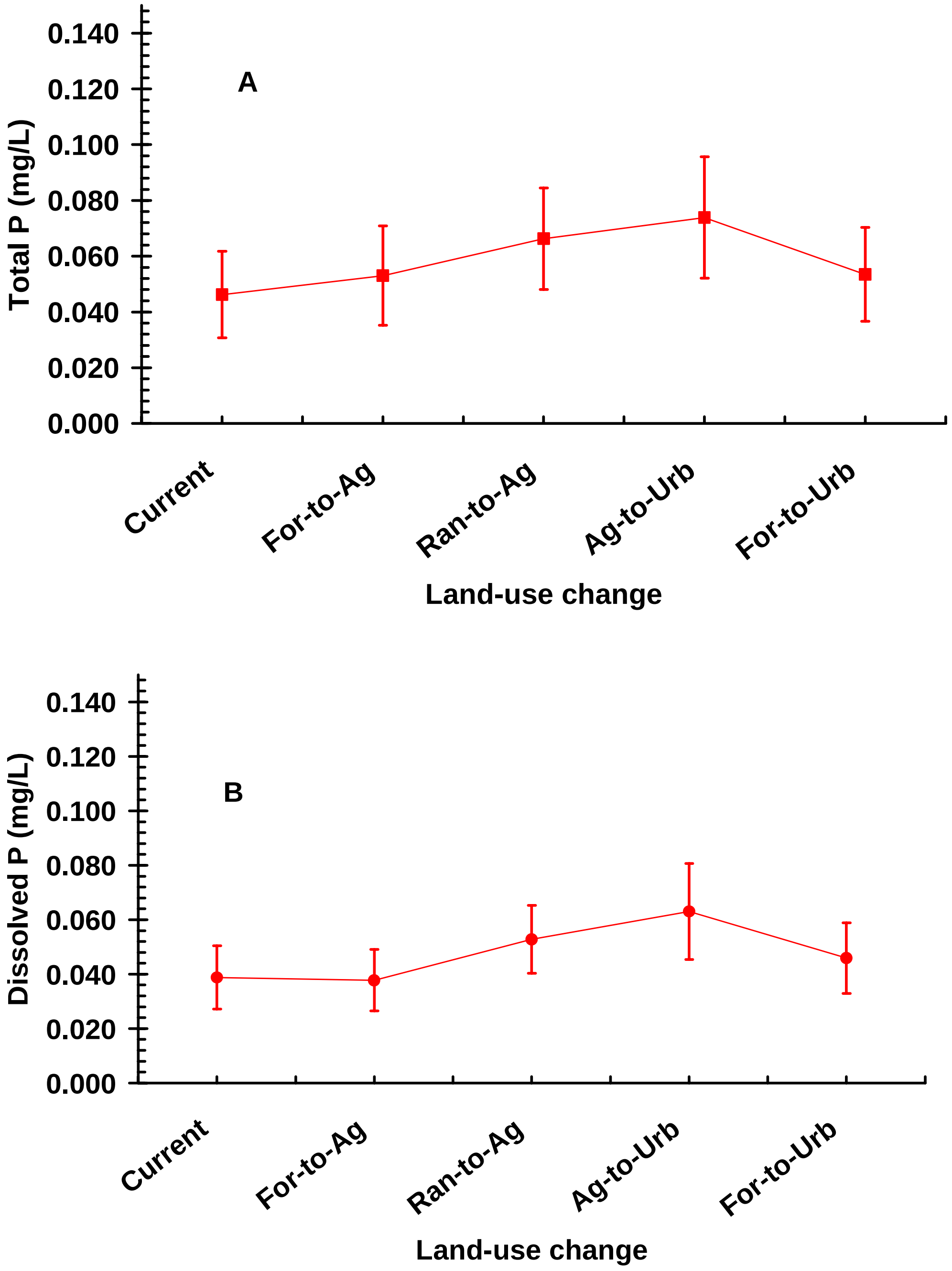
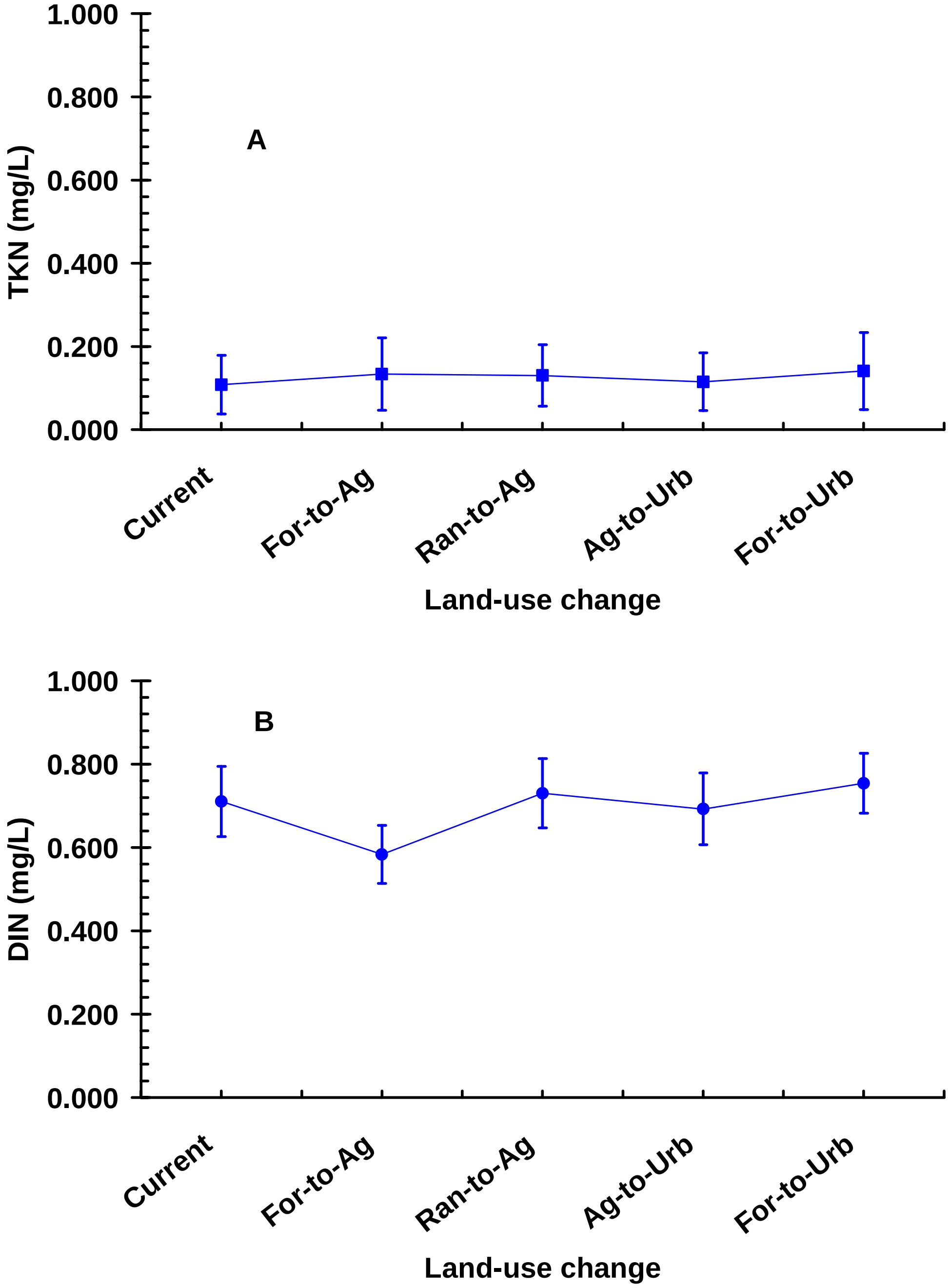


Figure 4.2. Predicted in-stream (A) total Kjeldahl N and dissolved inorganic N (DIN) (B) after converting 10% of the current land area.



5.0 HYDROLOGIC AND NUTRIENT LOAD ANALYSIS DURING STORM (RUNOFF) EVENTS

5.1. SPECIFIC OBJECTIVES

- Quantify nutrient and sediment concentrations and discharges during storm events
- Relate storm runoff events to possible land-use
- Quantify annual nutrient loads and yields

5.2. METHODOLOGY

Nutrient annual loads were estimated from the combination of grab samples (See Section 4.0) and storm events monitored from January through December 2003 in two sub-watersheds (Miraflores and Cerro Gordo) of the Río Grande de Añasco basin (Figure 3.1). Data collected by Corvera-Gomringer (2004) included that collected from August 2003 to 31 August 2004. At the point of interest of two of the sub-watersheds, Cerro Gordo and Miraflores, an ISCO® 3700 automatic sampler and ISCO® 4220 flow meter (ISCO Corp. Lincoln NE) were installed to trace the runoff hydrograph following a rainfall event in the sub-watershed catchment area. Surface water elevation was continuously monitored with the ISCO 4200 flow meter equipped with a pressure transducer attached to the recorder. The equipment was programmed to sample composite samples in 9L Nalgene (Nalgene Corp. Rochester, NY) storage bottles. Each storm event sampled corresponded to 15 sub samples of 600 mL increments. The sampler was programmed to sample at specific stage heights which was programmed to vary based on the expected intensity and duration of the storm-runoff event. Threshold stage heights were at least 0.30 cm from heights observed at or near base-flow. Details are found in Corvera-Gomringer (2004). During the storm event, sampling continued until the stage reach the threshold elevation set up in the autosampler for that location (cross section).

After every storm event, the composite bottle was brought to the laboratory for preparation and analysis within a 24-hr period. Samples were processed as described in Section 4.0 and analyzed as described therein.

Hydrologic Analysis

Runoff hydrographs were generated from 15-min precipitation records at the San Sebastian weather station (Figure 5.1) operated by the US Geological Survey for the year 2003 and the SCS-Curve Number method implemented in the Hydrologic Modeling System computer program (HEC-HMS). Curve Number for soil and land use coverages of each of the sub-watersheds and the time of concentration were calculated from Digital Elevation Models (DEM) files and Digital Ortho Quad Quadrangle (DOQQ) photos that provided the background to delineate homogeneous land use polygons (See section 4.0) (Table 5.2).

Daily runoff synthetic hydrographs generated for 2003 were plotted along with instantaneous runoff measured during grab sampling events and storm events dates to match the recorded event with the estimated flow (Figures 5.2 and 5.3). Most times good match was observed although some other times there was no agreement, this is explained because most times the grab sample was taken mostly during low to medium flow conditions usually at the descending limb of storm events hydrographs when students could enter the stream with minimum risk of life or equipment.

Rating Curves

Rating curves for the two sub-watersheds were required to use the stage data taken by the flow meter and convert that reading to flow. A rating curve was developed for the two gauged sub-watersheds Miraflores and Cerro Gordo to relate stage at the sampling point and runoff flow. The rating curve was developed with a simple model of the river reach that contains the sampling instruments using the River Analysis System (HEC-RAS). Cross sections before and after the sampling station were taken with surveying equipment. All topographic data were linked to a grid coordinate system for Puerto Rico (NAD 83, rev 1997). Simulated flow was coupled to the stage elevation recorded by the flow meter at the site to generate the rating curve for each of the watersheds (Figures 5.4 and 5.5).

Base flow determination

Miraflores and Cerro Gordo watersheds as well as the other three sub-watersheds studied in this project are ungauged, therefore there is no existing record to perform a low-flow frequency analysis, instead the gauged station USGS 50144000 on Río Grande de Añasco near San Sebastian was used to generate the low-flow frequency analysis for estimating the annual seven days minimum average flow (Riggs, 1972). The low-flow for a recurrence of 25 years was established as the base flow $7Q_{25}$ (Table 5.1) in this study.

Base flow was estimated for the five un-gauged sub-watersheds, Miraflores, Cerro Gordo, Cerrote, Chamorro and Guaba by extrapolation using the ratio of drainage areas method (Gupta, 1989). Runoff flow is proportional to the drainage area and is expressed by *equation 5.1*:

$$Q_b = \frac{Q_X}{A_X} A_b \tag{5.1}$$

Where Q_b is the extrapolated base flow for the un-gauged watershed with drainage area A_b and Q_x is the baseflow of the Río Grande de Añasco at San Sebastian with a drainage area A_x. Table 5.1 shows the $7Q_{25}$ base flow for the five sub-watersheds of this study.

The flow data was separated into baseflow and runoff flow using the Green and Haggard (2001) criteria: “Samples collected on days when baseflow was greater than or equal to 70 percent of total flow were considered to be baseflow samples. Surface-runoff samples were defined as samples collected on days that base flow was less than 70 percent of total flow (surface runoff was greater than 30 percent of total flow)”.

Regardless of the sampling method; grab or storm events, all flows were classified as base flow or storm event flow. Grab samples were taken predominantly during low flow conditions, however, there were instances were flows were high and were classified as runoff events following the Green and Haggard rule discussed before in this section.

Estimation of mean daily volume

Mean daily runoff for the five watersheds was calculated using 15-min precipitation records from the USGS 501 4400 San Sebastian station fed into a HEC-HMS computer simulation model of the watersheds and other physiographic characteristics of the watersheds contained in GIS coverages (soil, topography and land use). The NRCS-Curve Number (CN) method was used to estimate mean daily flow. Table 5.1 shows the set of parameters used in each sub watershed in the HEC-HMS model. Mean daily flow hydrographs for the 2003 calendar year are shown in Figures 5.2 and 5.3 for Miraflores and Cerro Gordo watersheds.

Estimation of Mean Daily Loads

Using grab samples and storm events, a correlation between mean daily volume and mean daily load was developed for the species of TP, DP, and TKN. The observed plotted data were fit to a power function model and a polynomial model. In most cases, the power function of the form load (L) = aV^x much better predicted load as a function of mean daily volume (V) for Miraflores (Figures 5.6, 5.7, 5.8) and Cerro Gordo (Figure 5.9, 5.10, 5.11) for DP, TP, and TKN, respectively. The power function model was selected to produce estimates of nutrient mean daily load as a function of mean daily runoff volume for each sub watershed. Annual nutrient loads were calculated based on daily time-series integration.

5.3. RESULTS AND DISCUSSION

A total of 22 grab samples were taken bi-weekly at all five of the sub watersheds. For two of the sub watersheds, Miraflores and Cerro Gordo, this was combined with samples collected during storm events during 2003. Nutrients and sediments move down the watershed as a result of dynamic forcing in the catchment area. This forcing is primarily due to concentrated runoff during storm events and in a minor percentage to base flow. Annual load was split between *base flow* and *runoff events* for the period January to December 2003 in the five sub-watersheds considered in this study.

Annual load was calculated by integrating mean daily volume (V) times nutrient concentration (C_i) for the calendar year 2003. The total annual load estimation (Table 5.3) was compared with the total annual load calculated after separating the load contribution from base-flow conditions (Table 5.4a) and runoff-flow conditions (Table 5.4b). As expected most of the nutrients move out of the catchment area into the observation point by storm events that occur less frequently in the watershed. Baseflow, although important, contribute only a minor fraction to the total nutrient yield in the five sub-watersheds estimated to range from 6 to 11% for DP. The nutrient contribution during storm events for the sub watersheds Cerrote, Chamorro, and Guaba may be underestimated because the large events were not sampled.

In general, annual yields (kg/ha) ranged from 0.018 to 0.61 for DP, 0.20 to 1.61 for TP, and 0.75 to 7.12 for TKN. The highest DP and TKN yields were obtained for Miraflores subwatershed, yet the highest TP yields were obtained for Cerro Gordo subwatershed. Since TP concentrations were significantly correlated to suspended sediments, sediment control in Cerro Gordo subwatershed, may probably reduce TP concentrations.

In samples collected during 2003-2004, suspended sediments, TP, DP, and TKN concentrations were higher than values collected during grab samplings (See section 3.0). Cerro Gordo sub watershed had significantly higher SS and DP concentrations than Miraflores, and TP and TKN concentrations were similar between Cerro Gordo and Miraflores sub watersheds (Table 5.5) (Corvera-Gomringer, 2005). Suspended sediment concentrations significantly influenced TP and TKN concentrations in Miraflores and Cerro Gordo sub watersheds. As expected, the correlations between hydrologic flow and nutrient and sediment loads were significant, yet correlation coefficients were significantly higher for phosphorus than for nitrogen.

Table 5.1 Calculated base flow ($7Q_{25}$) for USGS gauging station in San Sebastián (USGS 50144000) and five sub watersheds of the Rio Grande de Añasco watershed.

	Area (Ha)	Base Flow (cfs)
San Sebastian	24,291.92	68.00
Miraflores	224.00	0.63
Cerro Gordo	714.70	2.00
Cerrote	293.30	0.82
Chamorro	397.30	1.11
Guaba	1320.00	3.70

Table 5.2 Input parameters for hydrologic simulation with HEC-HMS in the five sub watersheds of this study.

	Curve Number	SCS Lag (hr)	Base Flow (CFS)
Miraflores	81.4	0.27	0.63
Cerro Gordo	83.1	0.49	2.00
Cerrote	70.3	0.40	0.82
Chamorro	78.0	0.36	1.11
Guaba	71.2	1.54	3.70

Table 5.3. Nutrient annual load for the entire observation period between January and December 2003.

	Annual Yield (kg/ha)		
	DP	TP	N
Miraflores	0.61	0.66	7.12
Cerro Gordo	0.33	1.51	4.10
Cerrote	0.39	0.61	0.65
Chamorro	0.56	0.61	0.37
Guaba	0.18	0.20	0.75

Table 5.4. Nutrient annual load separation by baseflow (A) and runoff-storm (B) events.

	Base Flow (A)		
	Annual Yield (kg/ha)		
	DP	TP	N
Miraflores	0.04	0.05	0.09
Cerro Gordo	0.03	0.06	0.10
Cerrote	0.03	0.06	0.09
Chamorro	0.04	0.06	0.07
Guaba	0.02	0.02	0.05

	Runoff Flow (B)		
	Annual Yield (kg/ha)		
	DP	TP	N
Miraflores	0.57	0.60	7.03
Cerro Gordo	0.29	1.45	4.00
Cerrote	0.37	0.55	0.56
Chamorro	0.51	0.55	0.30
Guaba	0.16	0.18	0.70

Table 5.5. Mean values for water-quality parameters collected for storm events during 2003-2004 (data from Corvera-Gomringer 2004).

Parameter	Miraflores	Cerro Gordo	Significance ($P<0.05$)
Volume (m^3)	2.12×10^4	2.21×10^5	Yes
Suspended sediments (mg/L)	1551	2737	Yes
Total P (mg/L)	0.34	0.48	No
Dissolved P (mg/L)	0.06	0.02	Yes
Total Kjeldahl N (mg/L)	1.70	3.15	no

Figure 5.1. Precipitation at the USGS station 50144000 Río Grande de Añasco near San Sebastián. Jan- Dec., 2003.

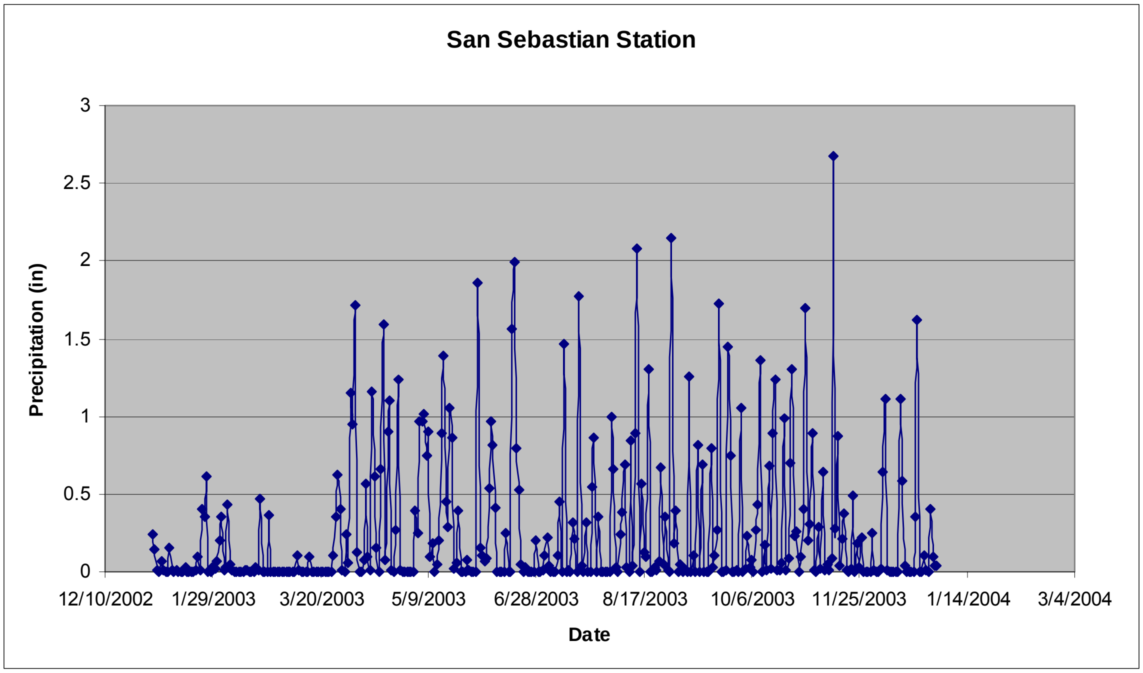


Figure 5.2. Estimated runoff flow from Miraflores sub watershed and flow measured at time of grab samples.

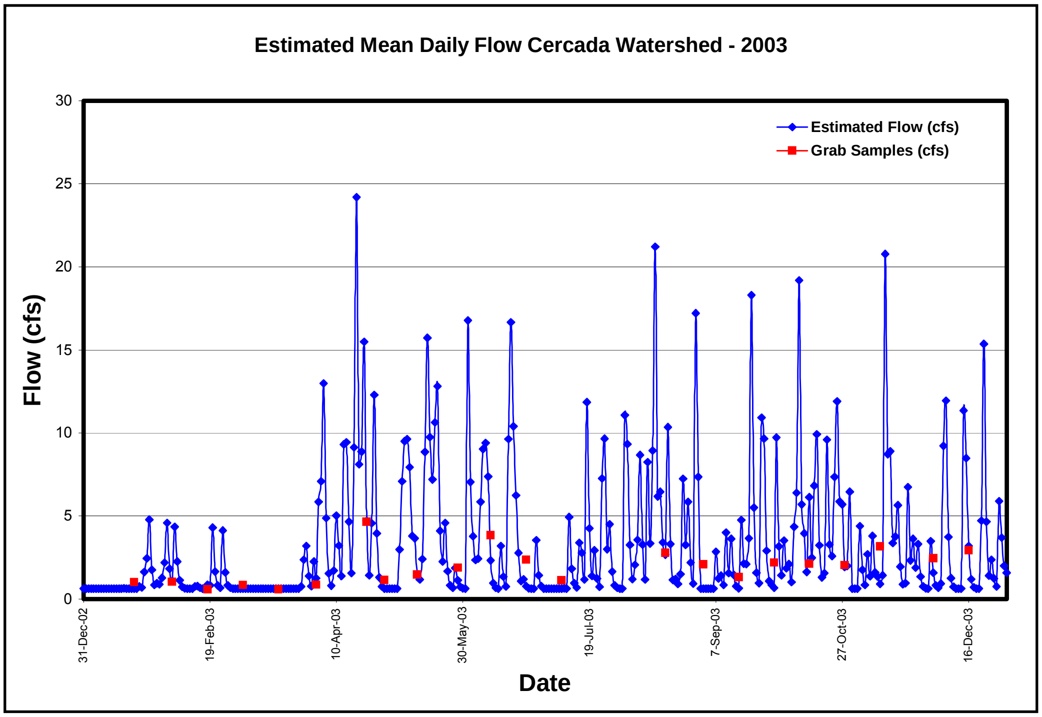


Figure 5.3. Estimated runoff flow from Cerro Gordo sub watershed and flow measured at time of grab samples.

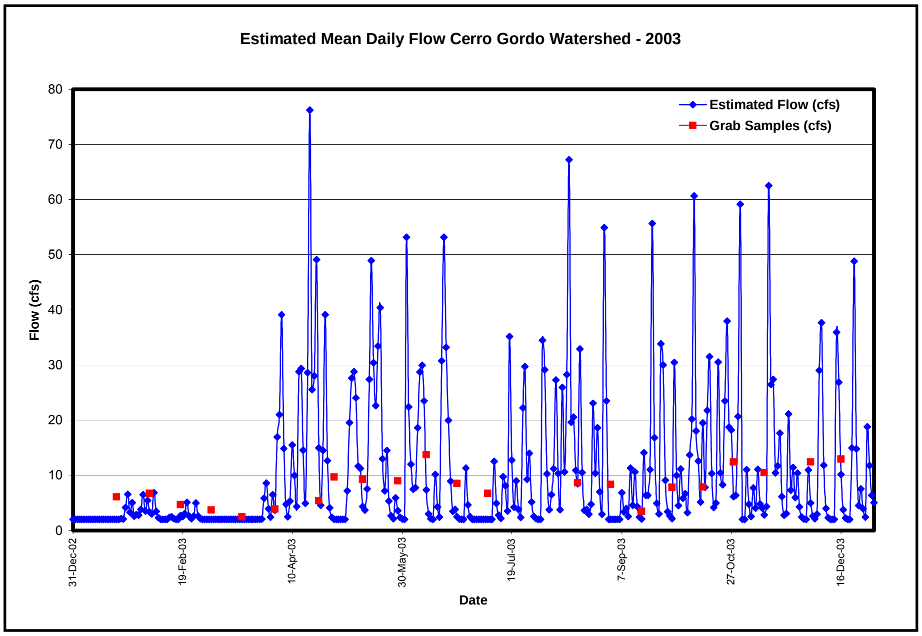


Figure 5.4. Rating curve for sampling point at Miraflores sub watershed.

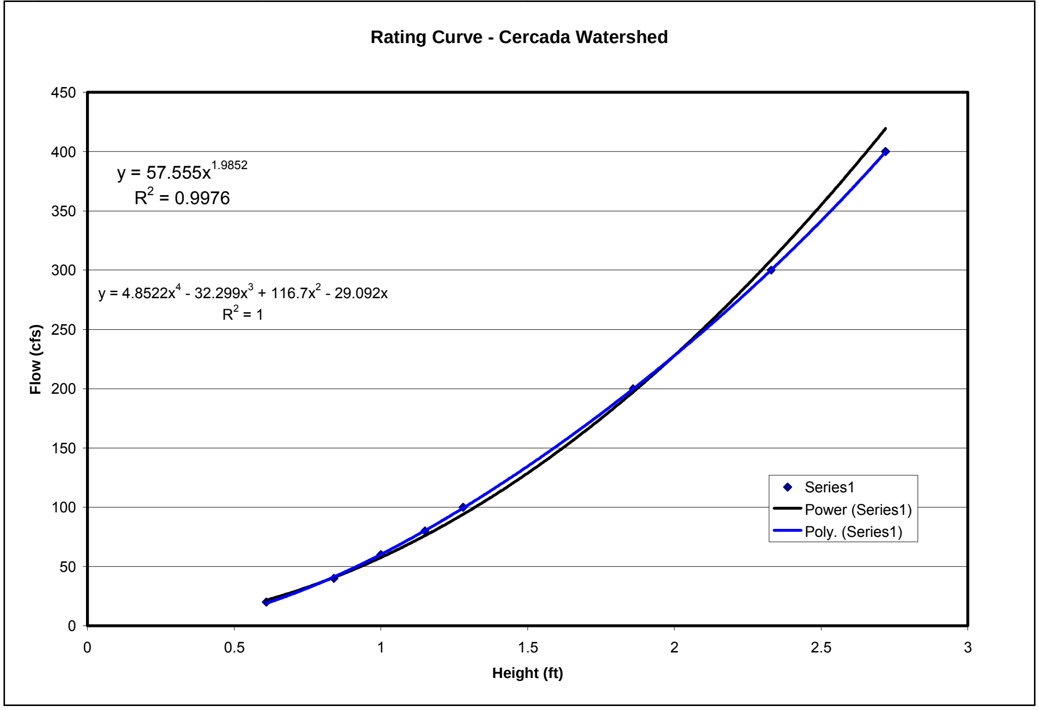


Figure 5.5. Rating curve for sampling point at Cerro Gordo sub watershed.

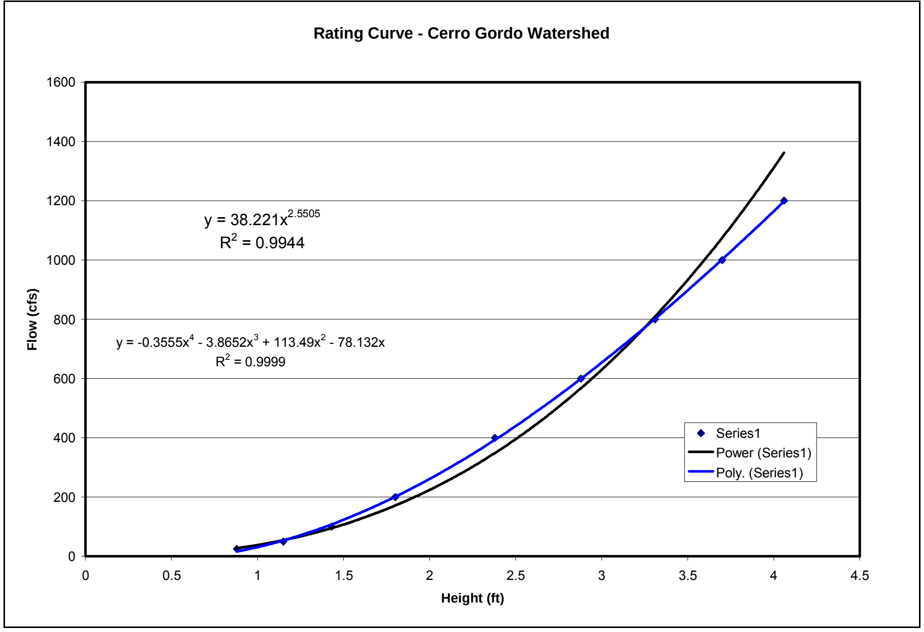


Figure 5.6. Dissolved P load and runoff volume from grab samples and storm events for Miraflores sub watershed in 2003.

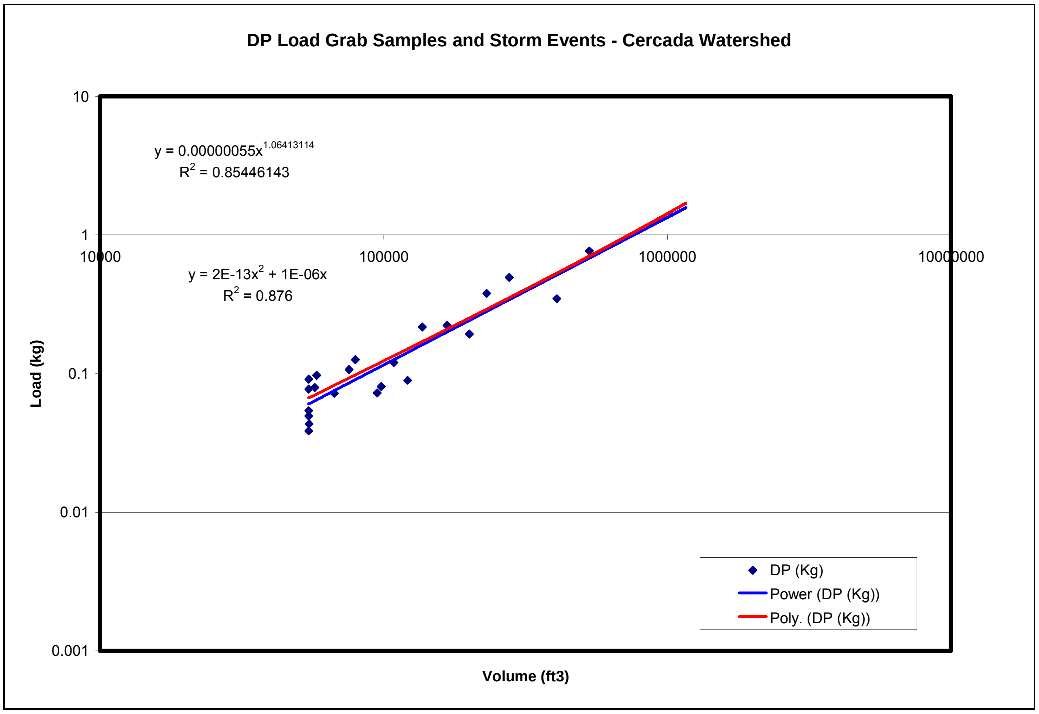


Figure 5.7. Total P load and runoff volume from grab samples and storm events for Miraflores sub watershed in 2003.

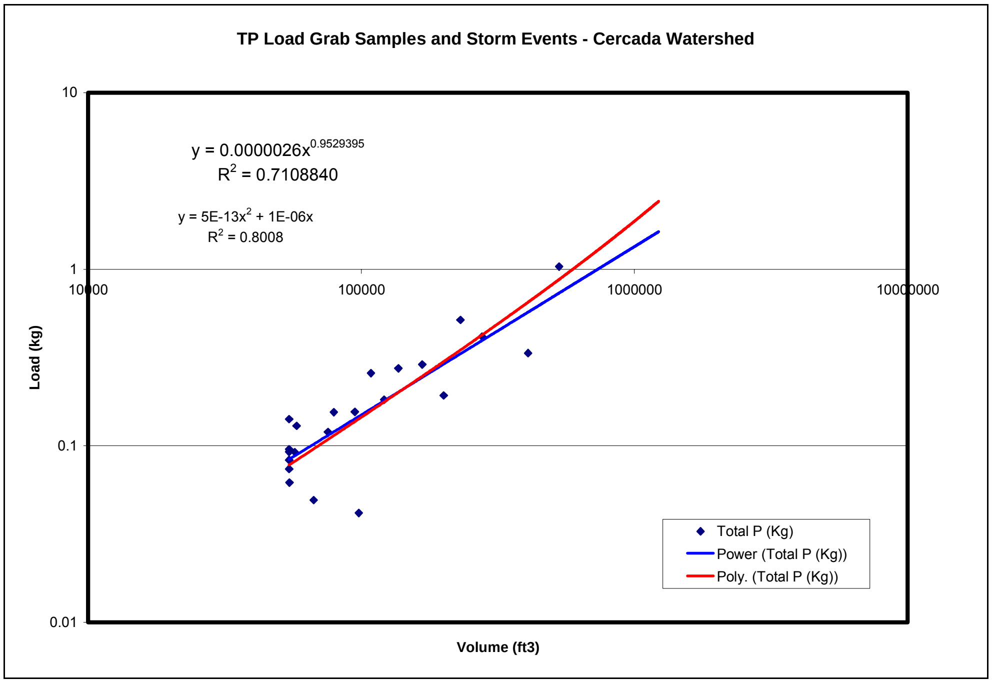


Figure 5.8. Total nitrogen load and runoff volume from grab samples and storm events for Miraflores sub watershed in 2003.

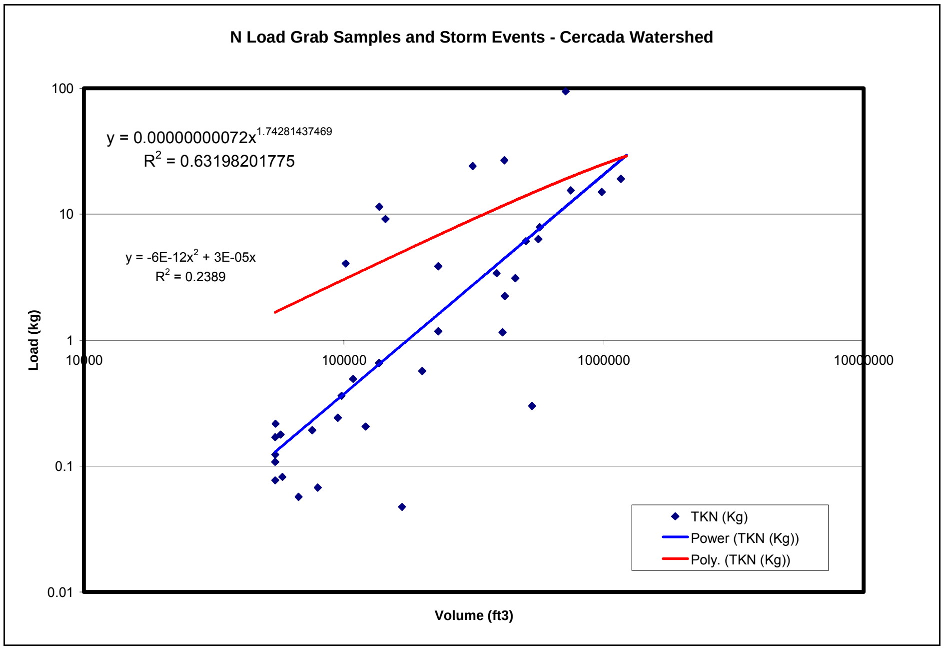


Figure 5.9. Dissolved P load and runoff volume from grab samples and storm events for Cerro Gordo Watershed in 2003.

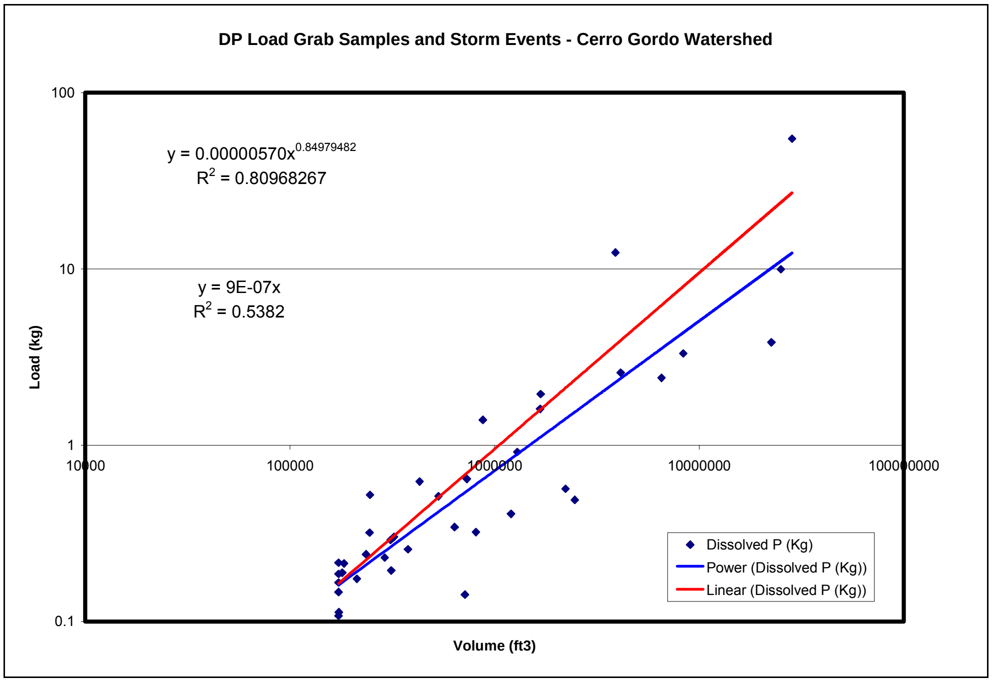


Figure 5.10. Total P load and runoff volume from grab samples and storm events for Cerro Gordo Watershed in 2003.

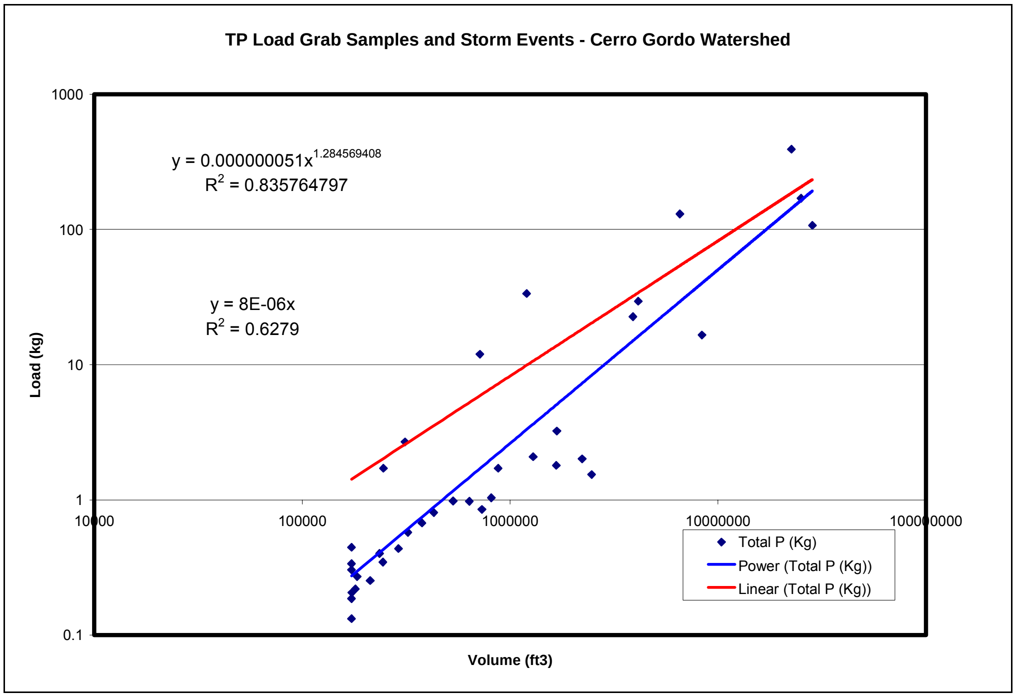
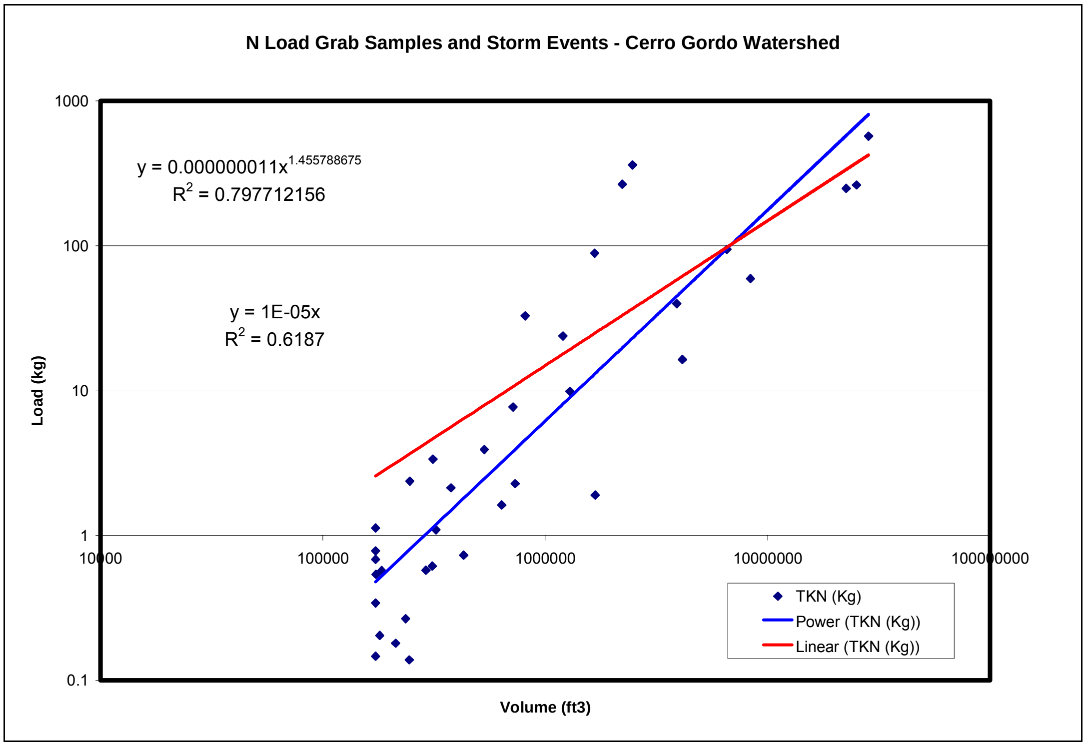


Figure 5.11. Total nitrogen load from grab samples and storm events for Cerro Gordo sub watershed in 2003.



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7.0 APPENDIX

Participating Students

- Undergraduate students

1. Miguel Santiago (Agronomy and Soils)
2. Lesly M. Colón Sánchez (General Agriculture)
3. Lisandra Torres (Agronomy and Soils)

- Graduate students

1. Ronald Corvera Gomringer (Agronomy and Soils)
2. Jairo Diaz Nelve (Civil Engineering)
3. Gustavo Suarez (Agric. Engineering)
4. Idarnis Gaztambide (Biology)

Publications Related to Proyect:

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1. Sotomayor-Ramírez, D., M. Alameda, G. Martínez, L. Pérez-Alegría, R. Corvera-Gomringer. 2005. Microbiological surface-water quality of the Rio Grande de Añasco Watershed in western Puerto Rico. Caribbean Journal of Science (In Preparation)

2. Sotomayor-Ramírez, G. Martínez, L. Pérez-Alegría, R. Corvera-Gomringer. 2005. Nutrient dynamics and exports from tropical watersheds. Journal of Environmental Quality (In Preparation)

3. Pérez-Alegría, L., D. Sotomayor-Ramírez, G. Martínez and R. Corvera-Gomringer. 2005. Hydrologic and nutrient loads during runoff events in tropical sub watersheds. (In preparation).

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