INVENTORY OF SEPTIC TANKS AS A SOURCE OF POLLUTION IN GROUNDWATER AND CORAL REEFS IN THE BELVEDERE NATURAL RESERVE IN WESTERN PUERTO RICO

Final Report submitted to the
Department of Natural Resources
of the Commonwealth of Puerto Rico

by

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I. Project Overview

Inventory of Septic Tanks as a Source of Pollution in Belvedere

A. Introduction and Rationale

This project overview presents the principal aspects of the study rationale, objectives and design. It also presents the principal findings of the study components, which are individually discussed in this Report's Appendices.

- **Septic tank pollution**

Coral reefs and coastal waters in Western Puerto Rico are subject to stresses due to water pollution from non-point sources. Septic tanks are one of the most important non-point sources of water-borne pathogens and several inorganic pollutants. Contamination by septic tank systems is a real problem that does not count with the attention that it really should have. Studies have demonstrated that some outbreaks of hepatitis, gastroenteritis and the Norwalk viruses have been cause by contamination of ground water by septic tanks. Badly designed and operated septic tank systems do not work effectively to remove pathogens, and can cause the dispersion of contamination factors such as total coliforms and fecal coliforms. Suspended solids carried by runoff from overflowed septic tanks during storm events and organic and inorganic chemicals from seeping sewage may also contribute to coral reef degradation. A comparison between the ecological health status of coral reefs near these onshore pollution sources and offshore coral reefs provides information on the ecological impact of septic tanks in the region.

In addition to loading of subsurface water, onsite disposal systems, such as septic tank and injection wells, are known to be source of microbial contamination of surface water. The maximum age for the best functioning of a septic tank is 20 years. Many of the septic tank systems in Puerto Rico are older than 20 years and an outflow of these systems can result in serious problems. Studies have demonstrated that bacteria and viruses can be transported through the subsurface and contaminate groundwater many hundreds of meters horizontally and survive for weeks. Another important point is that contaminants produced by septic tanks can be deposited in the bottom of rivers and lakes for several weeks before they die. For this reason some studies have found lower densities of bacteria in the water column but higher in sediments samples. Densities have been found of total and fecal coliforms between 6-45 MPN/100ml versus 150-2088 MPN/100ml in the water and sediments respectively (MPN = Most Probable

Number). These bacteria can live for weeks in the bottom and can be affect recreational activities.

- **Study site**

The Belvedere Natural Reserve was established in February 2003 by the Puerto Rico Planning Board. It is located in the municipality of Cabo Rojo on the West Coast of Puerto Rico, (approximately centered in 18°N 05' N and 67°W 11') and covers a coastal area of 256.36 acres of wetlands. The ecologically sensitive wetland of the Reserve is bordered by the Puerto Real settlement of Barrio Miradero. Figure 1 shows a United States Geological Survey aerial photo taken in November 1993 where we can observe the wetland area right next to a densely populated urban settlement that is encroaching on the ecologically sensitive land and water resources of the Reserve. According to the 2000 Census, Puerto Real had a population of 6,166 persons and 2,254 housing units (see Figure 2). To make the current situation more critical, old and substandard septic tanks have created a serious problem of water quality in the area, seriously damaging habitats within the Reserve and the coral reefs on the coastal zones surrounding the Reserve.

In this project the Department of Environmental Health (DEH), Graduate School of Public Health of the University of Puerto Rico Medical Sciences Campus conducted an Inventory of Septic Tanks as a Source of Pollution in the Belvedere Reserve, sampled and analyzed coastal waters near coral reefs, and evaluated the health of coral reef communities both inside and near the Reserve. The project was carried out in two phases of six months each.

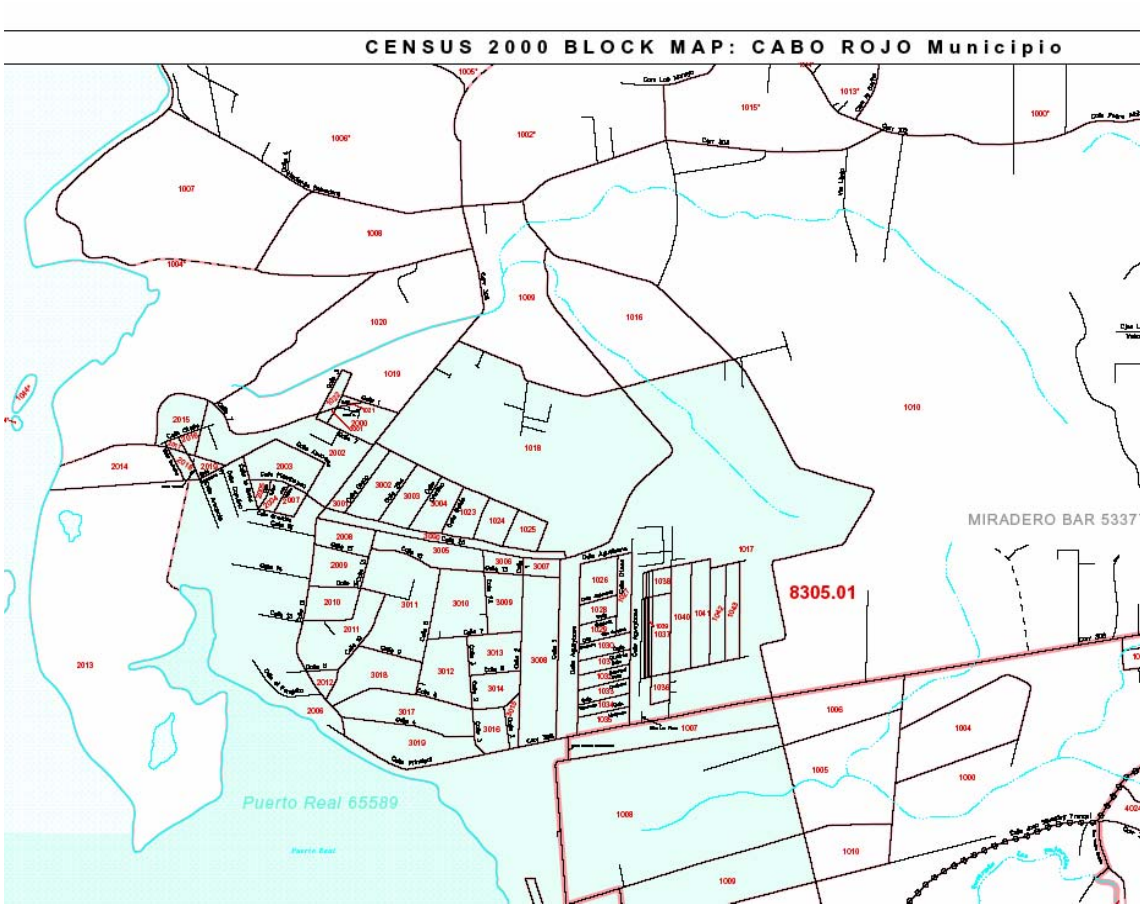
Figure 1

Belvedere Natural Reserve and Puerto Real Settlement, November 1993

Source: United States Geological Survey (USGS)

Figure 2

Census Tracts, 2000, Belvedere Natural Reserve and Puerto Real Settlement



B. Research Objectives

The objectives of this study were:

A. To identify and do an inventory of housing units in the watershed of the Belvedere Reserve using remote sensing and Geographic Information System techniques.

B. To classify housing units with septic tanks, sewage and sanitary sewage (i.e. with waste water treatment).

C. To conduct household interviews and onsite inspections of septic tanks facilities of a representative sample of housing units of those communities lacking sewage facilities. The interviews and onsite inspection will be carried out to determine the conditions of the septic tank, and the household interviews will measure knowledge of the environmental impact of septic tanks and attitudes toward this problem.

D. To determine the environmental health implications of this situation using digital georeferenced data on the phreatic level, geohydrology, water quality and location of known drinking water wells.

E. To develop an Environmental Education Program through seminars on the design and operation of septic tanks addressed to members of the communities, government agencies and other stakeholders.

C. Methodology/Approach

The study was carried out in two phases. During Phase 1 an inventory of septic tanks and households was completed. During Phase 2 the water column near coral reefs inside and near the reserve was sampled and analyzed to determine the quality of the coastal water in the coral reefs area.

In Phase, in order to carry out an inventory of septic tanks, Geographic Information Systems techniques were used to combine 1990 and 2000 Census data with data from the Puerto Rico Aqueducts and Sewers Authority (PRASA). A household physical survey of the Puerto Real community was also carried out. The household interviews to measure knowledge of the environmental impact of septic tanks and attitudes toward this problem were substituted with interviews with local environmental activists from the Fishermen’s Association. This was done due to the fact that mainly households lying below the street level near shore were operating problematic septic tanks. Fishermen were aware of this problem since the construction of sewer lines along Puerto real’s main shore street around 1990.

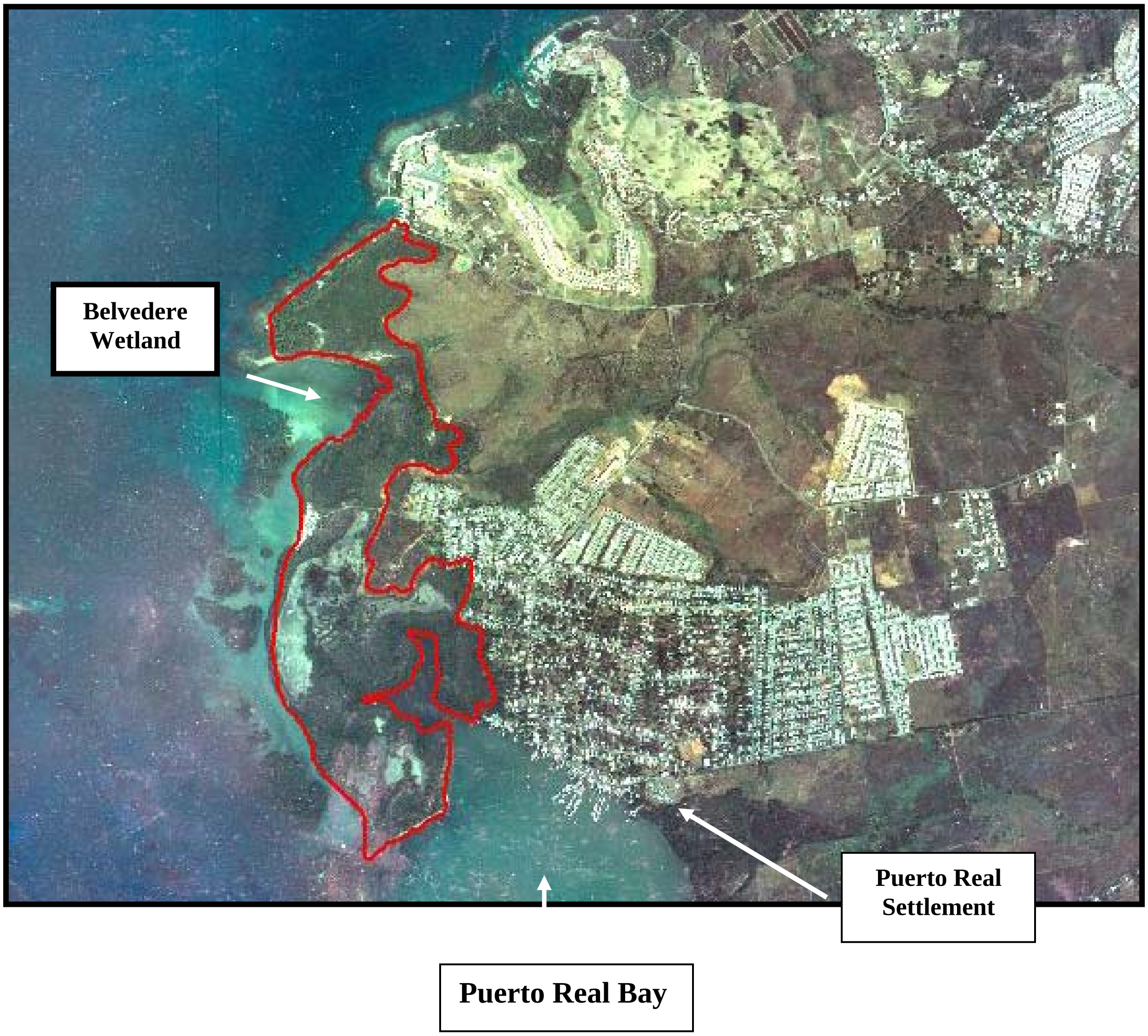
The planned Environmental Education Program through seminars on the design and operation of septic tanks addressed to members of the communities, government agencies and other stakeholders was substituted by other activities. These activities were providing scientific information and education to the Puerto Real Fishermen's Association which was already involved in community action to clean the Puerto Real Bay. Information and advice on the septic tank problem, general microbial and physicochemical water quality in the study site, and general status of coral reefs was provided during the period leading up to its participation in the central government's legislative public hearings regarding cleanup of Puerto Real Bay in March 2006. Afterwards, actions were taken by PRASA to clean sewer lines along Puerto Real's main shore street and improve sewage pumps, which were provoking coastal sewage pollution events from overflow through bypass valves when malfunctioning.

Once the first phase was completed, in Phase 2 identified precisely the area of the Belvedere Reserve using recently obtained copies of legal documentation and GIS mapping techniques (see Figure 3 and Report Appendices). The water quality impact of onshore nonpoint pollution sources was precisely measured using microbiological, physicochemical and ecological coral reef parameters in the study area (see Figure 4).

A geological study of the area was carried out using Puerto Rican government databases and Geographic Information System techniques. The results are found in Appendix C of this report. The following appendices contain, in detailed form, the results of each component of this study.

Figure 3. Belvedere Natural Reserve Limits.
USGS Puerto Real Topographic Map based on 1966 Data

Figure 4. Study Area
2005 Aerial Photo of Belvedere Wetland and Puerto Real Settlement



D. Summary of Results

- **Land based human activity results**

In the area surrounding the Belvedere Wetland a total of 2,060 residential units and commercial establishments were identified with 435 septic tanks. It is of concern that most of these septic tanks, 301 (69 percent), are located in close proximity to the Wetland and in the area next to the Puerto Real Bay (see Figure 3). A serious problem of septic tank discharges that reach the Wetland and the Bay area was found.

- **Remote sensing results**

Remote sensing analysis of the study area confirms that suspended sediment identified in Puerto Real nearshore waters apparently comes from land sources by runoff. Human activities such as construction and land cover modification in Puerto Real could be related with major sediment discharge to nearshore areas. There is also evidence that discharges from the Guanajibo River are having a major impact in the area of the Reserve. A qualitative evaluation of Guanajibo River suspended sediment plume was done using remote sensing techniques. Ortophotos with 4 and 2 meters spatial resolution collected by NOAA (National Oceanic Atmospheric Administration) and USDA (United States Department of Agriculture) were evaluated to identify the Guanajibo River plume and suspended sediment in nearshore areas for the 1999 to 2004 period.

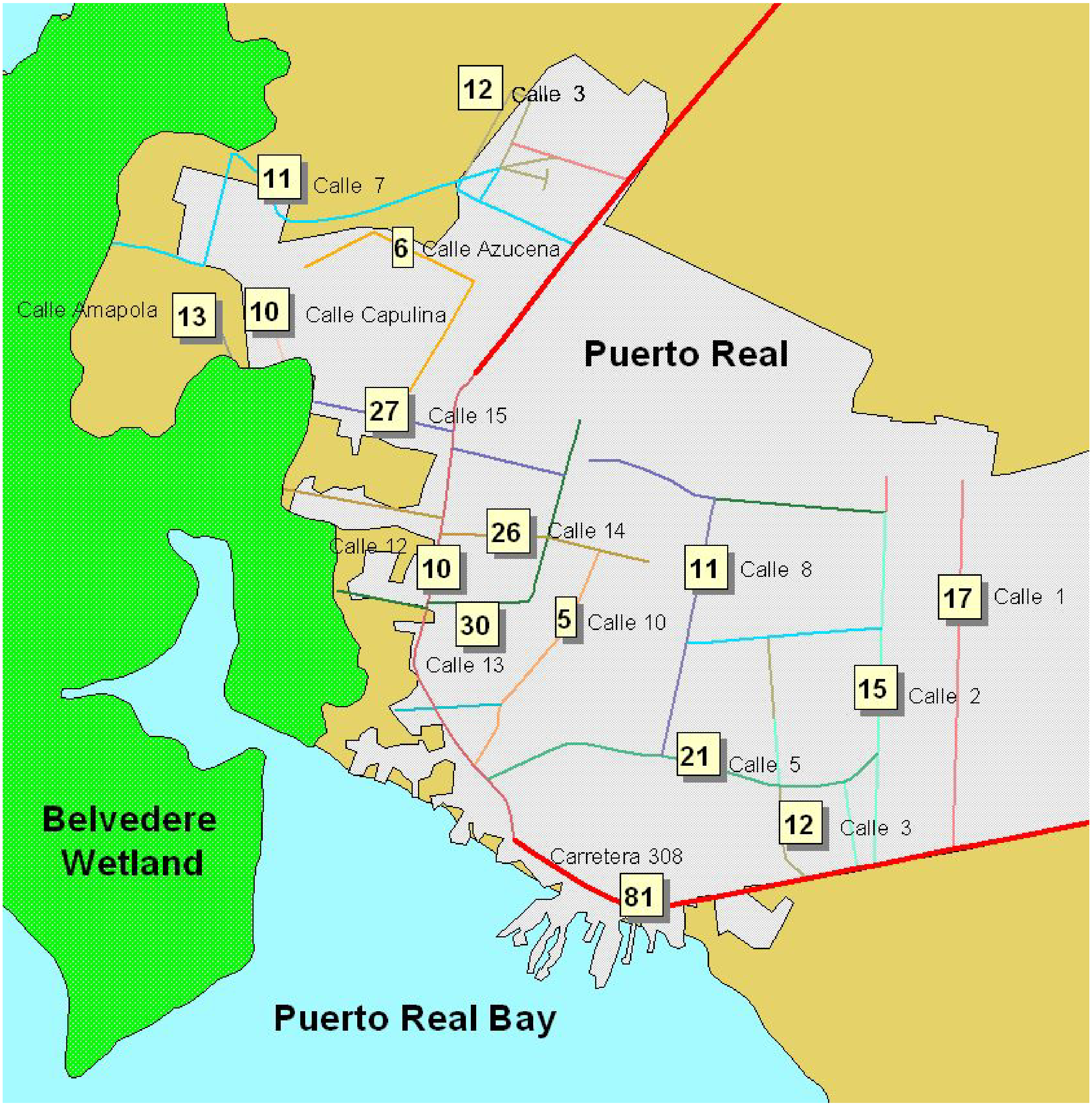
Landsat Enhancement Thematic Mapper (ETM) images with 30 meters of spatial resolution was used to identify the river plume and suspended sediment for 2001 and 2003. Spectral bands 3 (red) and 2 (green) were used to discriminate suspended sediments in nearshore waters. Unsupervised classification procedure was used to conduct suspended sediment categories. General results showed that: 1) the Guanajibo River plume was identified in all images for 1999, 2001, 2003 and 2004 period; 2)the river plume varies in morphology and direction for each period; 3) major river plume direction is to the west and south. 4) major southward movement of river plume was identified for 2004 arriving close to Belvedere coastline; 5) a second major suspended sediment plume was identified in Puerto Real Bay at Cabo Rojo for all periods; 6) a major sediment plume distribution was identified for 2004.

- **Geologic Study Results**

The area of study, with respect to the infrastructure using septic tanks, contains soil types Cabo Rojo clay (CaC), Tidal swamp (Td) and the Voladora silty clay loam (VoD2). The CaC has a percent slope of 2-12, a moderate permeability, a medium runoff and a hazard for erosion, so this kind of soil can bring pollution as sediments to the marine ecosystem. Also, this kind of soil in structure is a mix of clay, with sticky texture of high plasticity. The movement of water or other substances in it, will be slow. The Tidal swamp, is covered mostly with mangroves. It is most of the time under salt water, it has a high water table and any substance can be transported to the marine ecosystem. By this extrapolation it can be said but not established that the soil in the study area represents a route of exposure of contaminant related to water quality in slow movement or a direct one, as with the tidal swamp. It can be established from the soil type analysis that the area can be a source of pollution in terms of sedimentation. For a precise explanation of the behavior of substance in the soils within the area of study survey of it is needed. Also it will help the contour lines of underground water. There are no previous studies in the area of focus in terms of underground water movement.

Figure 5.

Number of Septic Tanks by Street Location in Area Next to the Wetland and Puerto Real Bay, 2006



Número de pozos sépticos en cada calle

- **Physicochemical analysis results**

This study showed that water quality along the western coast of Puerto Rico is significantly influenced by land-derived activities that produce frequent non-point source pollution pulses. This has resulted in a nearly permanent state of high inshore water turbidity. Ten out of the thirteen study sites (77%) showed at any given moment violations to the existing water turbidity standard of 10 NTU for SB classified marine waters, as set by the PR Environmental Quality Board (EQB). Also, 50% of the time there were sampling sites showed turbidity violations. Oceanographic dynamics may often carry out turbid polluted waters offshore impacting significant coral reef and seagrass habitats that function as essential fish habitats for fish and macroinvertebrate species of high commercial value. Gradient patterns were not completely clear due to oceanographic variable dynamics during the course of the study, but evidence of overall habitat quality degradation suggest that there is a definite long-term trend of environmental degradation associated to human-derived water pollution.

Data shows evidence that turbid waters may significantly influence inshore waters, and often affect habitats located up to 10 km offshore, such as El Ron. Turbid and contaminated runoff from the Guanajibo River plume, as well as from non-point sources from Puerto Real Bay, Joyuda Bay, and possibly from Playa Buyé and Boquerón Bay, are negatively impacting inshore waters at a platform-wide scale.

- **Microbiological analysis results**

This region receives the direct and consistent impact of fecal contamination from local non-point sources that include: illegal crude discharges from residences and local businesses, infiltration of septic tanks, discharges without treatment that enter small streams that end in Río Guanajibo, possible waste dumping from boats and house-boats, and discharges of cattle and other animal farms. The influence of Guanajibo River represents one of the main sources of sediments and sewage pollution that cause increasing turbidity levels in the region. This situation affects the measurements of microbial indicators.

High fecal indicator counts revealed that the sites sampled received periodical influence of various non-point pollution sources. In several sampling sites fecal indicators detected were above the recognized standards by the Environmental Quality Board of Puerto Rico (EQB). For beaches these are 200 cfu/100mL, for fecal coliforms and 35 cfu/100mL for enterococci (EQB, 2006). These waters are mainly coastal and estuarine water used for primary and secondary contact.

The sampling stations were clustered into two groups according to the distance from the shoreline. Sites that were clustered into the "inshore" category (<0.5 km off the coast) showed violations to the EQB fecal indicator standards when all the sampling periods were included in that particular cluster. These sites are closer to point and non-point sources of fecal pollution.

The variability of the results suggests that presence of indicator bacteria varies notably during short periods of times. Clearly, the sites that went over the standards previously stated and are not suitable for recreational purposes.

The sites were clustered as Marine Protected Areas (MPA) and Non-Marine Protected Areas (Non-MPA). Sites that are included in MPA are Ron (Tourmaline Area), CR (Cayo Ratones and adjacent water resources), AF, PC and PRP (all part of Finca Belvedere Natural Reserve Marine Extension). The rest of the sites are Non-MPA. Based on the results obtained for FC and ENT for all sampling stations, the sites that were part of Non-MPA violated standards for the microbial fecal indicators tested. This could be a result of management practices for those sites. If this is the case, management practices need to be implemented, since some of these sites are used for commercial fishing and harbor coral reef and seagrass habitats.

Fecal coliform concentrations were overall higher in inshore sampling stations when compared to offshore sites. However, they showed fluctuations that seemed to be associated to factors such as: 1) tidal flow; 2) turbidity; 3) wind direction and speed; 4) heavy rainfall; and 5) Guanajibo river stream flow. These fluctuations are suggested by the presence of higher concentrations of fecal coliforms in samples collected during strong winds, ebbing tides followed by heavy rainfall runoff and after being impacted by the Guanajibo river plums. A similar pattern is evident in enterococci counts for these sites.

It is expected that some environmental parameters, such as water salinity can influence the presence of indicator bacteria. In this study the variations in salinity occurred in sites where estuarine or freshwater conditions persisted (GREB and GRM). However, fecal pollution could be detected for these two sites for all the months sampled. These results could indicate that this area is constantly polluted with non-point fecal contamination sources.

It is evident that Guanajibo River and other major bodies of water from the region have influences in water quality of the western shelf area of Puerto Rico. The peak values of Guanajibo River stream flow was registered in October 2005, but it was not possible to sample in this date due to weather conditions. However, during moderate but significant stream flow peaks (July 2005, August 2005, September 2005, and November 2005), microbial counts in Ron, PO, AF, PC, PLOB, PLIB, PRFV, and PRP were variable, being September 2005 the month with the highest values for fecal coliforms.

Sites close to sources fecal contamination like GREB and GRM registered the highest values for fecal coliforms and enterococci. This probably occurred because the river could be influenced by tributary streams where temperature, light, and/or nutrient conditions favor enhanced bacterial survival. Also non-point sources of contamination discharge into this river and pollutants are eventually carried out to the estuary.

Non-point source fecal pollution was detected along vast areas of the southwestern Puerto Rico platform. This is a threat to public health, since HF183 MST marker revealed fecal pollution coming from a human source. In this context, it is important to determine non-point sources of fecal pollution in order to prevent diseases, especially within MPAs used for fishing and recreational purposes. Moreover, human fecal pollution was found within MPA systems that support significant coral reef, seagrass and mangrove communities. These areas function as nursery, shelter, feeding and/or spawning grounds for a wide variety of fish and macroinvertebrate species of commercial significance. Chronic sewage pollution and eutrophication, in addition to other anthropogenic causes of stress (i.e., turbidity, sedimentation, overfishing), could have significantly contributed to the overall environmental degradation and ecological decline of these communities.

- **Coral reef evaluation results**

This study has shown evidence that coral reefs along a significant portion of the southwestern Puerto Rico shelf are being severely impacted by non-point source sewage pollution, mostly from human origin. The combination of historic natural factors (i.e., hurricanes) with long-term non-point source pollution, and other potential anthropogenic reef degrading factors, such as sedimentation and overfishing, have contributed to a dramatic phase shift in coral reef community structure. Phase shifts have favored dominance by macroalgae and non reef-building taxa. Such changes are generally irreversible at least in a human generation time scale (Knowlton, 1992; Hughes, 1994; Bellwood et al., 2004). Most often this is due to a phenomenon known as hysteresis, which is a phenomenon where a recovering community follows a different trajectory from that observed during the decline (Hughes et al., 2005). For instance, a declining reef previously dominated by massive corals might have shifted towards other community dominated by macroalgae or other non-reef building taxa. In the long-term, it may show some signs of recovery towards another alternate state such as dominance by octocorals. This is the case of Arrecife Fanduco, and most probably Punta Ostiones and Punta Arenas.

Declining coral reefs by sewage pollution along the western Puerto Rico shelf have resulted in significant declines of entire coral assemblages. This has resulted in a dramatic loss of functional redundancy (sensu Bellwood et al., 2003). The most dramatic example is the extirpation of Elkhorn coral from most inshore coral reefs. *Acropora palmata* constitutes a functional mono-specific group responsible of constructing an entire reef zone. Losing the *palmata* zone might have most probably resulted in a major biodiversity decline at the entire reef ecosystem level due to the net loss of nursery, shelter and feeding grounds of a myriad of fish and invertebrate species.

Another significant finding is that local MPAs had no significant impact on the status of coral reef benthic communities. Current management activities by the PR Department of Natural and Environmental Resources (DNER) at Tourmaline Natural Reserve, Cayo Ratones and Adjacent Waters Natural Reserve, and at Finca Belvedere Natural Reserve marine extension are largely limited. There are no specific management actions implemented either by DNER or the

PR Environmental Quality Board (EQB) to address the imminent negative impacts of non-point source sewage pollution in coral reef ecosystems.

This study showed that coralline communities along the entire southwestern Puerto Rico coast are being constantly impacted by sewage pollution. Even offshore remote reefs are being often impacted by pollution pulses from the Guanajibo River plume, and during heavy sediment resuspension associated to variable oceanographic dynamics. According to ISRS, this could have paramount long-term negative impacts in reef communities by: (a) reducing coral larval production; (b) reducing coral recruitment; (c) increasing incidence of coral disease; (d) reducing coral skeletal density; (e) increasing coral mortality; (f) reducing coral species diversity; (g) producing a community phase shift; (h) enhancing bioerosion; (i) enhancing macroalgal growth and biomass; and (j) and by probably enhancing coral predators abundance.

It is probable that most inshore coral reefs along the southwestern Puerto Rico shelf have degraded beyond recovery within a human time scale. Further, water quality degradation is of such magnitude that recovery may never occur. Stronger efforts are needed from the government of Puerto Rico to prevent further degradation of remote reefs through the region. There is an immediate need to implement a sound management strategy to reduce and/or prevent non-point source sewage pollution impacts in coral reef habitats before we witness a reef ecological and socio-economic collapse within the next few years.

E. Conclusions and Recommendations

Actions must be taken to reduce septic tank and sewer bypass outflows into coastal waters of the Belvedere Reserve region. All households should be connected to existing sewers, constructing pumping facilities. Households located near shore should be relocated if possible. Sewer cleanup and pumping station improvements should be carried out regularly by the Puerto Rico Aqueducts and Sewers Authority to prevent outflows of raw sewage to coastal waters though bypass valves. All nearshore business operations must be connected to sewers for all their discharges. Houseboats should be relocated elsewhere to places where they do not represent an ecological hazard. These recommendations are based on the conclusion that significant sewage discharges are reaching coastal waters in the region. The worst effect is in Puerto Real Bay, where water-based fishing and recreational activities are severely hampered. This study also showed that human waste discharges are affecting coral reefs, with the associated ecological impacts

F. Project Participants

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Appendix A 1

Water quality dynamics across a non-point source sewage stress gradient along the southwestern Puerto Rico shelf.

Edwin A. Hernández-Delgado

A1. Water quality dynamics along a suspected non-point source sewage stress gradient across the western Puerto Rico shelf.

Introduction.

Waterborne diseases have been among tropical countries greatest public health problems, particularly because there is a higher diversity and severity of these diseases in comparison to temperate zones. The combination of large population densities and often lack of wastewater treatment may result in greater waterborne disease effects in tropical countries (Santiago-Mercado and Hazen, 1987). In addition, lack of wastewater treatment often results in another threat, non-point source sewage pollution, which implies a wide spatial distribution of fecal pollution from a wide variety of sources. These may include human settlements, cattle grazing areas, farms, malfunctioning septic tanks, sewers and treatment facilities, etc. In addition, high rainfall rates in many tropical countries may result in frequent pulses of high concentrations of allochtonous nutrients and microbes, which in combination with constantly high temperature, may result in nearly constant favorable conditions for microbial growth under environmental conditions (Hazen and Toranzos, 1990). Thus, many studies in tropical countries have found a widespread presence of point and non-point source fecal pollution indicators in all kinds of source water and soil samples (Santiago-Mercado and Hazen, 1987; Hazen, 1988; Hernández-Delgado and Toranzos, 1990, 1995; Hardina and Fujioka, 1991; Hernández-Delgado, 1991; Hernández-Delgado et al., 1991; Toranzos, 1991; Alvarez et al., 1993), as well as marine habitats (Toranzos and Hernández-Delgado, 1992). Further, it has been shown that survival and growth of enteropathogenic microbes may directly depend upon water quality (Carrillo et al., 1985; Hazen et al., 1987; López-Torres et al., 1987; Bermúdez and Hazen, 1988; Pérez-Rosas and

Hazen, 1988). Thus, non-point source sewage pollution in tropical islands often results in rapid contamination of estuarine and coastal waters, and that water quality of runoff sources, including estuarine waters, may play a significant role in determining the fate of allochthonous microbes arriving to the marine environment.

Non-point source sewage pollution is one of the most significant causes of concern in tropical coastal waters, particularly from the public health standpoint. Increasing chronic non-point source sewage pollution may result in the permanent degradation of water quality impairing most human direct (i.e., swimming, other types of direct contact recreation) and indirect (i.e., fishing) uses. In this context, it is important to identify non-point sources of fecal contamination in order to prevent diseases and improve water quality (Scott, at al., 2002), as well as to manage human uses of coastal waters. From the standpoint of marine ecosystem conservation, chronic water quality degradation associated to non-point sewage pollution can have profound long-term irreversible effects in the integrity and community structure of coral reefs, seagrass communities, mangroves and other associated coastal systems (Cloern, 2001), as well as in the composition of marine food webs (Livingston, 2001).

Effects of coastal water quality degradation associated to non-point hypertrophic pollution sources have been reviewed by McComb (1995), Kennish (1997), Cloern (2001), Livingston (2001) and Szmant (2002). Impacts may typically result in hypereutrophication, increasing biological oxygen demand, hypoxia and anoxia (Kennish, 1997). Also, it may include increasing water turbidity, declining sunlight penetration and deterioration of sediment quality (Livingston, 2001). Further, it may result in the development of phytoplankton blooms (McComb, 1995), a general decline of fisheries (Hodgkiss and Yim, 1995), and in a decline in seagrass communities (Duarte, 1995) and coral reefs (Hernández-Delgado, 2000, 2005; Cloern, 2001; Kaczmarsky et al., 2005). Water quality degradation is particularly a concern in shallow coastal habitats due to sediment accumulation of nutrients and contaminants (McComb, 1995). Sediment resuspension as a function of oceanographic (i.e., tidal cycles, surface currents, wave action) and atmospheric dynamics (i.e., winds), as well as a result of navigation activities, can be a major factor in nutrient accumulation and loading processes as it has been previously found in temperate environments (de Jong, 1995; de Jong and van Raaphorst, 1995). Nutrient-rich tropical marine sediments may also function as an enterotoxigenic pathogen reservoir. Further, natural localized natural oceanographic processes, such as upwelling, can also result in increasing nutrient concentrations that may trigger ecosystems responses that could be easily confounded with anthropogenic eutrophication effects (Szmant, 2002). Therefore, water quality dynamics are of paramount importance in understanding microbial population dynamics in coastal marine environments and should be an intrinsic part of standard microbial water quality and long-term ecological monitoring programs aimed at understanding causes and effects of water quality degradation in coastal communities.

This study was aimed at characterizing the dynamics of physical water quality parameters at 13 sampling sites distributed along the western Puerto Rico coast in the Cabo Rojo area (Figure 1.1). We tested the general null hypothesis of lack of significant geographic gradients in water quality across a distance gradient from known non-point sewage pollution sources in the western coast of Puerto Rico.

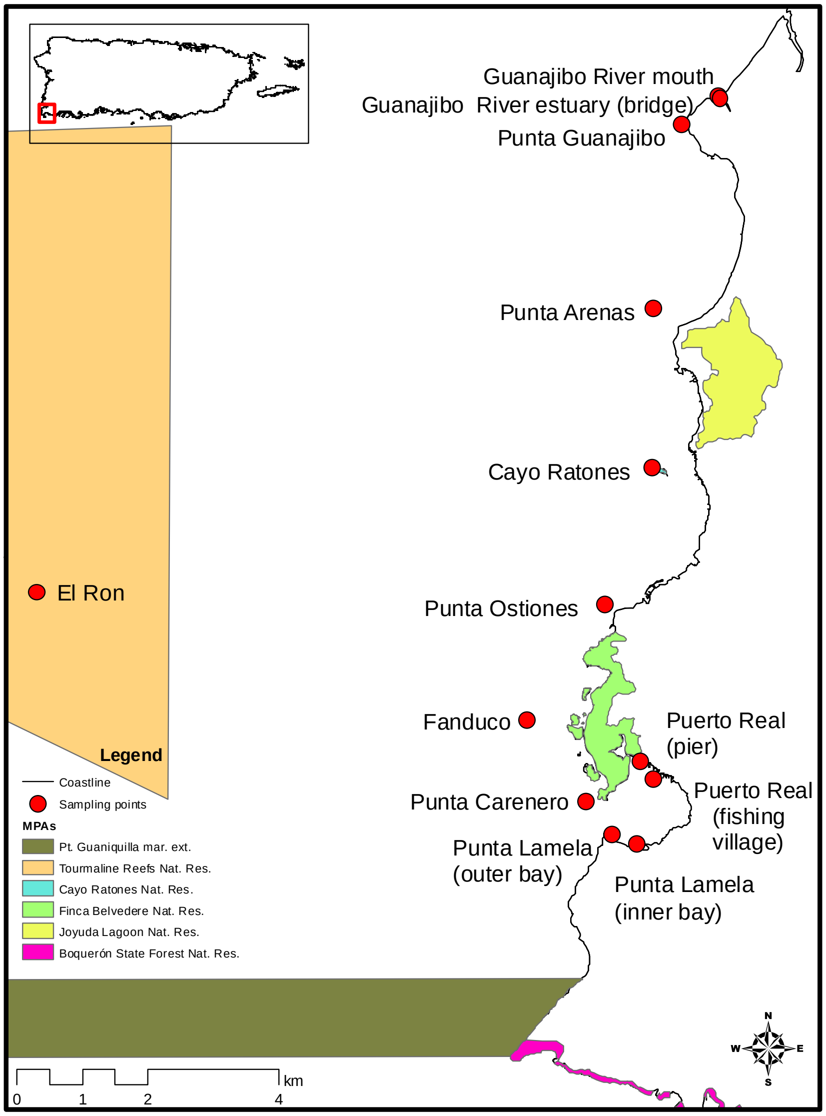

FIGURE 1.1. Water quality sampling sites and their relationship to the existing local PRDNER-managed natural protected area network.

Methodology.

Water quality sampling sites.

Studies were carried out at 13 sampling sites located off the Cabo Rojo coast in the western Puerto Rico shelf (Figure 1.1). These included the following: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites were representative of predominant benthic habitat types through the region: coral reef and/or coralline communities (sites 1, 2, 3, 4, 10, and 11), seagrass communities (sites, 1, 2, 3, 4, 5, 8, 9, 10, 11, and13), and mud bottom (sites 2, 3, 4, 5, 6, 7, 8, 9,10, 11, 12, and 13).

Water quality parameters.

Monthly measurements of water quality data such as sea surface temperature, pH, salinity (ppt), conductivity (ms/cm), and dissolved oxygen concentration (mg/L) were obtained in triplicates during each monthly visit to study sites using portable instrumentation (Horiba, Co.). Ten replicate lectures of water turbidity were obtained during each monthly visit to study sites using a Orbeco 866 portable turbidimeter. Turbidity data was expressed in nephelometric turbidity units (NTU). Data from sites 1-8 was collected from June 2005 to June 2006, with the exception of October, 2005. Data from sites 9-13 was collected between December 2005 and June 2006.

Hypothesis testing.

We tested the null hypothesis of no significant difference in water quality parameters among sites. Temporal data was pooled for each site and tested for normality (Shapiro-Wilk test) using Statistix Software 8.0 (Analytical Software). Data was analyzed using a one-way analysis of variance (ANOVA). *Site* was used as main variable and replicate samples through time as error term. We tested a second null hypothesis of no significant difference in water quality parameters between geographical locations. Locations were subdivided into *Inshore* (<0.5 km) and *Offshore* (>0.5 km). Inshore sites included sites 5, 6, 7, 8, 9, 12, and 13. Offshore sites included sites 1, 2, 3, 4, 10, and 11. Locations were arbitrarily selected based on distance of sampling site from known or suspected non-point source sewage pollution. Temporal data was pooled for each geographical location and tested for normality as above. Data was analyzed using a one-way ANOVA. *Geographical location* was used as main variable and replicate samples through time as error term.

We also tested a third null hypothesis of no significant difference in water quality parameters through time. Spatial data was pooled for each month and tested for normality as above. Data was analyzed using a one-way ANOVA. *Time* was used as main variable and replicate spatial samples as error term. Finally, we tested the null hypothesis of no significant effect of management regime (i.e., marine protected area, MPA) on water quality parameters. Estuarine sampling sites 12 and 13 were excluded from this analysis because of significant departure from normality due to their natural estuarine conditions in comparison to marine sampling sites. Sampling sites were grouped as follows: *Within MPA sites*: Arrecife El Ron, Punta Carenero, Puerto Real-pier, Cayo Ratones), with all remaining sites as *Control Outside MPA sites*.

Management was used as main variable and replicate samples through time as error term. We included within the MPA category Punta Carenero and the Puerto Real-pier sites because they were located in close proximity (<200 m) to Finca Belvedere Natural Reserve boundaries (Figure 1.1).

A Pearson correlation analysis was performed to test relationships between individual water quality parameters and geographic distance from the Guanajibo River mouth, Puerto Real Bay and the coastline. Regression analysis were specifically conducted between water turbidity and geographic distance from the Guanajibo River mouth, Puerto Real Bay and the coastline to test for any gradient effect.

Rainfall (inches) and streamflow (ft^3/s) data from the Guanajibo River were obtained from the U.S. Geological Survey Guanajibo River sampling station readily available in the internet (http://waterdata.usgs.gov/pr/nwis/uv?50138000. Daily data was obtained from June 1, 2005 to June 30, 2006. Daily means were calculated and temporal trends were analyzed using one-way ANOVA. Also, linear regression analysis was used to test for the relationship between Guanajibo River dynamics and water turbidity across the western Puerto Rico shelf.

Results.

Null hypothesis 1: ***No significant difference in water quality parameters among sites.***

Mean water turbidity values ranged from as low as 0.86±0.25 NTU at Arrecife El Ron to 8.89±0.25 NTU at the Guanajibo River (Figure 1.2). Water turbidity at Puerto Real bay pier site was statistically higher (p=0.0260) than in other sites (Table 1.1). Turbidity values showed wide fluctuations at each study site as a result of runoff pulses that were coincident with mean higher rainfall and stream flow peaks (see below). Mean surface water temperature ranged from 27.02±0.72ºC at the Guanajibo River mouth to 28.05±0.65ºC at Puerto Real-pier site (Figure 1.3). However, no significant differences among sites were detected. Mean pH ranged from 7.83±0.09 at the Guanajibo River estuary (bridge) to 8.18±0.07 at Punta Lamela inner bay site (Figure 1.4). But no significant differences among sites were detected. Mean dissolved oxygen concentration ranged from 6.98±0.37 mg/L at the Puerto Real fishing village to 7.78±0.25 mg/L at Arrecife Fanduco (Figure 1.5). However, no significant differences among sites were detected. Water salinity showed mean fluctuations from 0.54±0.27 ppt at the Guanajibo River estuary (bridge), and 12.53±5.35 ppt at the Guanajibo River mouth, to 34.03±0.14 ppt at Punta Arenas (Figure 1.6). Salinity at the Guanajibo River estuary and mouth was significantly lower (p<0.0001) than at other sites (Table 1.1). A similar trend was documented with conductivity with mean low values of 1.22±0.50 ms/cm at the Guanajibo River estuary (bridge), and 20.34±8.40 ppt at the Guanajibo River mouth, to 51.90±0.25 ms/cm at Punta Lamela inner bay (Figure 1.7). Wider parameter fluctuations associated to runoff pulses and highly variable oceanographic conditions (discussed below) at the moment of each sampling visit prevented us

TABLE 1.1. Summary results of one-way ANOVA analysis of water quality parameters among sites.

Parameter	**Unit**	**D.F. (within, between)**	**F statistic**	***P* value**
Turbidity*	NTU	12,116	2.05	**0.0260**
Temperature	ºC	12,78	0.22	0.9972
pH	pH units	12,78	1.71	0.0807
Dissolved oxygen	mg/L	12,65	0.55	0.8764
Salinity	ppt	12,78	47.7	**<0.0001**
Conductivity	ms/cm	12,78	43.3	**<0.0001**

*Data from June 2005 to June 2006. Other parameters, data from December 2005 to June 2006.

to detect significant differences in most of the above parameters.

Null hypothesis 2: ***No significant difference in water quality parameters between inshore and offshore geographic locations.***

Mean water turbidity values ranged from as low as 5.86±0.89 NTU at offshore sites to as high as 7.81±0.94 NTU at inshore sites. This difference was statistically significant ($p<0.0001$) (Table 1.2). There were no significant differences in mean surface water temperature between offshore (27.55±0.18ºC) and inshore sites (28.56±0.21ºC). Mean pH showed non-significant fluctuations from 8.09±0.03 at inshore sites to 8.15±0.03 at offshore sites. Mean dissolved oxygen concentration showed similar non-significant fluctuations from 7.35±0.14 mg/L at inshore sites to 7.46±0.12 mg/L at offshore sites. However, water salinity was significantly higher ($p=0.0004$) at offshore sites (33.86±0.10 ppt) than at inshore sites (25.95±1.97 ppt) (Table 1.2). Conductivity was also significantly higher ($p=0.0004$) at offshore sites (51.58±0.18 ms/cm) than at inshore

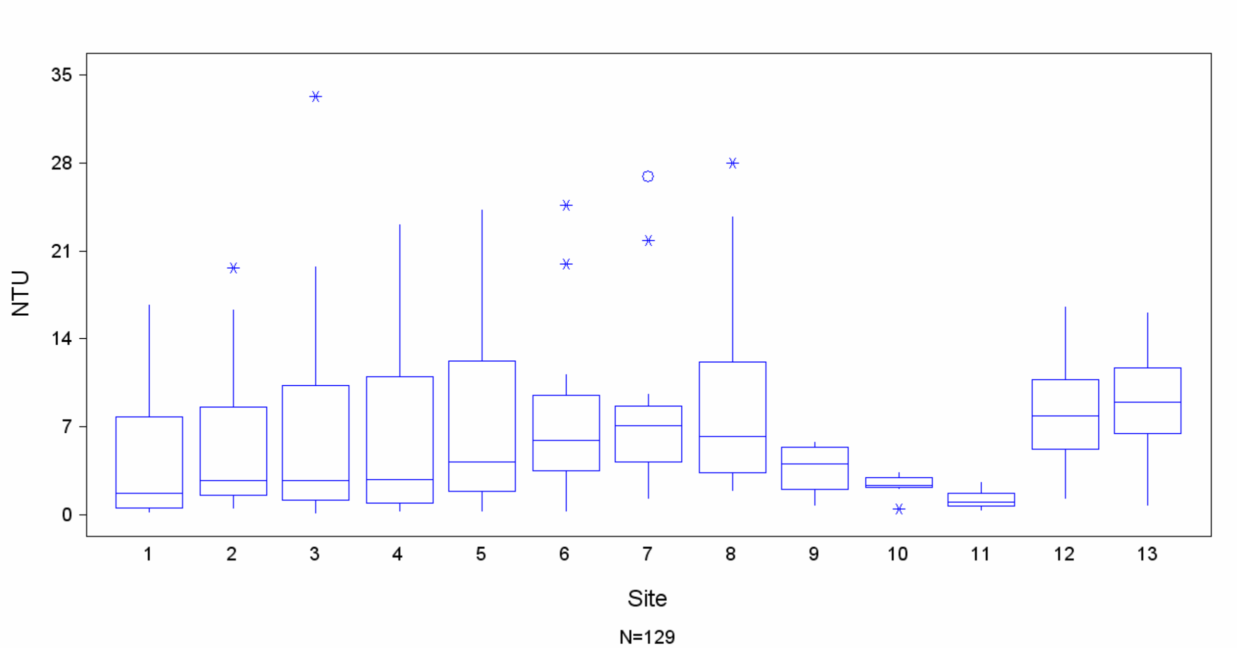

FIGURE 1.2. Box and whisker plot for turbidity values across sites (Sites 1-8 based data from 13 months; Sites 9-13 based on data from 7 months). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Presence of long whiskers and outlier points suggest presence of turbid water pulses from non-point source runoff. Sites: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites 1, 2, 3, 10, and 11 corresponded to coral reef areas where benthic sampling was also conducted in this study. Site 4 also supports coralline communities. With the exception of sites 6, 7, and 12, which are dominated by mud bottoms, all other sites also support seagrass communities.

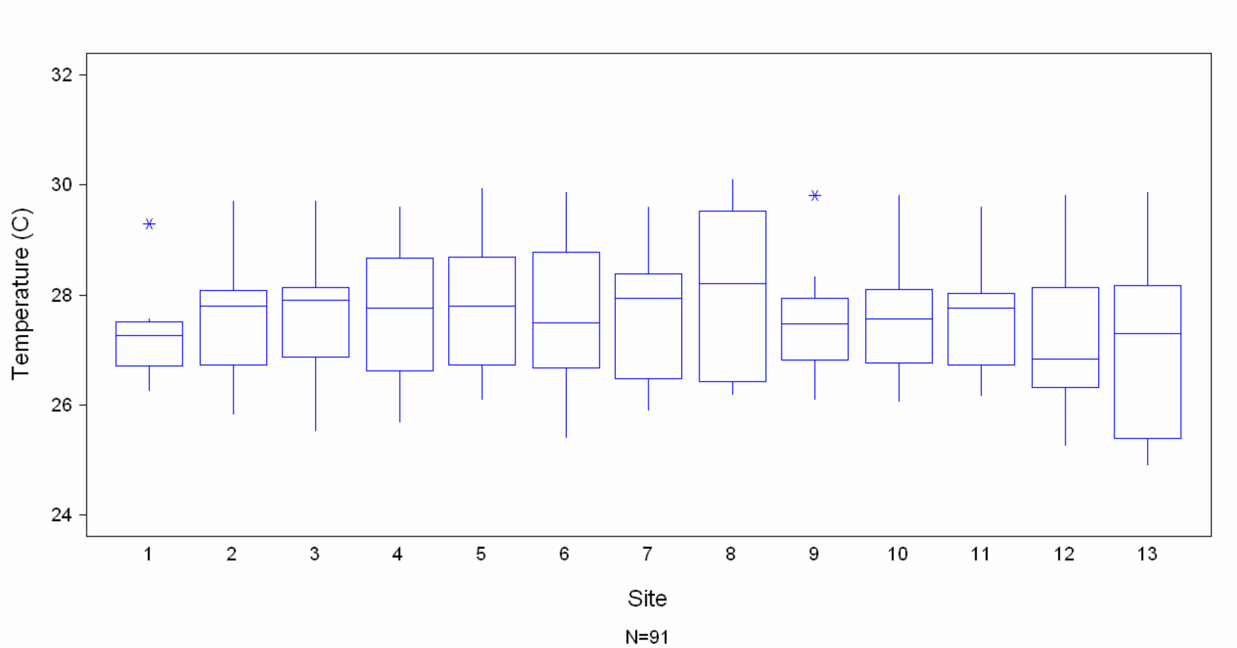

FIGURE 1.3. Box and whisker plot for water temperature values across sites (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Sites: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites 1, 2, 3, 10, and 11 corresponded to coral reef areas where benthic sampling was also conducted in this study. Site 4 also supports coralline communities. With the exception of sites 6, 7, and 12, which are dominated by mud bottoms, all other sites also support seagrass communities.

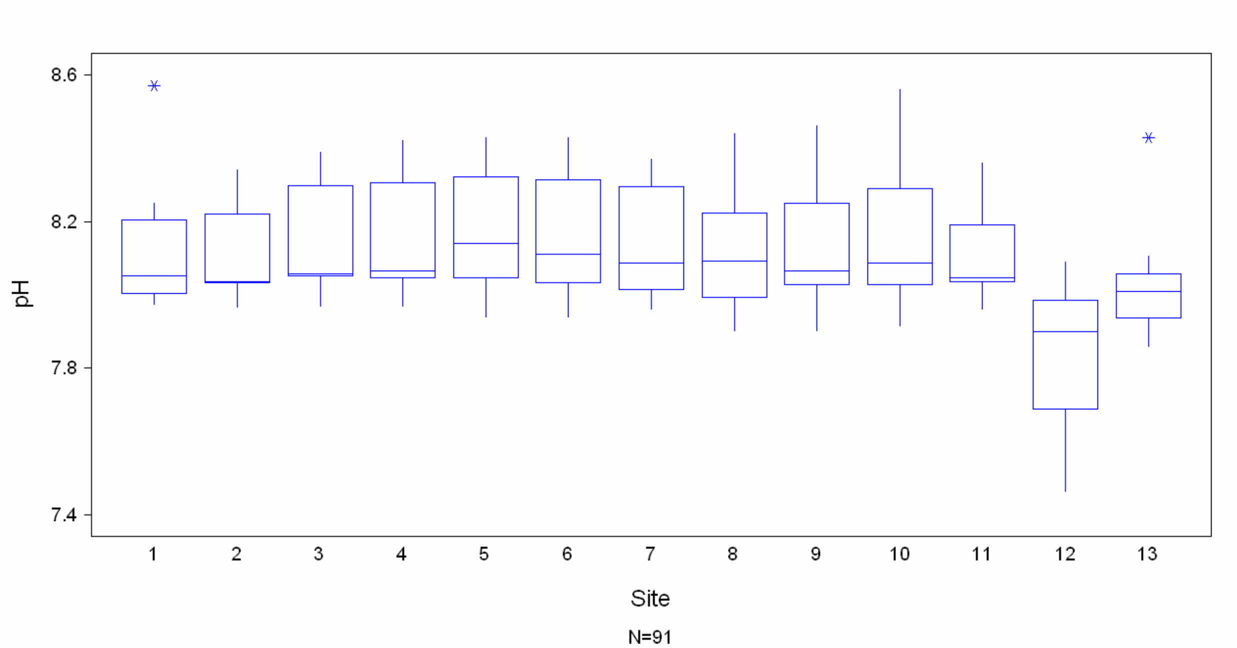

FIGURE 1.4. Box and whisker plot for pH values across sites (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Mean lower values were documented at the Guanajibo River estuary (12) and Guanajibo River mouth (13). Sites: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites 1, 2, 3, 10, and 11 corresponded to coral reef areas where benthic sampling was also conducted in this study. Site 4 also supports coralline communities. With the exception of sites 6, 7, and 12, which are dominated by mud bottoms, all other sites also support seagrass communities.

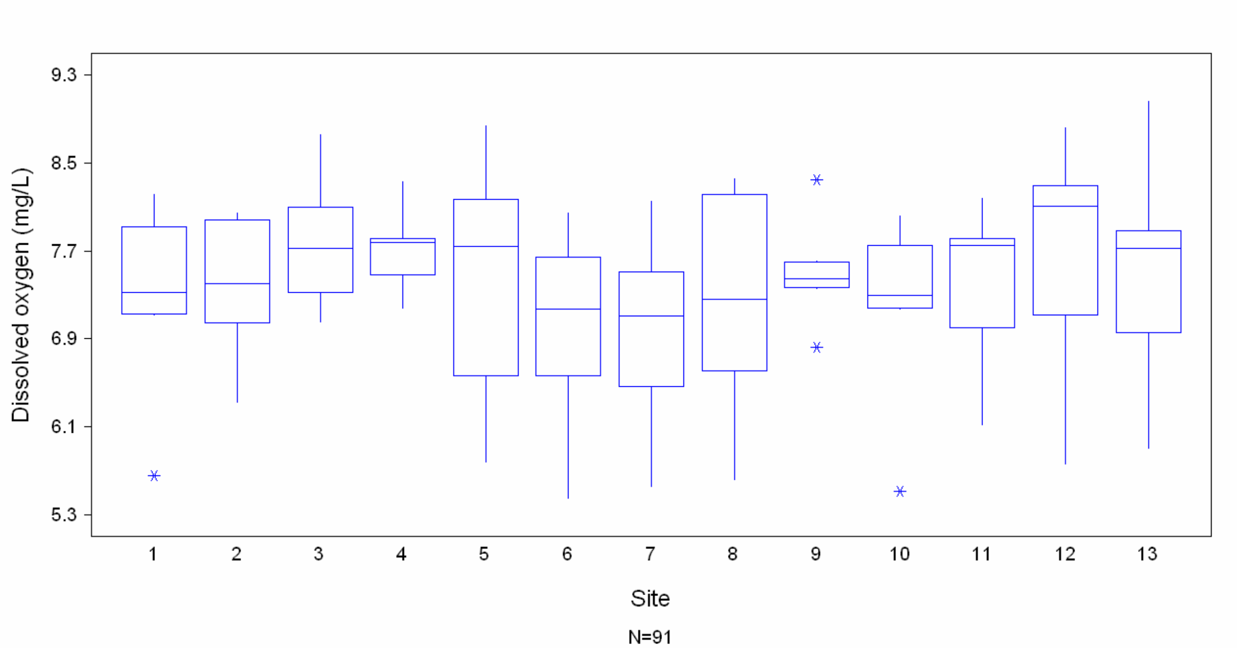

FIGURE 1.5. Box and whisker plot for dissolved oxygen values across sites (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Presence of lower long whiskers and outlier points suggest presence of lower-dissolved oxygen water pulses from non-point source runoff. Upper long whiskers represent stronger oxygen-rich water mixing due to incoming tides and strong winds. Sites: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites 1, 2, 3, 10, and 11 corresponded to coral reef areas where benthic sampling was also conducted in this study. Site 4 also supports coralline communities. With the exception of sites 6, 7, and 12, which are dominated by mud bottoms, all other sites also support seagrass communities.

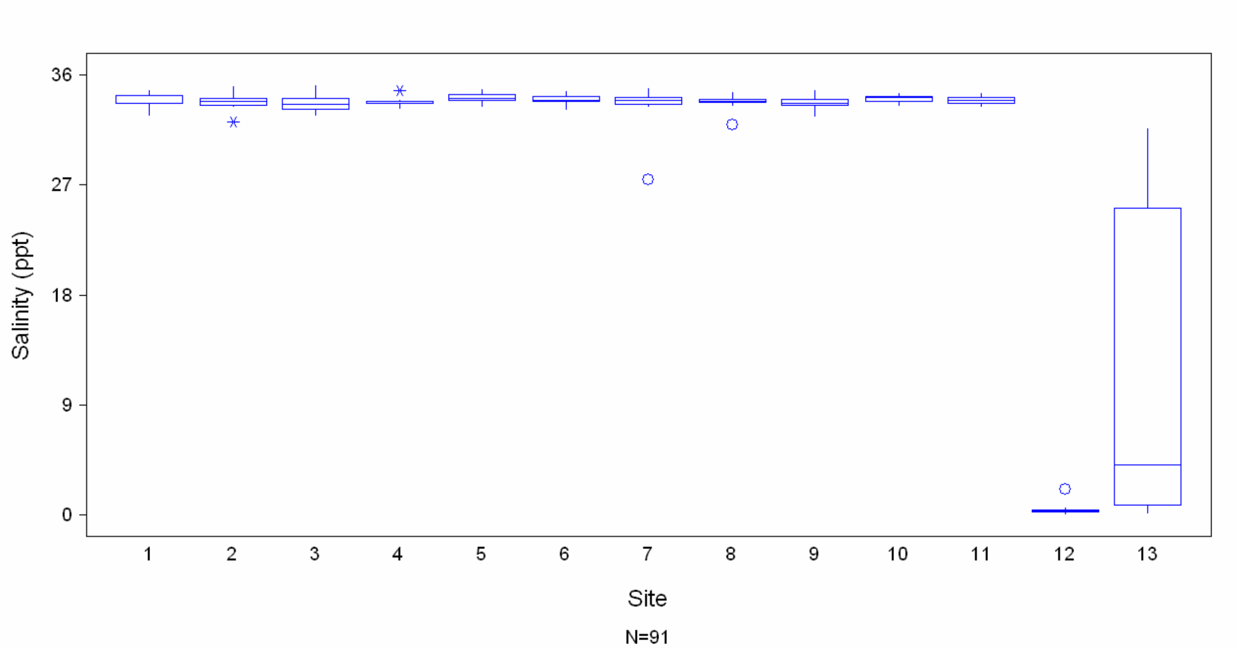

FIGURE 1.6. Box and whisker plot for salinity values across sites (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Presence of lower long whiskers and outlier points suggest presence of lower-salinity water pulses from non-point source runoff. Mean lower values were documented at the Guanajibo River estuary (12) and Guanajibo River mouth (13). Sites: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites 1, 2, 3, 10, and 11 corresponded to coral reef areas where benthic sampling was also conducted in this study. Site 4 also supports coralline communities. With the exception of sites 6, 7, and 12, which are dominated by mud bottoms, all other sites also support seagrass communities.

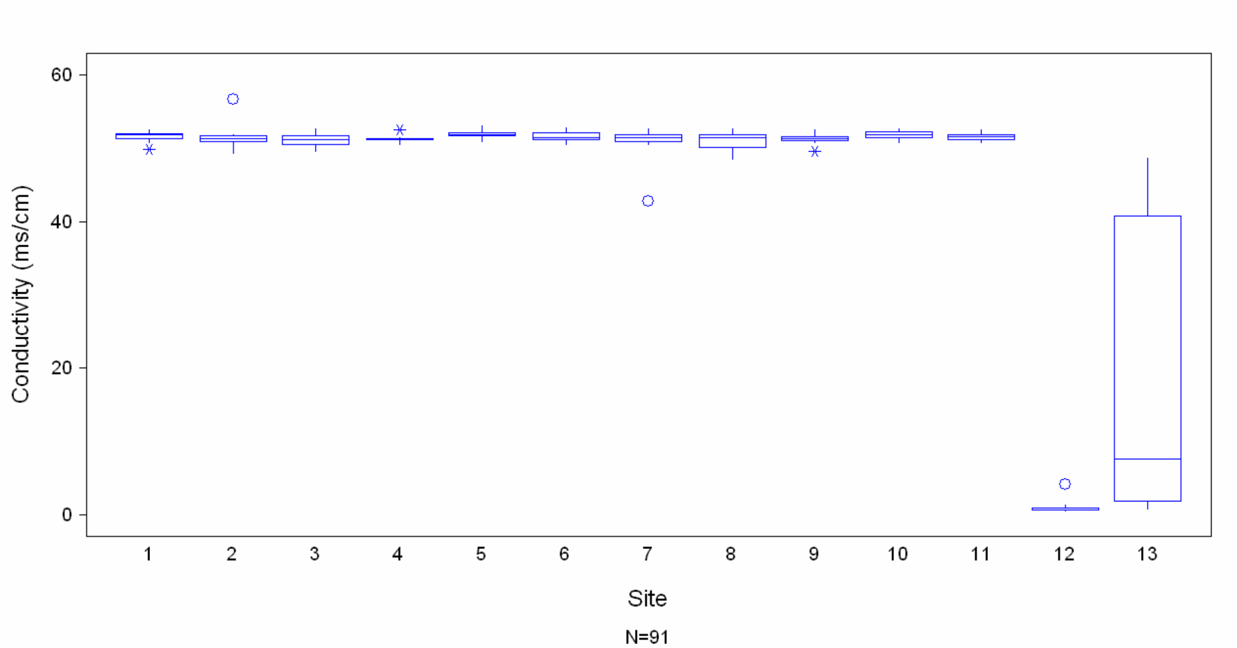

FIGURE 1.7. Box and whisker plot for conductivity values across sites (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Presence of lower long whiskers and outlier points suggest presence of lower-conductivity water pulses from non-point source runoff. Mean lower values were documented at the Guanajibo River estuary (12) and Guanajibo River mouth (13). Sites: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites 1, 2, 3, 10, and 11 corresponded to coral reef areas where benthic sampling was also conducted in this study. Site 4 also supports coralline communities. With the exception of sites 6, 7, and 12, which are dominated by mud bottoms, all other sites also support seagrass communities.

TABLE 1.2. Summary results of one-way ANOVA analysis of water quality parameters between inshore and offshore locations.

Parameter	**Unit**	**D.F. (within, between)**	**F statistic**	***P* value**
Turbidity*	NTU	1,127	7.59	**0.0067**
Temperature	ºC	1,89	<0.01	0.9657
pH	pH units	1,89	1.89	0.1731
Dissolved oxygen	mg/L	1,76	0.33	0.5647
Salinity	ppt	1,89	13.8	**0.0004**
Conductivity	ms/cm	1,89	13.6	**0.0004**

*Data from June 2005 to June 2006. Other parameters, data from December 2005 to June 2006.

TABLE 1.3. Summary results of one-way ANOVA analysis of water quality parameters through time.

Parameter	**Unit**	**D.F. (within, between)**	**F statistic**	***P* value**
Turbidity*	NTU	11,117	39.1	**<0.0001**
Temperature	ºC	6,84	99.5	**<0.0001**
pH	pH units	6,84	19.8	**<0.0001**
Dissolved oxygen	mg/L	6,84	10.6	**<0.0001**
Salinity	ppt	6,84	0.10	0.9958
Conductivity	ms/cm	6,84	0.12	0.9935

*Data from June 2005 to June 2006. Other parameters, data from December 2005 to June 2006.

sites (39.66±2.97 ppt) (Table 1.2). This suggests that non-point source runoff can affect specific water quality parameters mostly at nearby inshore locations.

Null hypothesis 3: No significant difference in water quality parameters through time.

Mean water turbidity values ranged from as low as 1.26±0.41 NTU during December 2005 to 22.72±1.38 NTU during September 2005 (Figure 1.8). Actually, water turbidity peaks were significantly higher ($p<0.001$) between August and November 2005 in comparison to other months through the study (Table 1.3). Thisi coincided with peak rainfall, turbid runoff and peak Guanajibo River streamflow (see below). Mean surface water temperature showed a highly significant increase (p<0.0001) from a lowest value of 25.84±0.10ºC during February 2006 to a highest of 29.74±0.05ºC during June 2006 (Figure 1.9). Mean pH showed a highly significant difference (p<0.0001) from a lowest value of 7.93±0.02 during April 2006 to 8.33±0.05 during December 2005 (Figure 1.10). Dissolved oxygen concentration also showed a highly significant difference (p<0.0001) from a lowest value of 6.67±0.34 mg/L during February 2006 to 8.19±0.10 mg/L during December 2005 (Figure 1.11). Water salinity showed non-significant fluctuations from 28.64±3.44 ppt during February 2006 to 31.26±2.47 ppt during June 2006 (Figure 1.12). A similar non-significant temporal trend was documented with conductivity with a mean low value of 43.56±5.17 ms/cm during February 2006 and highest value of 47.95±3.66 ms/cm during June 2006 (Figure 1.13). Although no significant rainfall or peak stream flows were documented in southwestern Puerto Rico during the months of December 2005 to June 2006, when most of the water quality parameters were documented, mean value fluctuations were largely the result of variable oceanographic dynamics (see below).

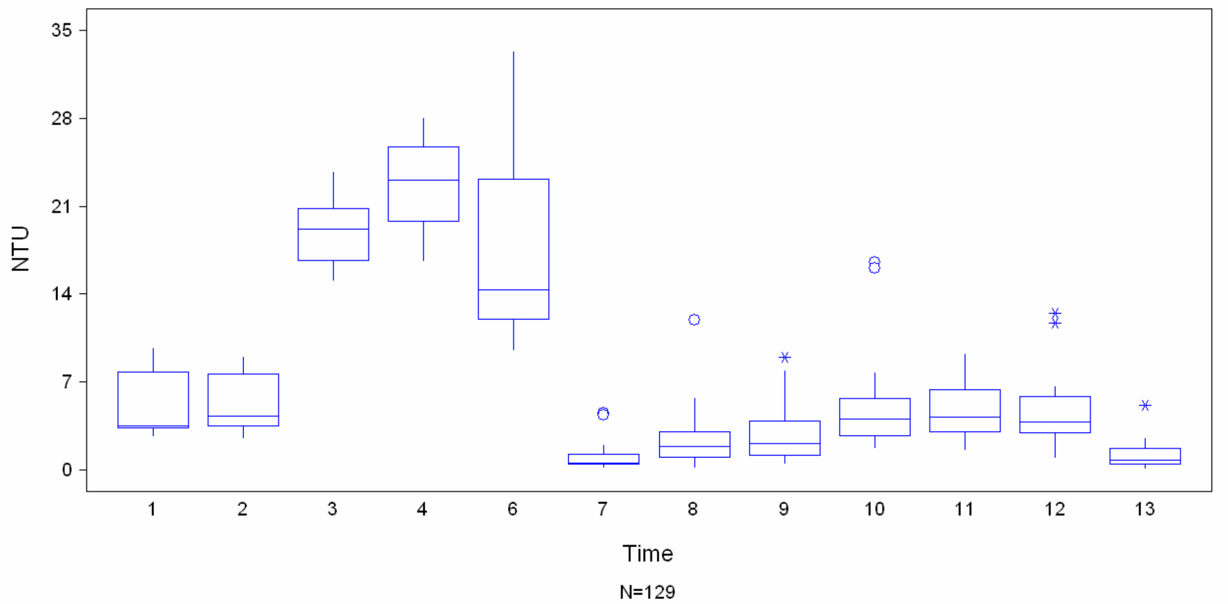

FIGURE 1.8. Box and whisker plot for turbidity values through time (13 month-time span, from June 2005 to June 2006, with the exception of October 2005). Presence of long whiskers and outlier points suggest presence of turbid water pulses from non-point source runoff at some of the inshore sampling sites. Mean higher values were documented during the wet months (August, September, November 2005).

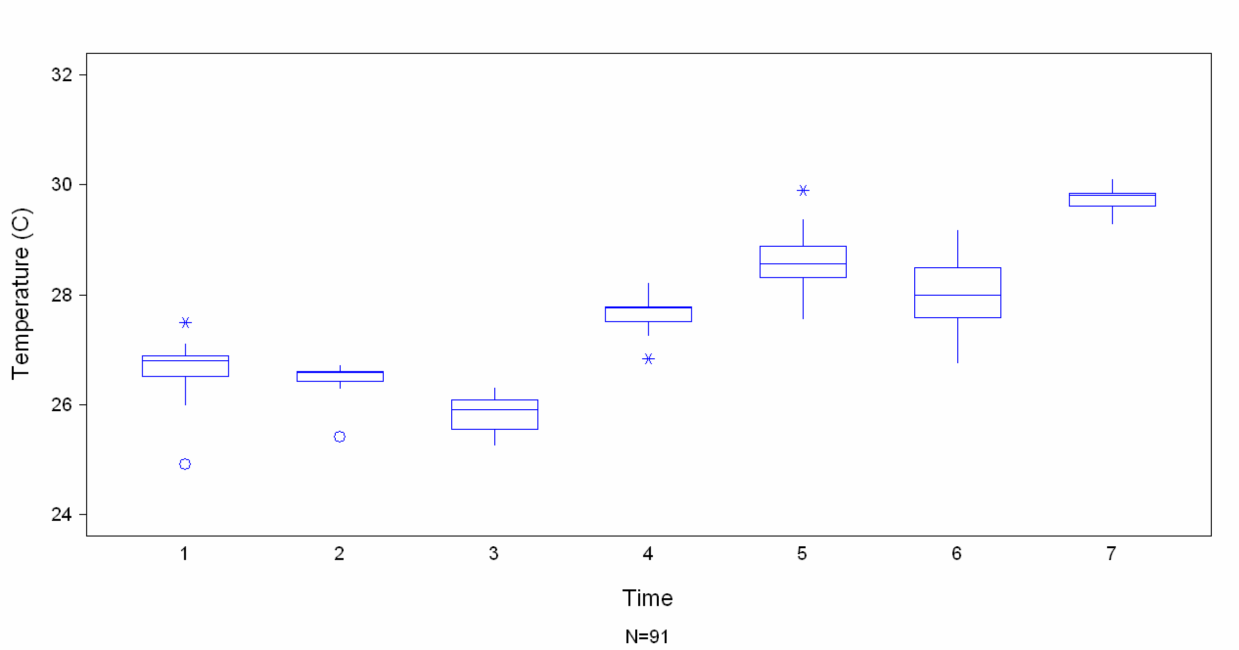

FIGURE 1.9. Box and whisker plot for water temperature through time (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003).

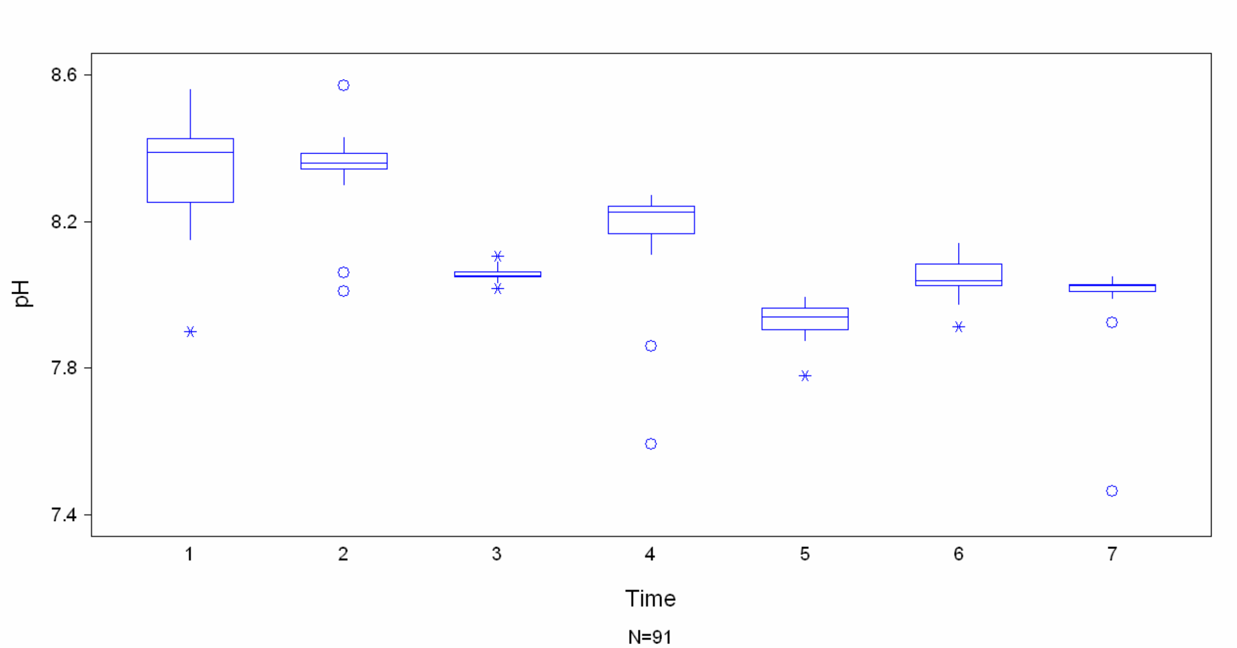

FIGURE 1.10. Box and whisker plot for pH through time (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Mean lower values corresponded to months with higher mean rainfall.

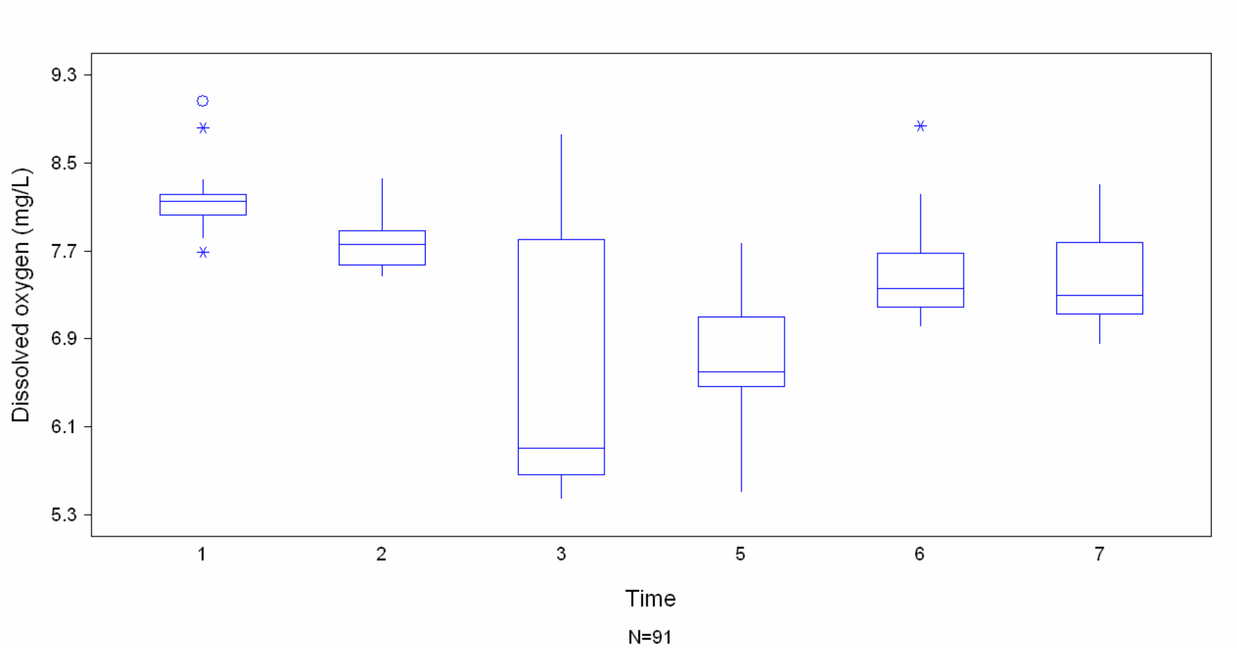

FIGURE 1.11. Box and whisker plot for dissolved oxygen through time (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Presence of lower long whiskers and outlier points suggest presence of lower-dissolved oxygen water pulses from non-point source runoff. Upper long whiskers represent stronger oxygen-rich water mixing due to incoming tides and strong winds.

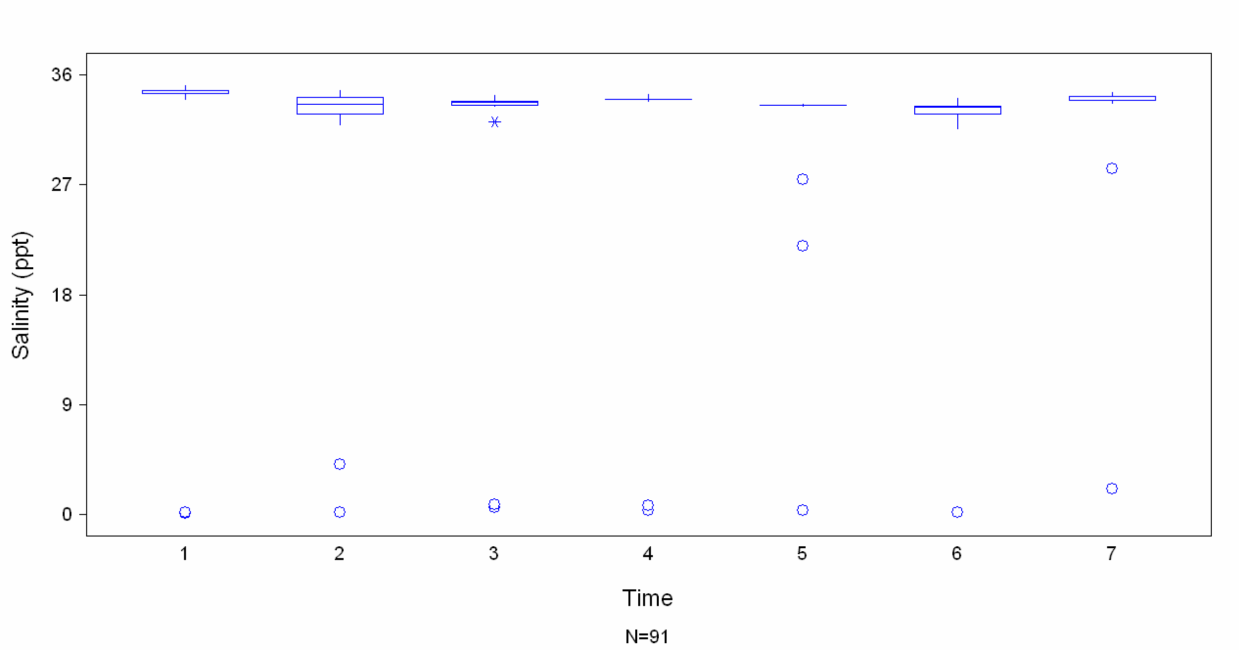

FIGURE 1.12. Box and whisker plot for salinity through time (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Presence of lower long whiskers and outlier points are due to low-salinity waters at the Guanajibo River estuary and river mouth.

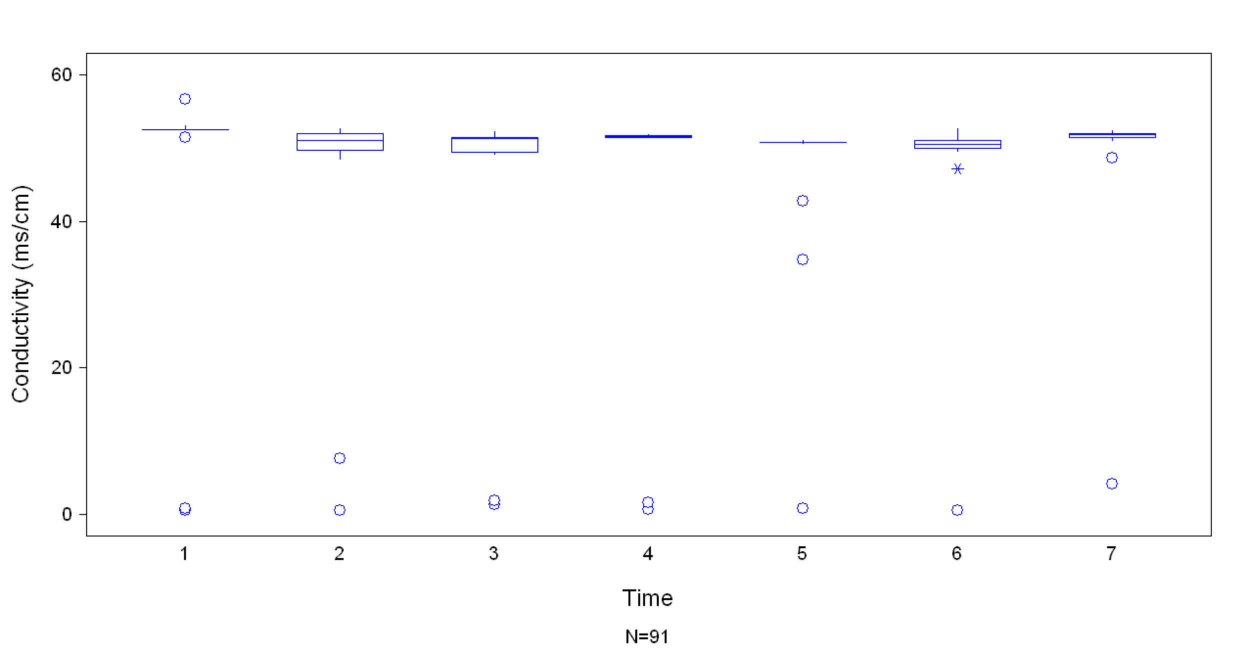

FIGURE 1.13. Box and whisker plot for conductivity through time (based on data from 7 months, December 2005 to June 2006). Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Presence of lower long whiskers and outlier points are due to low-salinity waters at the Guanajibo River estuary and river mouth.

TABLE 1.4. Summary results of one-way ANOVA analysis of water quality parameters between management regimes (MPA vs. non-MPA).

Parameter	**Unit**	**D.F. (within, between)**	**F statistic**	***P* value**
Turbidity*	Log_{10} NTU	1,75	4.15	**0.0451**
Temperature	ºC	1,75	0.01	0.9204
pH	pH units	1,75	0.18	0.6731
Dissolved oxygen	mg/L	1,64	0.16	0.6865
Salinity	ppt	1,75	0.14	0.7086
Conductivity	ms/cm	1,75	0.03	0.8645

*Data from June 2005 to June 2006. Other parameters, data from December 2005 to June 2006.

Null hypothesis 4: ***No significant difference in water quality parameters between management regimes.***

The Log_{10} mean water turbidity values were significantly higher (p=0.0451) outside of MPAs (1.96±0.33 NTU) in comparison to sites within MPAs (2.84±0.29 NTU) (Table 1.4). None of the remaining parameters showed any significant difference between management regimes. Temperatures averaged 27.62±0.18ºC outside MPAs and 27.65±0.25ºC within MPAs, while pH averaged 8.14±0.03 within MPAs and 8.16±0.02 outside MPAs. Dissolved oxygen concentration averaged 7.33±0.12 mg/L outside MPAs and 7.41±0.16 mg/L within MPAs. Salinity averaged 33.76±0.16 ppt outside MPAs and 33.85±0.11 ppt within MPAs, while conductivity averaged 51.34±0.19 ms/cm within MPAs and 51.40±0.24 ms/cm outside MPAs. Mean water quality parameter fluctuations were largely the result of variable oceanographic dynamics (see below).

TABLE 1.5. Summary results of one-way ANOVA analysis of mean daily Guanajibo River stream flow and rainfall.

Parameter	**Unit**	**D.F. (within, between)**	**F statistic**	***P* value**
Stream flow	$feet^3/s$	12,373	77.5	**<0.0001**
Rainfall	inches	12,379	2.81	**0.0011**

*Data from June 1 2005 to June 30, 2006.

Spatial patterns of water turbidity gradients.

Water turbidity was subjected to a sort of regression analyses to test for any spatial pattern in relationship to the geographic distance from the Guanajibo River mouth (Figure 1.14), Puerto Real bay (Figure 1.15), and the closest shoreline (Figure 1.16). In the first case, sampling sites within Puerto Real bay were excluded from the first regression analysis due to local water quality influences. Similarly, Gunajibo River estuary, river mouth and Punta Guanajibo sites were excluded from the second regression analysis due to the local water quality influences by the Guanajibo River. Although all three regressions were significant ($p<0.0006$), no spatial patterns were evident as a result of widely fluctuating turbidity values through the study. These were the result of variable oceanographic conditions and occasional non-point source runoff pulses.

Guanajibo River influences in water quality of the western Puerto Rico shelf.

Guanajibo River stream flow showed a significantly higher ($p<0.0001$) peak value during the month of October 2005 (Table 1.5). Stream flow also showed moderate but significant peaks during the months of July, August, September and November 2005 (Figure 1.17). Stream flow

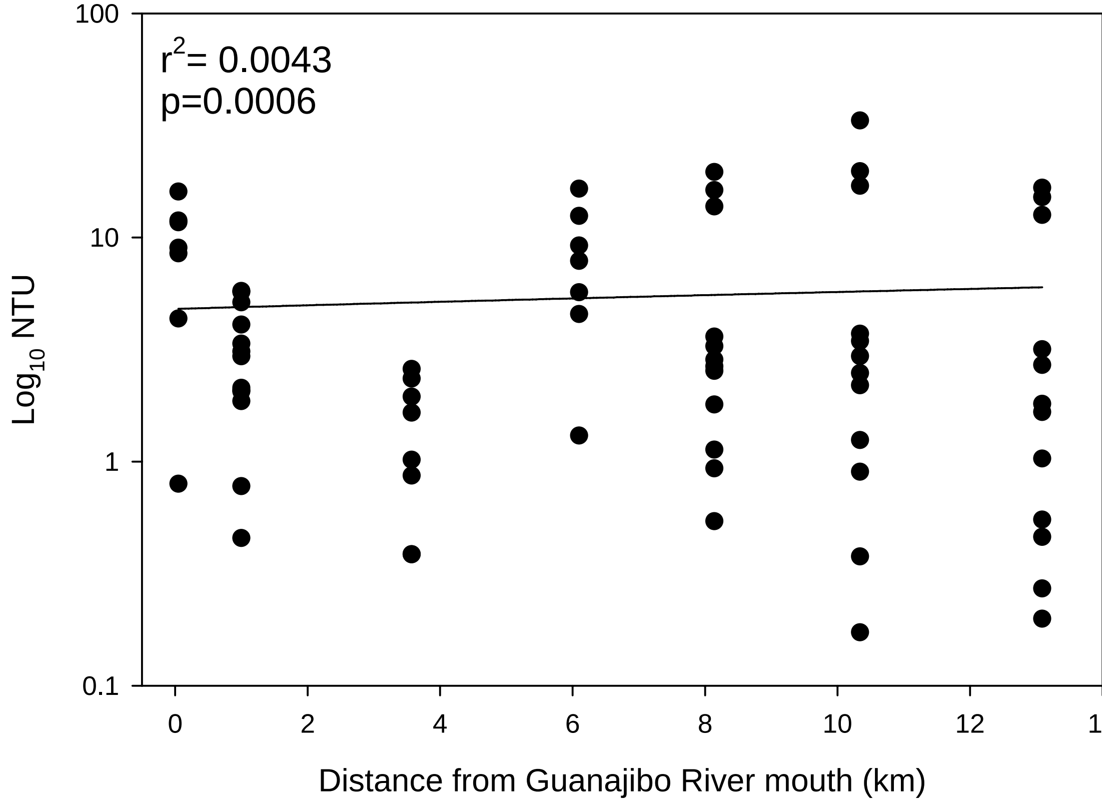

FIGURE 1.14. Linear regression analysis between water turbidity (log_{10} NTU) and distance (km) from the Guanajibo River mouth. This river represents the most significant source of turbid runoff and pollution to the western platform off the Cabo Rojo coast. Lack of a linear pattern results from data "noise", which in turn is a reflection of frequent turbid runoff pulses. Such trends are difficult to catch within short temporal scale sampling efforts (i.e., one year) and may require long-term sampling efforts. Data only from sites known to be directly or potentially affected by the Guanajibo River plume following significant rainfall and flooding events.

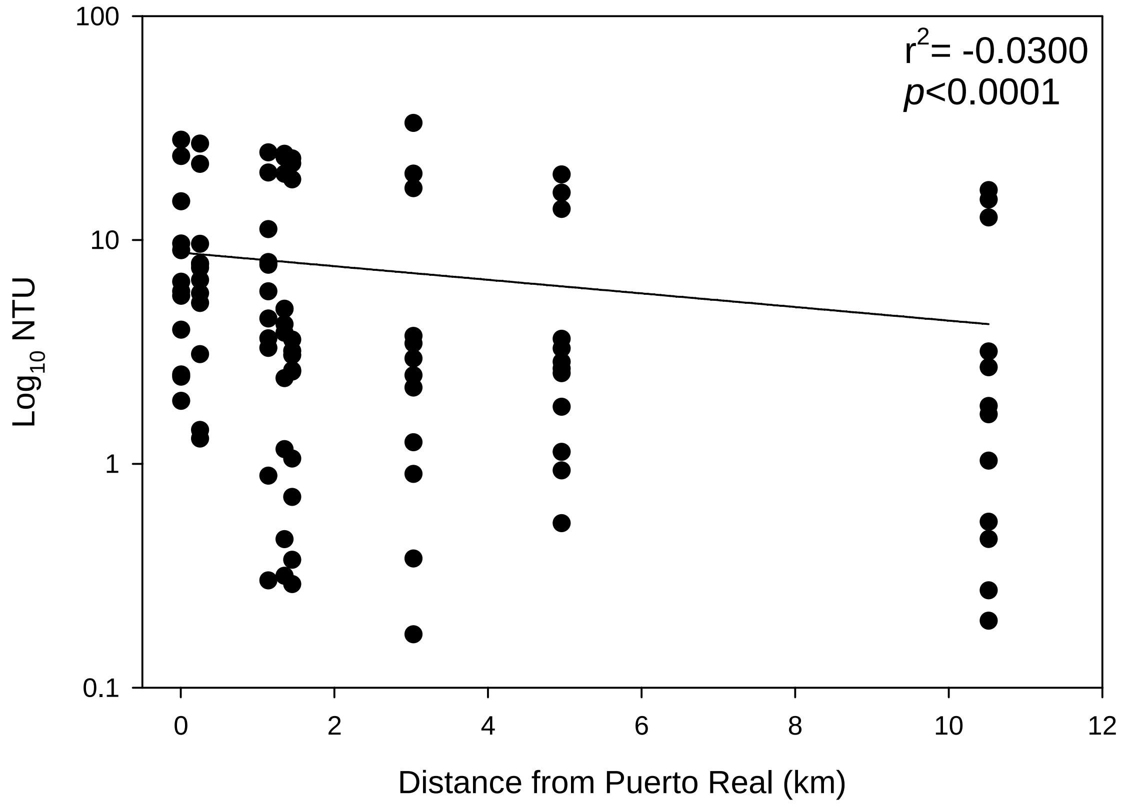

FIGURE 1.15. Linear regression analysis between water turbidity (log_{10} NTU) and distance (km) from Puerto Real bay. This bay represents one of the most significant non-point sewage and turbid runoff sources in the western coast. Lack of a linear pattern results from data “noise”, which in turn is a reflection of frequent turbid runoff pulses. Such trends are difficult to catch within short temporal scale sampling efforts (i.e., one year) and may require long-term sampling efforts. Data only from sites known to be directly or potentially affected by Puerto Real bay outflow following heavy rainfall and runoff.

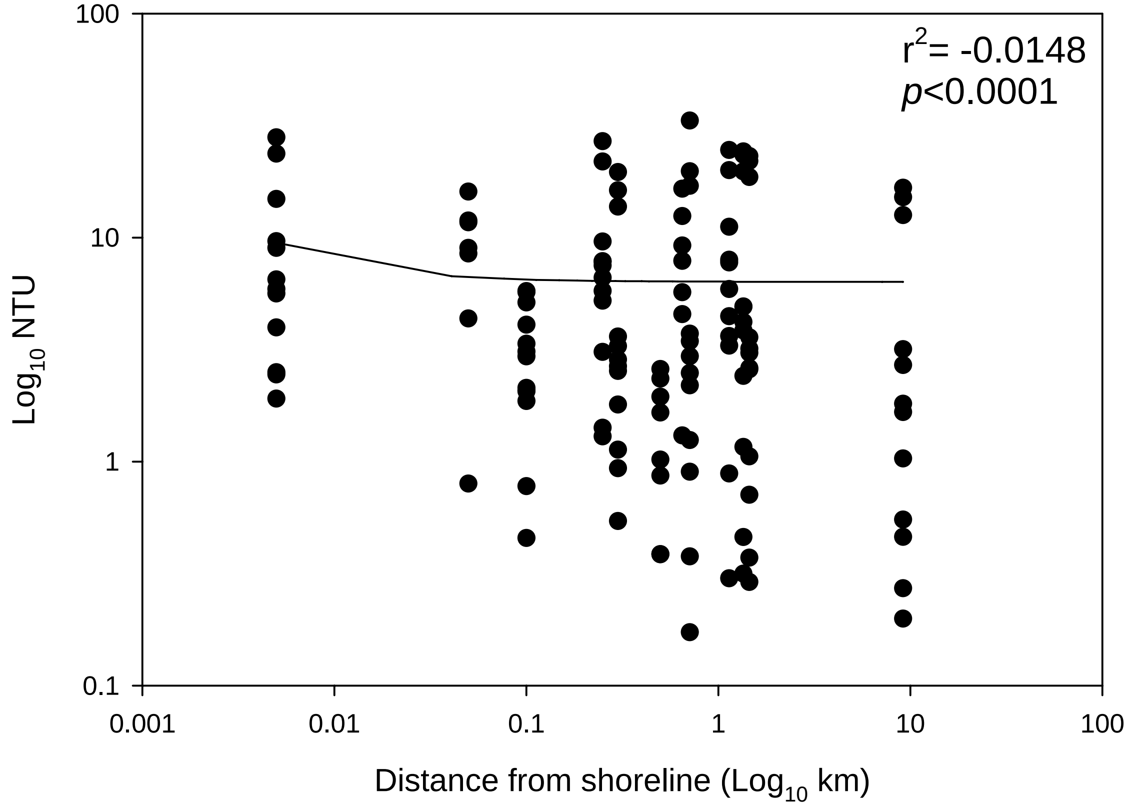

FIGURE 1.16. Linear regression analysis between water turbidity ($\log_{10}$ NTU) and distance ($\log_{10}$ km) from the shoreline. Lack of a linear pattern results from data "noise", which in turn is a reflection of frequent turbid runoff pulses. Such trends are difficult to catch within short temporal scale sampling efforts (i.e., one year) and may require long-term sampling efforts.

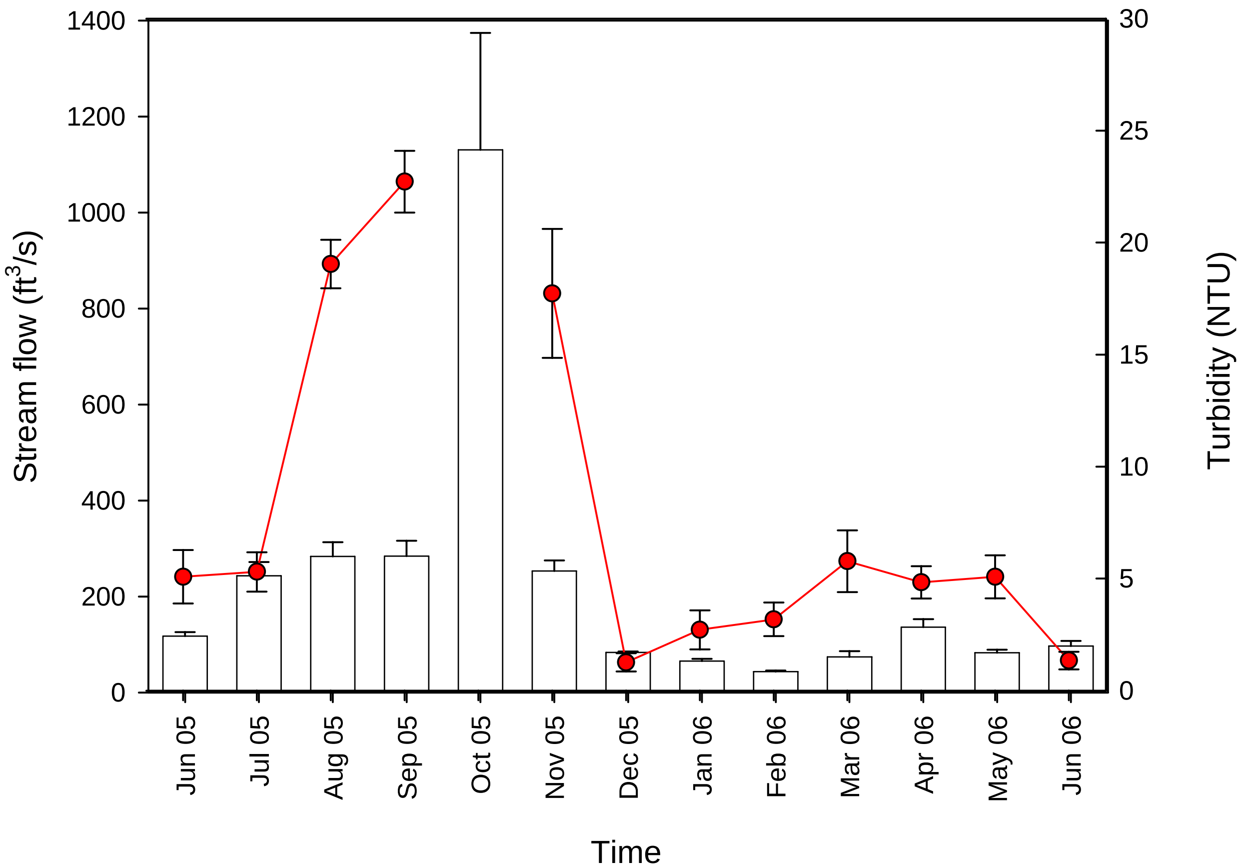

FIGURE 1.17. Guanajibo River mean daily stream flow (mean±one standard error). Data source: http://waterdata.usgs.gov/pr/nwis/uv?50138000. Red dots represent mean monthly water turbidity data averaged across study sites.

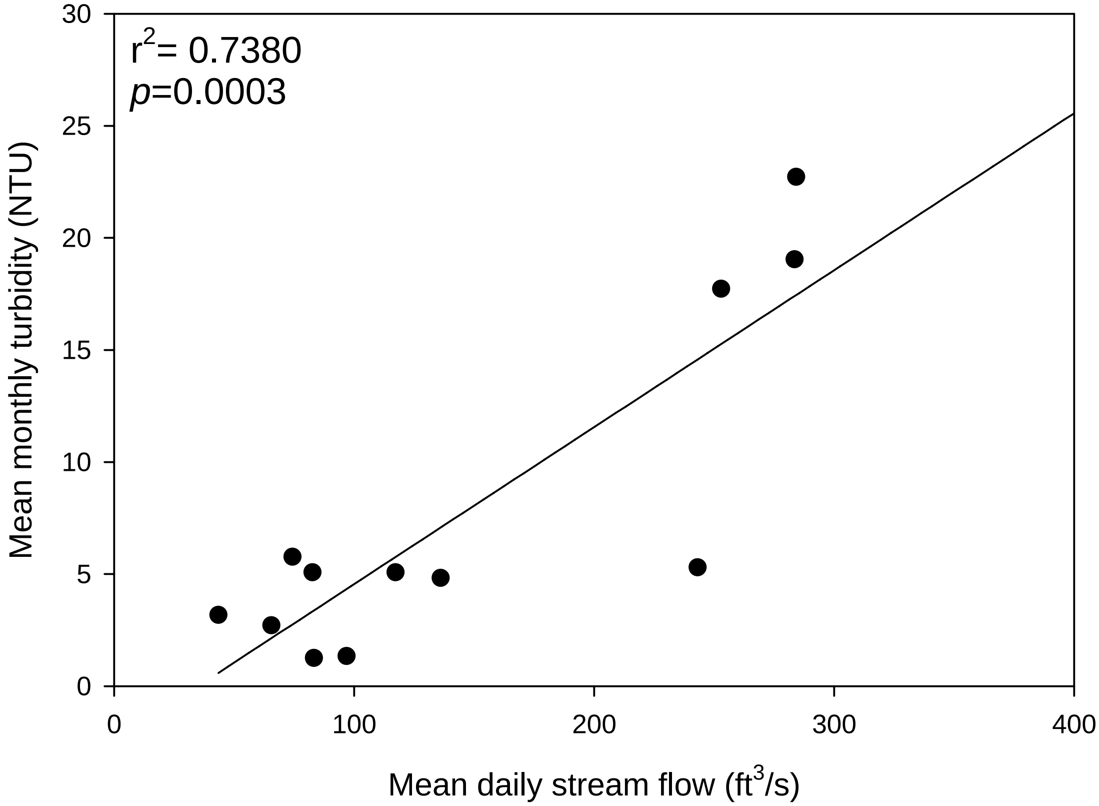

FIGURE 1.18. Linear regression between mean daily Guanajibo River stream flow and mean monthly water turbidity across all study sites. Stream flow data source: http://waterdata.usgs.gov/pr/nwis/uv?50138000. Stream flow data from October 2005 was excluded from the analysis because no water quality sampling was conducted in that month.

was significantly lower during February 2006. Stream flow peaks in the Guanajibo River coincided with peak water turbidity values across the western Puerto Rico shelf (Figure 1.17). Linear regression analysis showed a very strong relationship among these (r^2=0.7380, p<0.0003), which further suggest a possible strong influence of the Guanajibo River plume across some of the study sites (Figure 1.18). Mean rainfall at the Guanajibo River watershed was similarly plotted against mean turbidity across the shelf (Figure 1.19). Rainfall showed a significantly higher peak (p=0.0011) during the month of October, 2005, with significantly lower values between December 2005 and the months of January and February 2006 (Table 1.5). However, rainfall data showed no linear relationship (r^2=0.0030, p=0.1474) with mean turbidity across the shelf (Figure 1.20). Stream flow showed a quadratic relationship with rainfall in the Guanajibo River (r^2=0.7739, p=0.0500) (Figure 1.21).

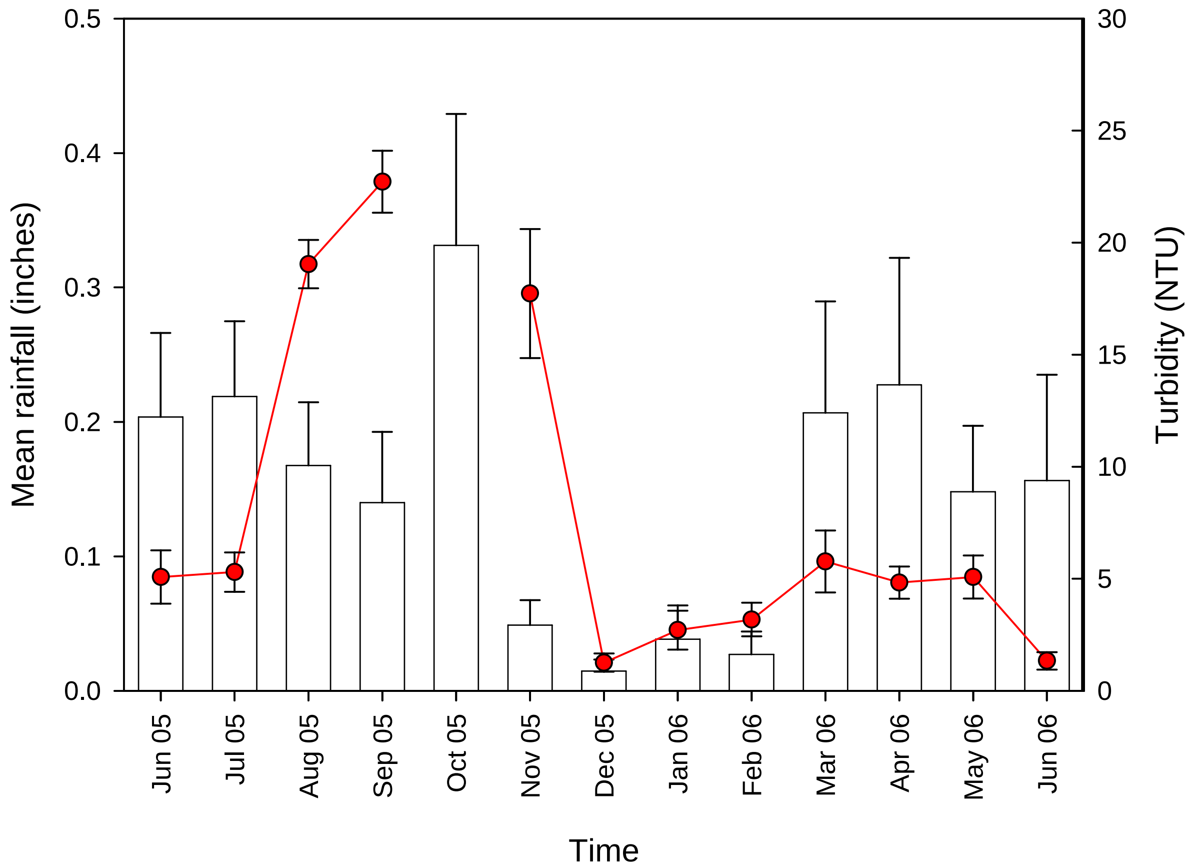

FIGURE 1.19. Guanajibo River mean daily rainfall (mean±one standard error). Data source: http://waterdata.usgs.gov/pr/nwis/uv?50138000. Red dots represent mean monthly water turbidity data averaged across study sites.

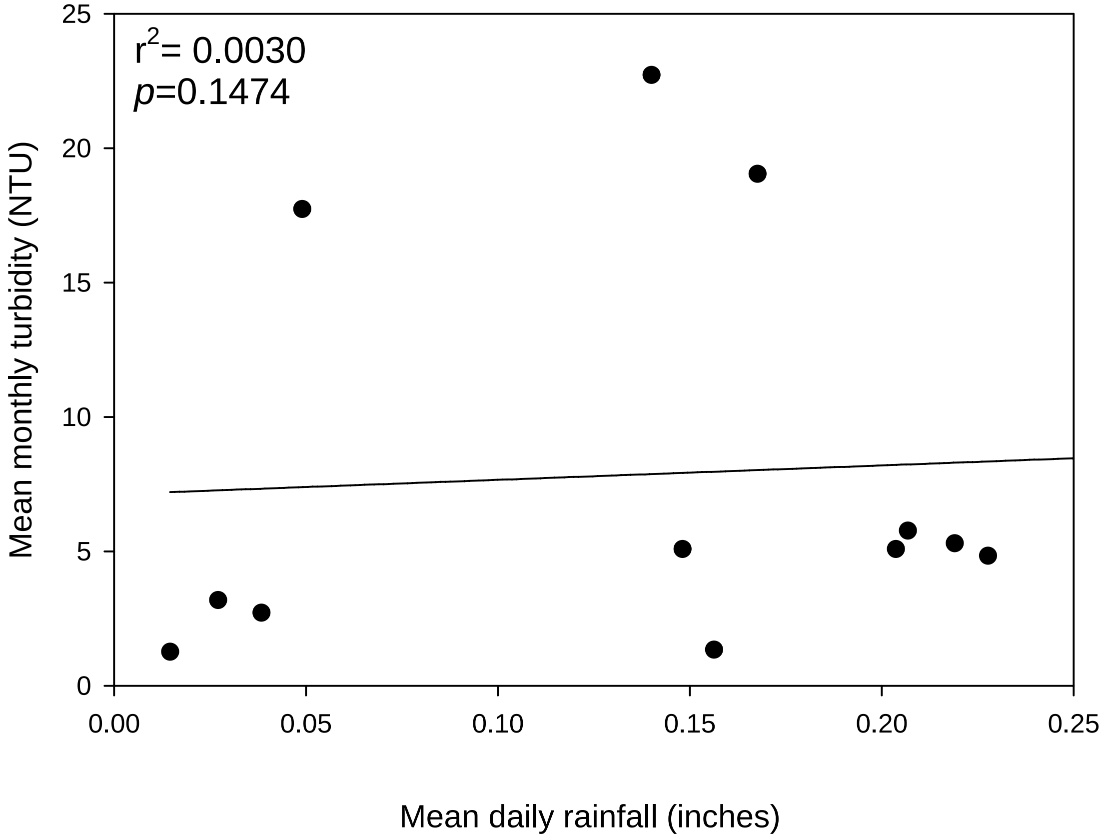

FIGURE 1.20. Linear regression between mean daily Guanajibo River rainfall and mean monthly water turbidity across all study sites. Stream flow data source: http://waterdata.usgs.gov/pr/nwis/uv?50138000. Rainfall data from October 2005 was excluded from the analysis because no water quality sampling was conducted in that month.

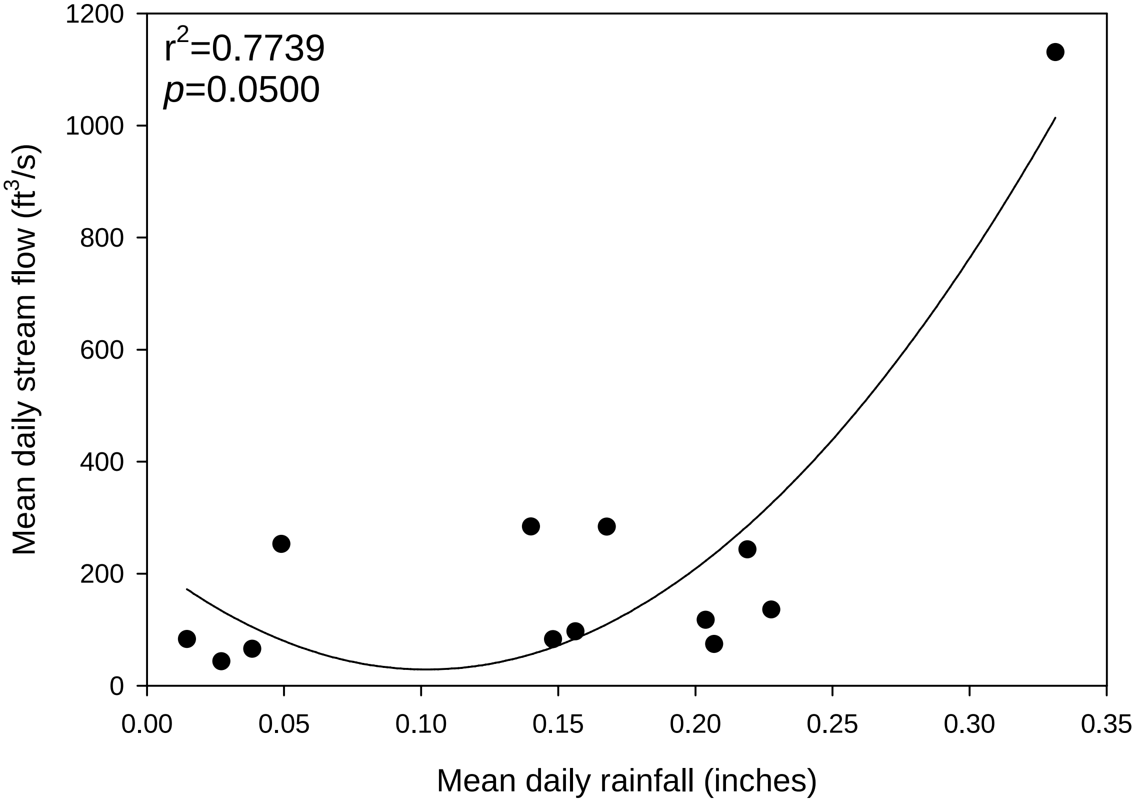

FIGURE 1.21. Quadratic regression between mean daily Guanajibo River rainfall and mean daily stream flow. Data source: http://waterdata.usgs.gov/pr/nwis/uv?50138000.

Discussion

Non-point source pollution is a cause of major concern along the western Puerto Rico shelf. Long-term non-point source pollution influences have resulted in permanent environmental degradation of inshore waters along the coast, particularly in declining water transparency, increased concentration of solid suspended material and declining sunlight penetration. Pollution sources identified in this study included, but might have not been limited to, illegal sewage discharges to local rivers and creeks, illegal sewage discharges from illegal house boats in mangrove areas at Finca Belvedere Natural Reserve, malfunctioning septic tanks and sewer lines, restaurants grey waters, sport marinas, boat fuel gas stations, runoff from agricultural lands and cattle grazing grounds, and turbid runoff from cleared lands.

Some water quality parameters showed wide spatial and temporal fluctuations through this study. Most of these fluctuations were caused by a combination of non-point source runoff pulses as well as by highly variable oceanographic conditions during sampling (Table 1.6). These fluctuations, in combination with a monthly-basis sampling scale, prevented us to adequately document spatial and temporal gradients in water quality parameters. Current environmental conditions of much of the local coral reefs and seagrass communities through the study sites suggest a long-term declining trend associated to environmental stress gradients largely as a result of non-point source pollution. However, such pulses generally operate at largely variable temporal and spatial scales. For example, historical stream flow (Figure 1.22) and rainfall data (Figure 1.23) at the Guanajibo River watershed have shown largely temporally spaced out peaks. Pulse effects associated to such peaks are phenomena that can operate at temporal scales that can

TABLE 1. Summary oceanographic conditions during sampling visits.

Month	Wind	Tide	Runoff
June 2005	Calm, SE	Low	No
July 2005	Calm, SE	High	No
August 2005	Moderate, E	Low	Yes
September 2005	Calm, E	Low	Yes
November 2005	Strong, E	Low	No
December 2005	Calm, NW	High	No
January 2006	Calm, NW	High	No
February 2006	Calm, NW	High	No
March 2006	Calm, NE	High	No
April 2206	Calm, NE	High	No
May 2006	Calm, NE	Low	No
June2006	Calm, NE	Low	No

fluctuate from months to years. Therefore, such effects can be potentially difficult to detect within year-long temporal scales such as the one used in this study. As a matter of fact, peak stream flow and rainfall during the course of this study in the Guanajibo River watershed fell within their normal distribution (Figures 1.23 and 1.24).

Spatial and temporal patterns of water quality parameters, such as turbidity, varied as function of the distance from known coastal pollution sources, increasing turbidity, tidal flow, sediment resuspension associated to wind patterns and/or boating activity, presence of northwestern swells, presence of sediment-ladden and polluted non-point source stormwater runoff, movement of the Guanajibo River plume, and the presence of non-point raw sewage sources along the shoreline. There were variable combinations of the above factors through the study, but three basic patterns were identified as the most influencing that could help explain some of the observed patterns of turbidity and fecal pollution along the western Puerto Rico coast: (1) oceanographic conditions dominated by high-pressure strong southeast winds and ebbing tide;

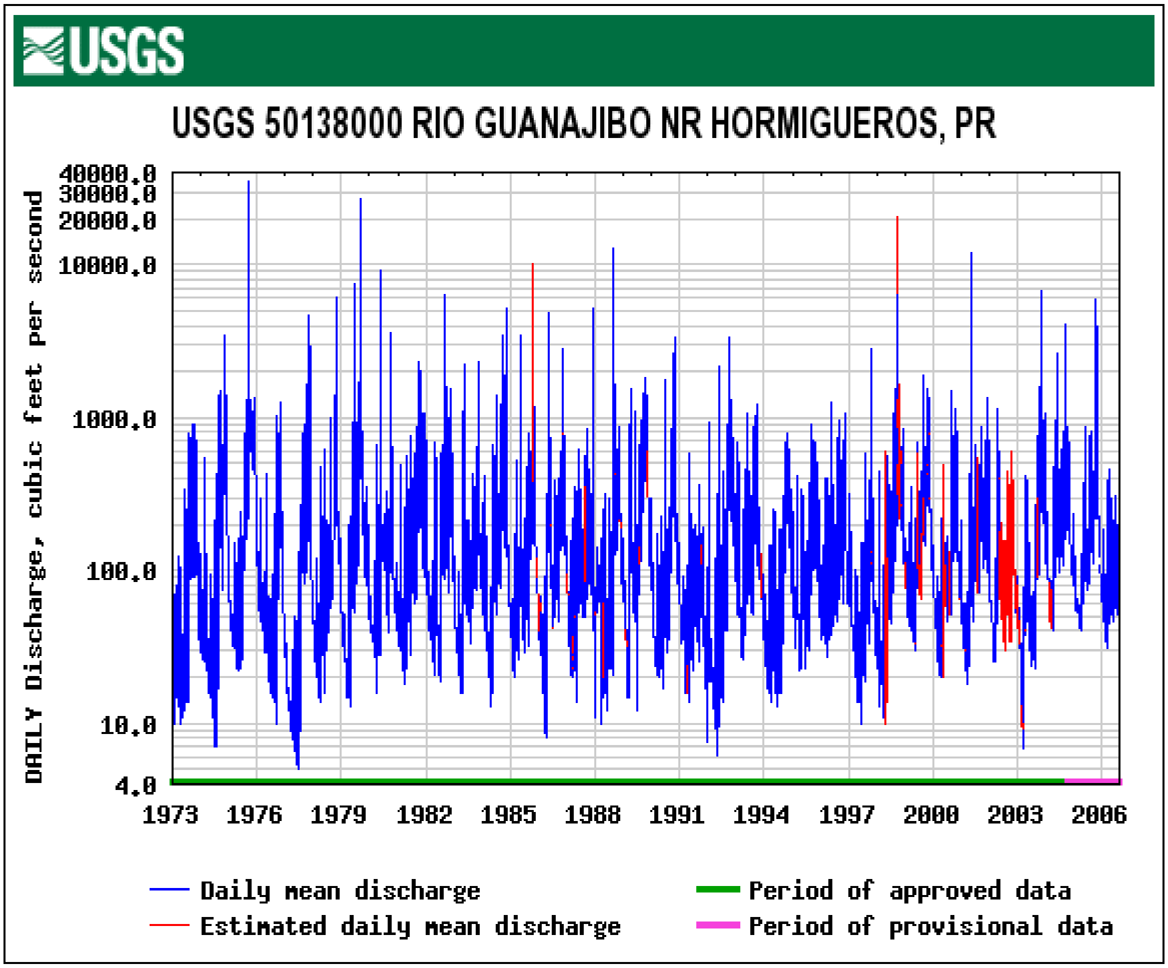

FIGURE 1.22. Historical Guanajibo River stream flow data through 33 years showing large temporal spacing of peak events. There were approximately 24 peak events exceeding 1,000 ft3/s between 1973 and 1980, 28 during the 1980s, 19 during the 1990s, and approximately 12 during the period of 2000 to 2006. Data source: http://waterdata.usgs.gov/pr/nwis/uv?50138000.

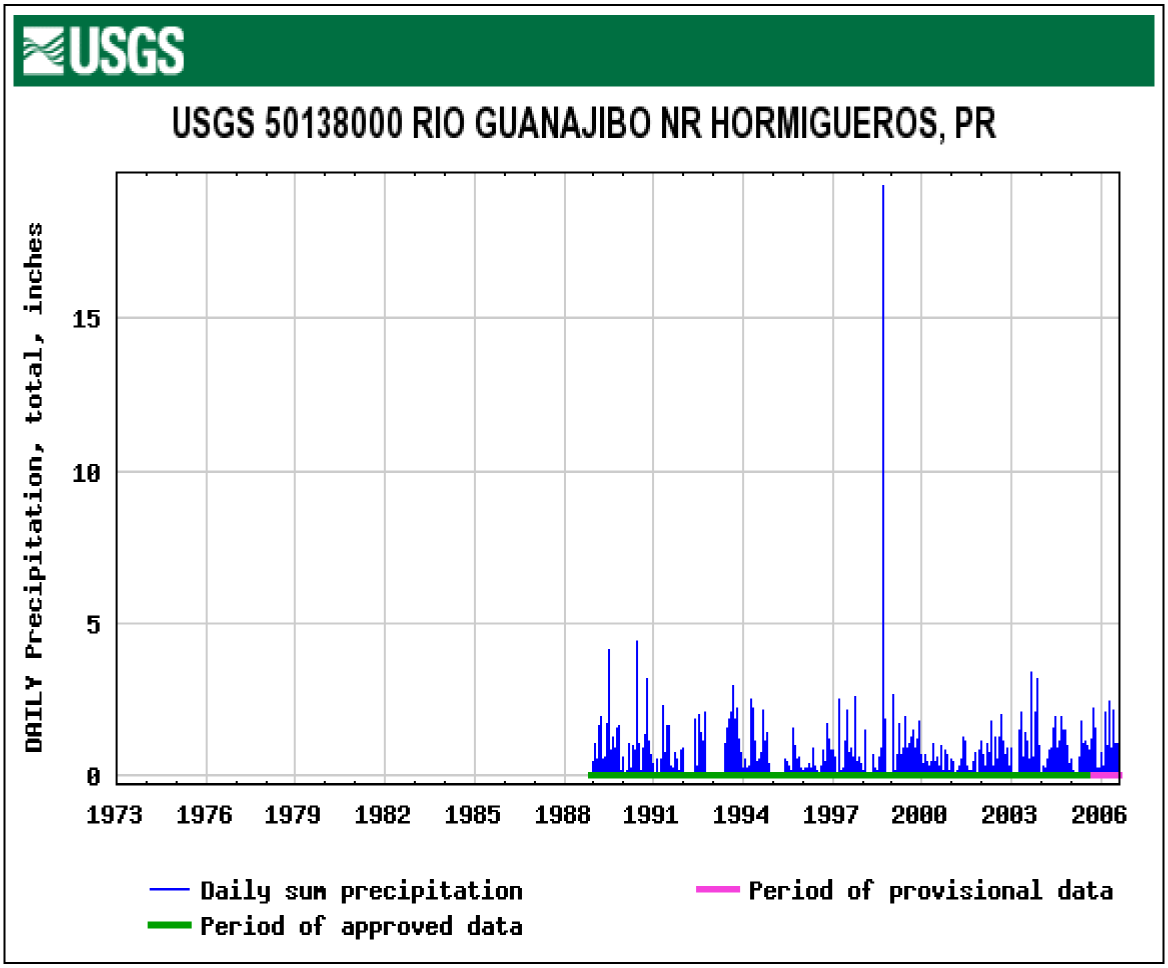

FIGURE 1.23. Historical Guanajibo River rainfall data through 17 years showing large temporal spacing of peak events. Data source: http://waterdata.usgs.gov/pr/nwis/uv?50138000.

(2) oceanographic conditions dominated by northeast winds and ebbing tide; and (3) oceanographic conditions dominated by northwest winds and incoming tide.

Under environmental scenario #1 (Figure 1.24), oceanographic conditions were dominated by high-pressure strong southeast winds. Ebbing tide, in combination with strong winds, caused significant wind-driven and tidal-driven sediment resuspension, as well as a net offshore displacement of turbid and polluted nearshore waters with ebbing tidal flow. This often resulted in increasing water turbidity levels and sometimes in slightly declining dissolved oxygen concentrations. Net surface water movement under such conditions was south-north and murky waters from Puerto Real Bay washed offshore towards Punta Carenero, Arrecife Fanduco, Punta Ostiones, and often to Arrecife El Ron. Similarly, non-source point pollution from Joyuda Bay flows towards Cayo Ratones and Punta Arena, and the Guanajibo River plume moves toward the west and northwest towards Mayagüez Bay, affecting Los Carrilones spur and groove reef systems, Escollo Rodríguez, Manchas Exteriores, and Arrecife Tourmaline. However, Mayagüez Bay was not sampled during this study.

Under environmental scenario #2 (Figure 1.25), oceanographic conditions were dominated by northeast winds and ebbing tide. Ebbing tide, in combination with strong northeast winds, also caused significant wind-driven and tidal-driven sediment resuspension, as well as a net offshore displacement of turbid and polluted nearshore waters with ebbing tidal flow. This also resulted in increasing water turbidity levels. Net surface water movement under such conditions was northeast-southwest and murky waters from the Guanajibo River plume washed offshore through the Guanajibo Channel and often to Arrecife El Ron, depending on the river stream flow level.

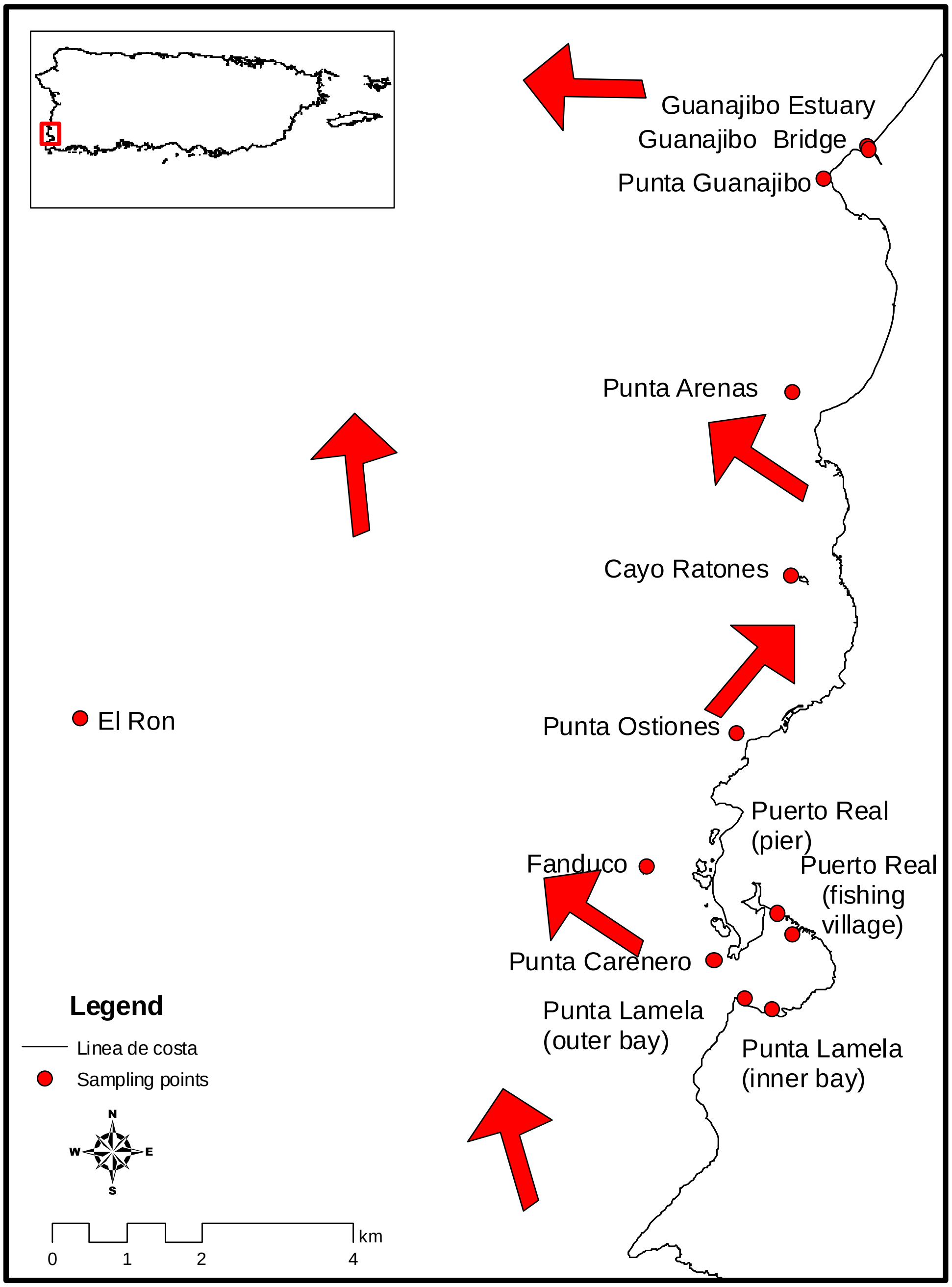

FIGURE 1.24. Scenario #1: Oceanographic conditions dominated by high-pressure strong southeast winds and ebbing tide. Characteristics include: significant wind-driven sediment resuspension, pulling of polluted nearshore waters with ebbing tidal flow and increasing water turbidity.

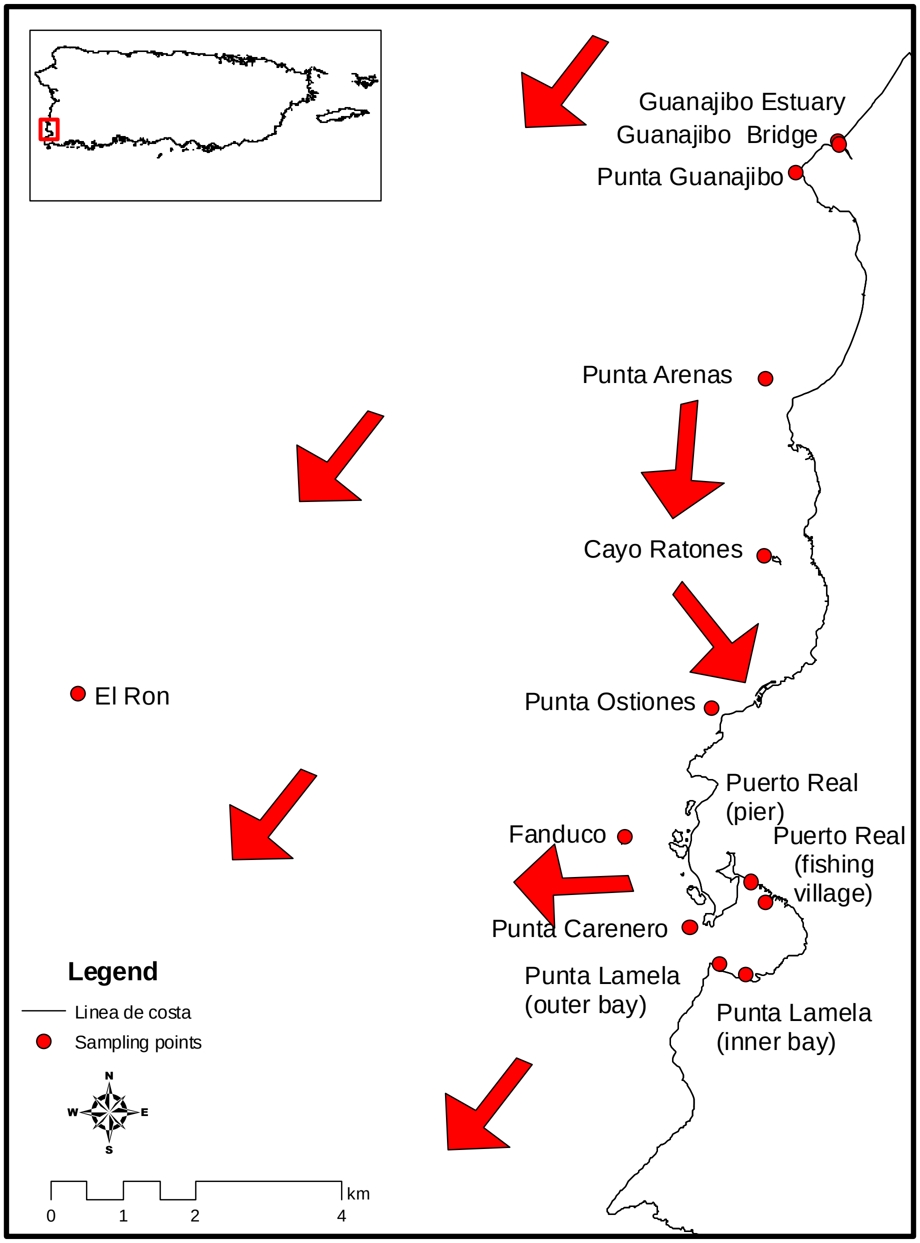

FIGURE 1.24. Scenario #2: Oceanographic conditions dominated by northeast winds and ebbing tide. Characteristics include: moderate to significant wind-driven sediment resuspension, pulling of the Guanajibo River plume with ebbing tidal flow (depending on stream flow), and increasing water turbidity (largely dependent on river plume movement and direction).

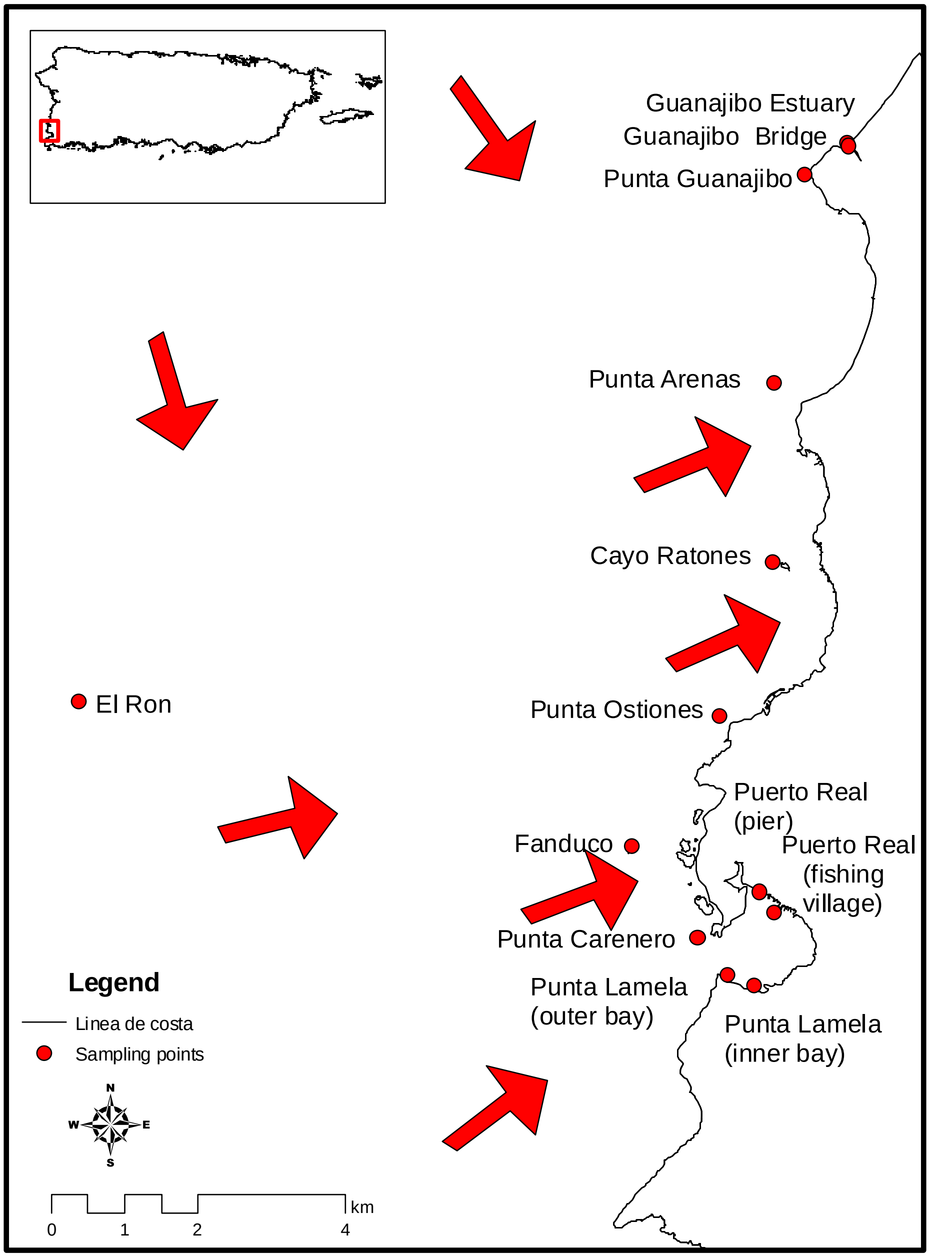

FIGURE 1.25. Scenario #3: Oceanographic conditions dominated by northwest winds and incoming tide. Characteristics include: offshelf clean waters move through the shelf, confining polluted waters to inshore areas, and reducing water turbidity.

Guanajibo Channel is a deeper water (approx. 20 m) basin draining the shallower coastal shelf areas and is characterized by strong tidal circulation often in a northeast-southwest direction. Non-source point pollution from Joyuda Bay also flows towards Cayo Ratones and westward towards Las Coronas reef system through the Guanajibo Channel. Finally, water from Puerto Real Bay move towards Punta Lamela, and eventually move parallel to the shore towards the south, impacting Playa Buyé and Punta Guaniquilla, which are also affected by polluted waters from Boquerón Bay. However, areas south of Puerto Real were not sampled in this study.

Under environmental scenario #3 (Figure 1.26), oceanographic conditions were dominated by weak northwest winds and incoming tide. These conditions triggered a net movement north-south or northwest-southeast of offshelf clean waters through the shelf, confining polluted waters to inshore areas, and increasing water transparency.

Oceanographic conditions often showed sudden dramatic variations (i.e., surface current direction and speed, wave height, swells) as a result of weather extreme events. Thus, conditions during the course of some of the sampling efforts were affected by such events.

Conclusions

This study showed that oceanographic dynamics can be highly variable in tropical environments. Such high spatial and temporal variation need to be taken into account when planning, designing and implementing water physico-chemical and microbiological water quality monitoring programs in tropical environments. Pollution pulses from non-point sources may trigger high variability in many parameters. This could result in high statistical noise and may prevent to identify spatial and/or temporal patterns under standard monthly monitoring efforts such as the one used in this study. Therefore, detecting such trends may require more frequent or automatized monitoring using strategically placed permanent digital data loggers. However, our data shows inequivocal evidence that turbid waters may significantly influence inshore waters, and often affect habitats located up to 10 km offshore, such as El Ron. This is highly significant because turbid and contaminated runoff from the Guanaijibo River plume as well as from non-point sources from Puerto Real Bay, Joyuda Bay, and possible from Playa Buyé and Boquerón Bay are negatively impacting inshore waters and local oceanographic dynamics move polluted waters offshore, affecting significant coral reef and seagrass communities.

Future studies of water quality impacts on coral reef and seagrass communities should include measurements of dissolved nutrients (i.e., Nitrate, Nitrite, Ammonium, Phosphate, H_2S), as well as measurements of N_{15} isotopes incorporated into seagrasses and/or selected ubiquitous algal species as an indicator of coastal eutrophication. In addition, sampling should be expanded to areas farther offshore across the shelf, and probably with a higher frequency, in order to test different hypothesis regarding spatial and temporal variation patterns, as well as their relationship to benthic habitat condition.

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Appendix A-2

Non-point source sewage pollution pulses along the southwestern Puerto Rico shelf.

Edwin A. Hernández-Delgado

Introduction

In 1972 the Clean Water Act established that US states, including its territories, had to adopt water quality standards that were compatible with pollution control programs in order to reduce pollutant discharges into waterways. The National Pollutant Discharge Elimination System (NPDES) has helped to significantly reduce loads from point sources. The US Environmental Protection Agency (USEPA, 2000) defined point sources as "*any discernable, confined and discrete conveyance, but not limited to any pipe, ditch or concentrated animal feeding operation from which pollutants are or may be discharged*". However, non-point pollution sources are mostly responsible for many water system impairments, especially following storm events. Most non-point sources are related to agricultural operations. Urban-associated contamination is also an important contributor due to increasing residential, commercial and industrial development, use of manure as fertilizers, persistence of combined sewer overflows, and malfunctioning septic systems. Other non-point source of fecal pollution is wildlife.

Marine ecosystems are vulnerable to the effects of wastewater as well as the latter sources of contamination. Potential human exposure to polluted coastal waters, particularly in the tropics, is a concern. The potential adverse impacts of wastewater pollution affect both the environment and human health (Lipp et al., 2002). There is a growing number of reports of both recreational and occupational users of marine waters developing gastrointestinal, respiratory, dermatologic, and ear, nose and throat infections (Henrickson, et al., 2001). Fecal microorganisms are the most useful biologic indicators to assess water fecal contamination, since they compose the human gastrointestinal tract microbiota (Carrillo, et al., 1985). For this matter, the USEPA has recommended the use of *Escherichia coli*, a member of the fecal coliform group, as a microbial indicator for recreational waters in freshwater bodies. The agency also recommends that members of the genus *Enterococcus* can be used as indicators for both freshwater and saltwater (USEPA, 2000). Some predictors of fecal coliform densities are: proximity to areas with septic tanks, as well as rainfall runoff from urbanized areas (Kelsey et al., 2004). In this context, it is important to determine sources of fecal contamination in order to prevent diseases and assure water quality (Scott, at al., 2002), since many coastal areas are used for fishing and recreational purposes.

Marine communities of the southwestern Puerto Rico platform are being affected by frequent non-point source pulse events that have degraded its water quality (discussed in Appendix A-1) and have caused a severe decline in coral reef communities (discussed in Appendix A-4). This region receives the direct and consistent impact of fecal contamination from local non-point sources that include: illegal crude discharges from residences and local businesses, possible infiltration of septic tanks, discharges without treatment that enter small streams that end in Río Guanajibo, possible waste dumping from boats and house-boats, and discharges of cattle and horse farms. The influence of Guanajibo River represents one of the main sources of sediments and sewage pollution that cause increasing turbidity levels in the region (discussed in Appenidix A-1). This situation affects the measurements of microbial indicators, because experiments have demonstrated that fecal coliforms in sediments decay more rapidly, though their survival is extended compared with that in the water column (LaLiberte and Grimes, 1982, Pommepuy et al., 1992).

There is a no data regarding the microbiological water quality of the southwestern Puerto Rico platform. Therefore, there is no way to determine what is the spatial distribution of non-point source fecal pollution impacts along the shelf. The first objective of this study were to document the spatial fluctuations of fecal pollution in marine waters using conventional microbiology methods (membrane filtering) around thirteen marine locations in the western Puerto Rico shelf. Second, results obtained from the fecal indicators were compared with parameters such as turbidity, rainfall and Guanajibo River stream flow. Finally, comparisons were made at different spatial scales, including sites, geographical location, and management regimes.

Materials and Methods

Sampling sites

Sampling was conducted monthly from June 2005 to June 2006, except October 2005, due to weather conditions. Representative water sampling sites were selected based on a geographical distance gradient from Puerto Real Bay to offshore representative coral reef/seagrass habitats (Figure 2.1). These included the following: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites were selected based on a geographical distance gradient from Puerto Real Bay towards offshore representative coral reef and seagrass habitats.

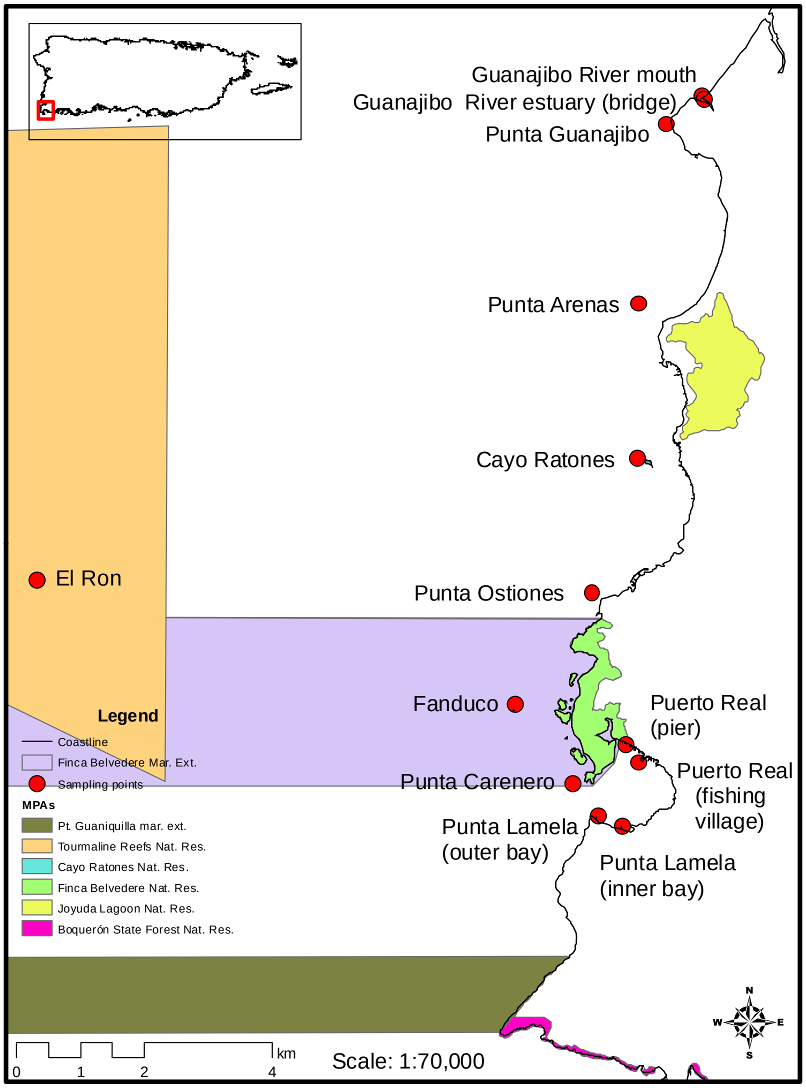

FIGURE 2.1. Water quality sampling sites and their relationship to the existing local PRDNER-managed natural protected area network.

Water sample collection

Grab water samples were collected at each study site using autoclaved Nalgene 1 L plastic bottles (Nalgene Co.). Samples were collected at 30 cm below surface using sterilized gloves and immediately placed on ice until analyzed within 6 hr.

Physicochemical Parameters

In situ analysis of physicochemical data such as temperature (°C), pH, salinity (‰), conductivity (ms/cm), dissolved oxygen (mg/L) were measured using portable instrumentation (Horiba). These parameters were taken in triplicate to submit for statistical analysis. Water turbidity was documented using an Orbeco 866 portable turbidimeter. Turbidity measurements were expressed in Nephelometric Turbidity Units (NTU).

Membrane Filtration

Samples were analyzed using standard membrane filtration techniques to quantify fecal coliforms and enterococcus (APHA). Water samples were filtered using cellulose acetate membranes, which have a porosity of 0.45 μm, using MicroFunnel™ filter funnels (Pall, Co., Ann Arbor, MI). Volumes analyzed were 1mL, 10mL, and 100mL, although variations were used as necessary, depending on water turbidity. Filters were washed with an autoclaved 3% saline buffer solution after the desired volume was filtered. Once the water was filtered, the membrane was transferred to a sterile Petri dish that contained selective media for the targeted microorganism. This technique, however, may result inconvenient for highly turbid water samples, due to mechanical obstruction of membrane surface area by sediment particles interfering with microbial growth.

Fecal coliforms were quantified using *mFC* agar. Confirmatory tests for both were carried out using *Azide Dextrose Broth* and *Lauryl Triptose Broth* (LTB). These tests were used to validate the presence of fecal microorganisms in the samples taken and to discard false positive results.

Media Cultures and Confirmatory Test

Fecal coliforms were quantified using *mFC* agar. Culture media was prepared by adding 52 g/L of purified water, set at pH of 7.4 ± 0.2, heated, agitated and mixed. After boiling, 10 ml/L of 1% rosolic acid solution was added to prevent growth by non-coliform microbiota. Rosolic acid was prepared by adding 0.5 g of rosolic acid/50 ml of 0.2 N NaOH. The final mixture was poured in culture plates. Plates were incubated at 44.5°C for 24 hr. A positive result for this media was the presence of blue colonies due to the fermentation of lactose. *Lauryl Triptose Broth* (LTB) was used to confirm positive results using culture tubes containing inverted Durham tubes. This culture media also contains lactose and was prepared by adding 35.6g/L of water, mixed and heated to dissolve the powder. LTB was autoclaved for 15 minutes at 121°C. Presumptive positive results were confirmed in LTB media and incubated at 35°C/24 h. Positive results produced turbidity and gas in the Durham tubes.

M Enterococcus agar was used to grow enterococci. Culture media was prepared by adding 42 g/L of purified water, set at pH of 7.2 ± 0.2, heated, agitated, mixed and poured on sterile plates. Plates were incubated at 35°C for 48 hr. Intense pink and brown colonies indicated a positive result. *Azide Dextrose Broth* (ADB) was used as a confirmatory test to validate the presence of enterococci and to discard false positive results. ADB was prepared by dissolving 34.7 g/1L of water. The solution was mixed and heated to dissolve the powder. This broth was autoclaved for fifteen minutes at 121°C. Its final pH was set at 7.2 ± 0.2. Microorganisms that were positive for the m Enterecoccus media were transferred to the tubes containing ADB and incubating for 24 hours at 35ºC. A positive result consisted in the presence of turbidity in the broth.

Bacteria Quantification

Colonies were counted directly in each dish. The equation to calculate the bacteria concentration was performed as follows:

$$\text{CFU (colony forming units)} = \frac{\text{number of counted colonies}}{\text{filtered volume}} \times 100$$

Hypothesis testing.

We tested the null hypothesis of no significant difference in the concentration of microbial indicators among sites. Spatial data was $\log_{10}$-transformed to standardize variances (Zar, 1984) and analyzed by means of a one-way analysis of variance (ANOVA) using Statistix Software 8.0 (Analytical Software). *Site* was used as main variable and replicate samples through time as error term. We tested a second null hypothesis of no significant difference in the concentration of microbial indicators between geographical locations. Locations were subdivided into *Inshore* (<0.5 km) and *Offshore* (>0.5 km). Inshore sites included sites 5, 6, 7, 8, 9, 10, 12, and 13. Offshore sites included sites 1, 2, 3, 4, 10. Locations were arbitrarily selected based on distance of sampling site from known or suspected non-point source sewage pollution. Data were treated and tested as above. *Geographical location* was used as main variable and replicate samples through time as error term.

We also tested the null hypothesis of no significant effect of management regime (i.e., marine protected area, MPA) on the response of individual microbial markers. Sampling sites were grouped as follows: *MPA sites*: Arrecife El Ron (Arrecifes Tourmaline Natural Reserve), Punta Carenero, Arrecife Fanduco and Puerto Real-pier (Finca Belvedere Natural Reserve marine extension), and Cayo Ratones (Cayo Ratones and Adjacent Waters Natural Reserve). All remaining sites were treated as *non-MPA controls*. *Management* was used as main variable and replicate samples through time as error term. Regression analyses were performed to test relationships between individual indicator bacteria, water turbidity, Guanajibo River stream flow and rainfall. In addition, our microbiological analysis results were analyzed against microbiological water quality violations of current standards to test efficiency of standard microbiological methods.

Results

Fecal coliform patterns among sites.

Fecal coliform (FC) data revealed that Guanajibo River estuary (bridge) (1262±455 cfu/100mL), Guanajibo River mouth (901±405 cfu/100 mL), and Puerto Real-pier (465±213 cfu/100 mL) showed significantly higher (p<0.0001) FC counts in comparison to other sites (Figure 2.2; Tables 2.1 and 2.2). Annual mean values at these sites resulted in permanent violations of current SB water classification standards for FC set by the PR Environmental Quality Board (EQB) at 200 cfu/100 mL. Figure 2.3 shows a box and whisker plot of FC counts that clearly illustrate the effects of non-point source sewage pollution pulses by the presence of long upper whiskers at most sites, and the particular presence of high outlier FC counts at Arrecife El Ron, Punta Ostiones, Punta Guanajibo and Cayo Ratones. The presence of non-point source sewage pollution pulses at Arrecife El Ron, located at 10 km offshore is alarming and suggests that a significant geographic area of the southwestern Puerto Rico shelf can be frequently impacted by turbid and nutrient-enriched sewage pulses.

There were a total of 21 violations to FC standards through time, accounting for 16% of the time (Table 2.3). However, Guanajibo River estuary (bridge) violated FC standards 100% of the time, while the Guanajibo River mouth showed violations 86% of the time. Puerto Real-pier site violated standards 33% of the time, while Punta Lamela-outer bay, Punta Carenero and Arrecife El Ron showed violations to FC standards only once during our survey.

TABLE 2.1. Mean FC counts by site.

Site	cfu/100 mL*
Arrecife El Ron	24.11±22.40
Punta Ostiones	17.16±12.64
Arrecife Fanduco	9.07±5.25
Punta Carenero	23.40±18.30
Punta Lamela-outer bay	8.14±5.60
Punta Lamela-inner bay	22.07±9.31
Puerto Real-fishing village	73.47±34.35
Puerto Real-pier	**465.46±213.28**
Punta Guanajibo	4.57±3.80
Punta Arenas	3.35±2.27
Cayo Ratones	0.14±0.14
Guanajibo River estuary (bridge)	**1261.7±455.17**
Guanajibo River mouth	**900.71±404.65**

*=Mean±one standard error. Bold figures represent mean violations to current FC standards (200 cfu/100 mL).

TABLE 2.2. Summary results of one-way ANOVA of microbial counts among sites.

Parameter	D.F. (within, between)	F	*P* value*
Fecal coliforms	12,116	16.5	**<0.0001**
Enterococci	12,118	20.9	**<0.0001**

*=Bold figures represent statistically significant results (p<0.0500).

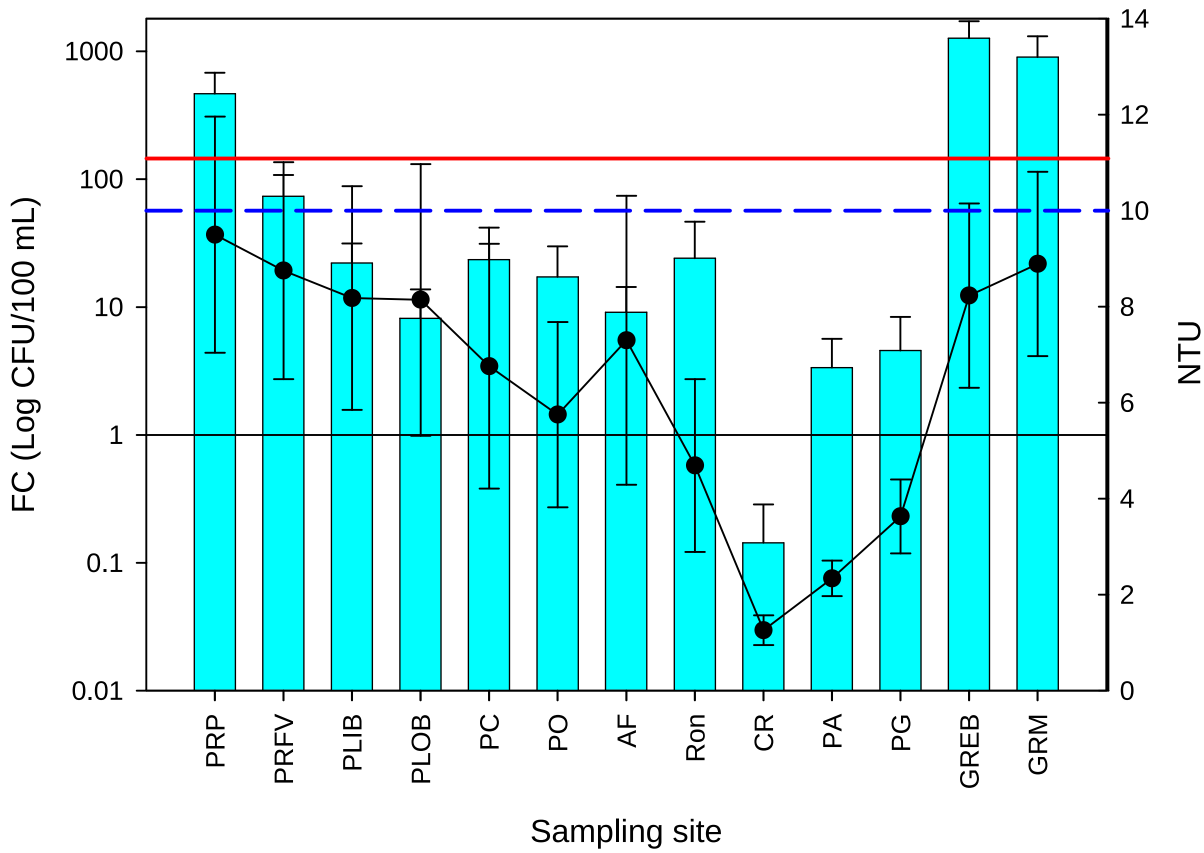

FIGURE 2.2. FC counts (turquoise bars) as a function of water turbidity by site (mean±one standard error). Red solid line represents FC standard (200 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

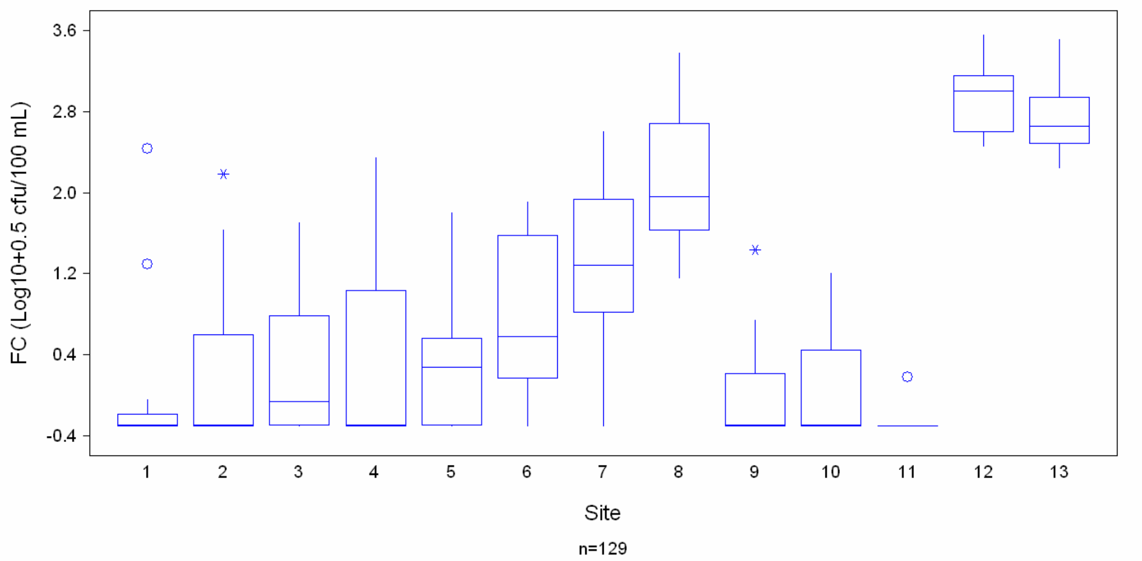

FIGURE 2.3. Box and whisker plot of FC counts (Log_{10}+0.5 cfu/100 mL) by site. Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). These outliers represent pulse events associated to non-point source runoff events or sediment resuspension associated to local oceanographic dynamics (described in Appendix A-1). Sites: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth.

TABLE 2.3. Microbiological water quality standard violations for FC for all sites sampled between June 2005 and June 2006.

Sample	Jun	Jul	Aug	Sept	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Ron	13	0	0	270	0	0	0	0	1	0	0	0
PO	1	1	0	28	11	151	0	0	0	0	0	0
AF	46	1	33	1	4	0	0	0	0	0	4	0
PC	5	0	0	23	15	222	0	1	0	0	0	0
PLIB	0	0	0	45	1	9	1	2	3	2	57	80
PLOB	0	0	1	11	336	42	1	0	1	1	0	4
PRFV	137	0	8	397	8	30	9	125	8	1	0	30
PRP	18	170	23	2370	1430	28	27	132	9	21	271	880
PA	**Ns**	**ns**	**ns**	**ns**	**ns**	2	18	0	0	0	0	0
CR	**Ns**	**ns**	**ns**	**ns**	**ns**	0	1	0	0	0	0	0
PG	**Ns**	**ns**	**ns**	**ns**	**ns**	0	5	0	1	10	0	0
GRE-B	**Ns**	**ns**	**ns**	**ns**	**ns**	1010	193	190	1020	523	2117	3587
GRM	**Ns**	**ns**	**ns**	**ns**	**ns**	730	457	117	1070	443	137	2817

NS= Site not sampled for that time period. Red color= violation to EQB class SB water quality classification FC standard (200 cfu/100 mL).

There was a consistent gradient of increasing mean water turbidity at inshore sites (Figure 2.2). When turbidity data was pooled across sites, no violations were detected in mean annual values. Further, higher mean turbidity values did not correspond always to high mean FC counts due to high variance in the data. This was the result of variable pulses of non-point source sewage pollution, pulses of turbid runoff from land-cleared areas, and rapid changing oceanographic dynamics. However, mean FC counts showed a moderate significant logarithmic increase as a function of water turbidity (r^2=0.2688, p<0.0001), particularly above 7.6 NTU (Figure 2.4). Data was pooled across time (Figure 2.5) and plotted against FC counts. There were continuous turbidity standard violations from August to November 2005, coinciding with the rainy season peak. Mean monthly FC counts violated FC standards during the months of September 2005, and May and June 2006 (Figure 2.5). Also, the months of November and December 2005, and March 2006 FC counts felt just short of standard violations. FC counts showed a non-significant temporal decline (r^2=-0.1683, p=0.2519) with increasing NTU (Figure 2.6), indicating possible

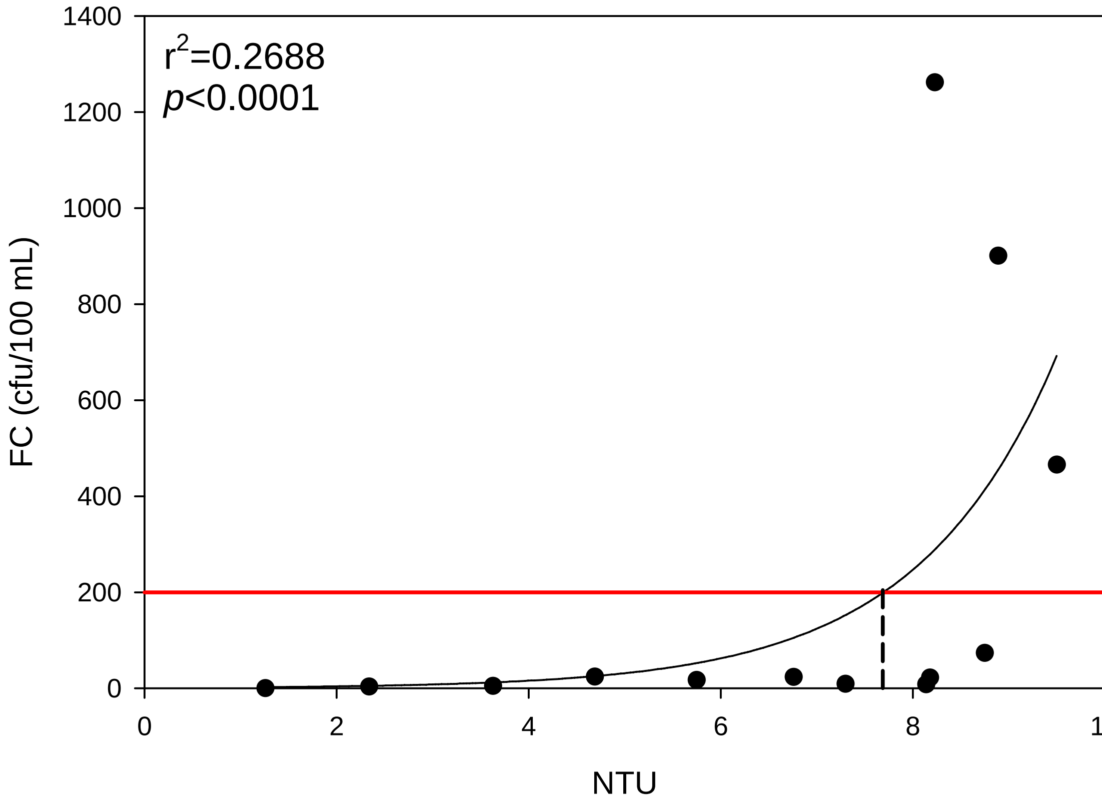

FIGURE 2.4. Logarithmic regression for FC counts and water turbidity by site. Red solid line represents FC standard (200 cfu/100 mL). Dashed line represents approximate turbidity level at which FC counts may exceed current standards.

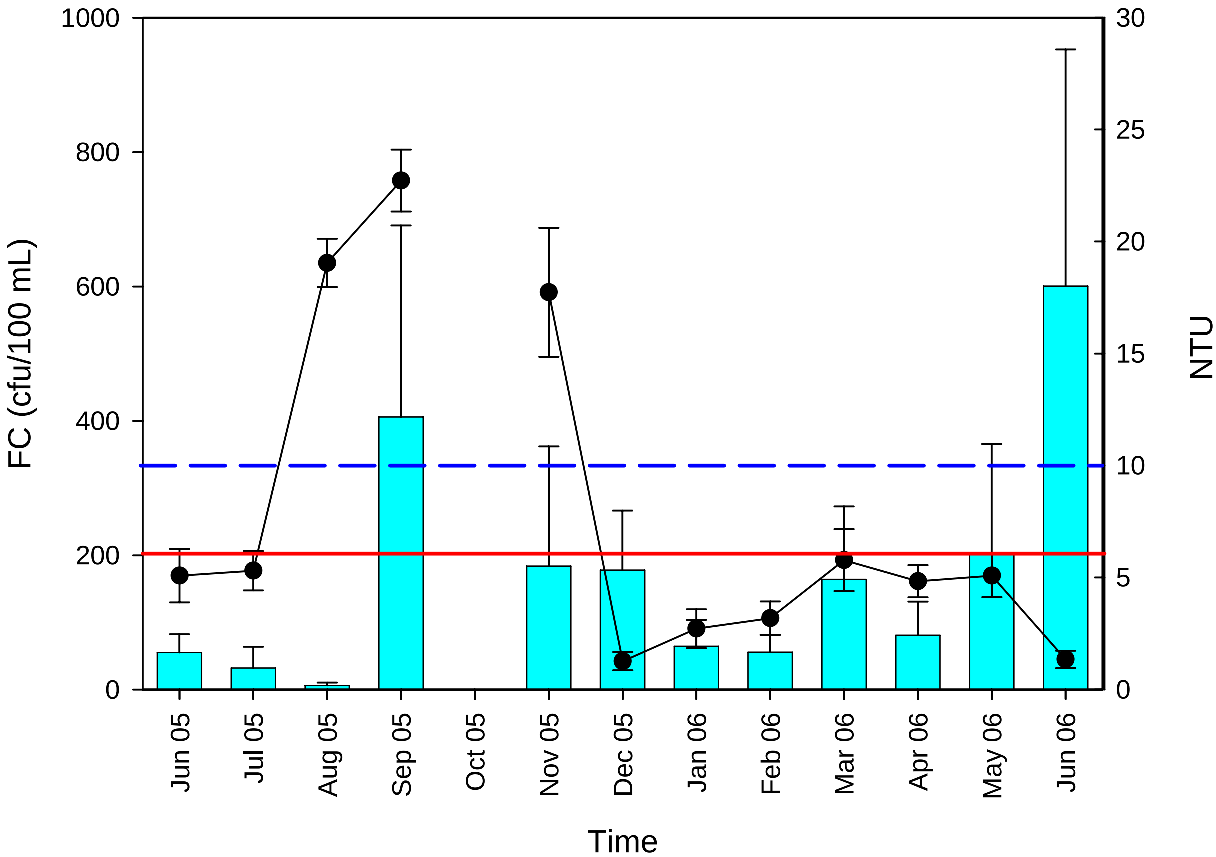

FIGURE 2.5 FC counts (turquoise bars) as a function of water turbidity through time (mean±one standard error). Red solid line represents FC standard (200 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

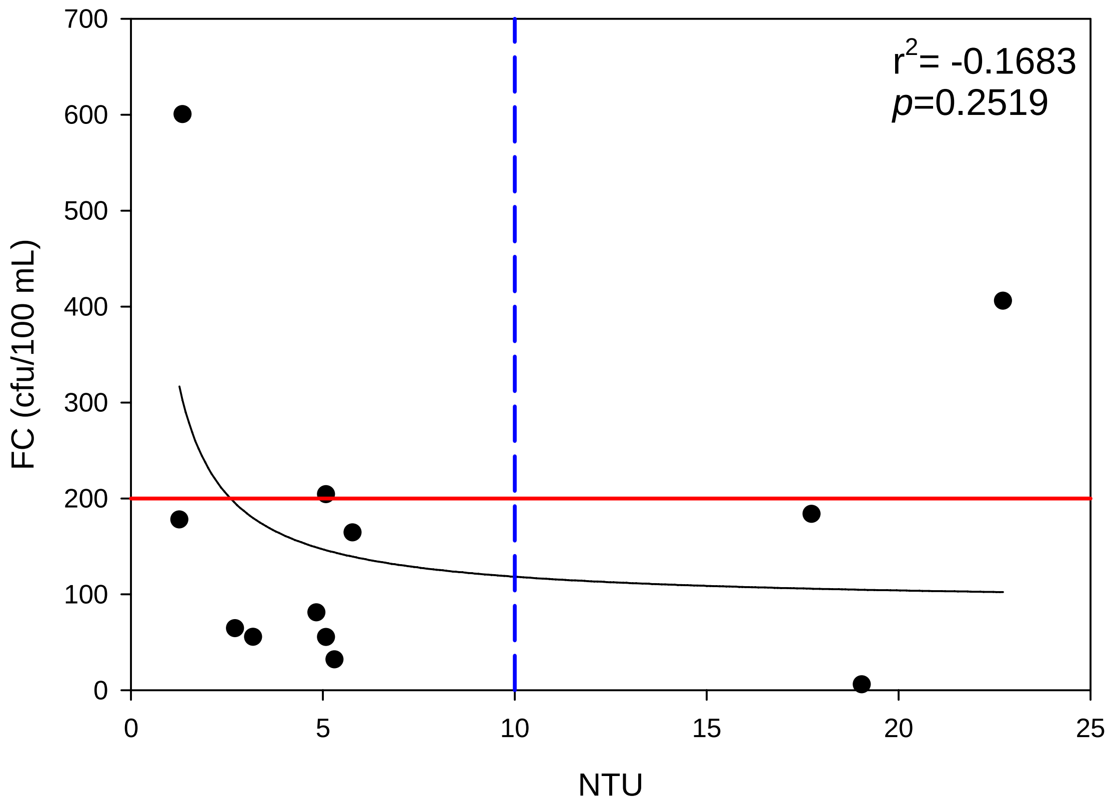

FIGURE 2.6 Logarithmic regression for FC counts and water turbidity by site. Red solid line represents FC standard (200 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

sediment interference in with plate counts, but also high variability as a result of non-point source pollution pulse events and variability in oceanographic dynamics.

TABLE 2.4. Mean FC counts by geographic location.

Location	cfu/100 mL
Inshore (1)	327.88±87.74
Offshore (2)	14.67±6.09

*=Mean±one standard error. Bold figures represent mean violations to current FC standards (200 cfu/100 mL).

TABLE 2.5. Summary results of one-way ANOVA of microbial counts between geographic locations.

Parameter	D.F. (within, between)	F	*P* value*
Fecal coliforms	1,127	45.3	**<0.0001**
Enterococci	1,127	40.4	**<0.0001**

*=Bold figures represent statistically significant results (p<0.0500).

Fecal coliform patterns between geographic locations.

FC data was pooled into two geographic location to test for spatial differences in FC counts between inshore (<0.5 km) and offshore (>0.5 km) waters. Tables 2.4 and 2.5 show that sites clustered within the *inshore* category had overall significantly higher (p<0.0001) mean FC counts that violated the current standard. The box and whisker graph shown in Figure 2.7 indicates that even though *offshore* sites had mean lower FC counts than inshore sites, they presented significant high outlier counts which were remarkably similar to the normal FC count variability observed at *inshore* sites. This suggests that non-point source sewage pollution pulses

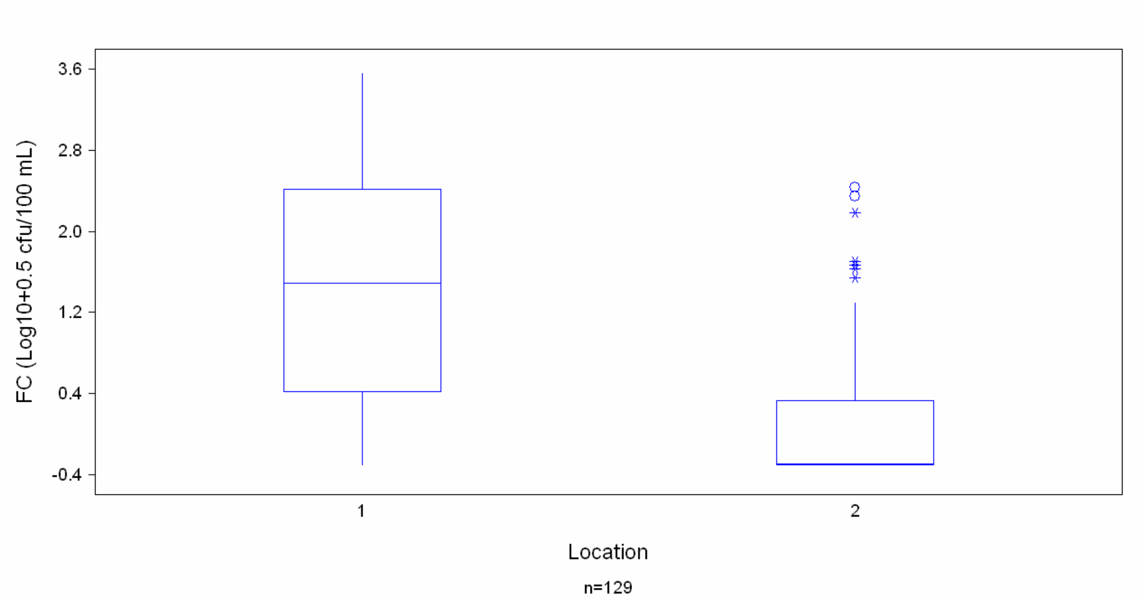

FIGURE 2.7 Box and whisker plot of FC counts (Log_{10}+0.5 cfu/100 mL) by geographic location. Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Outliers documented within location 2 represent pulse events associated to non-point source runoff events or sediment resuspension associated to local oceanographic dynamics (described in Appendix A-1). Locations: (1) Inshore (<0.5 km); (2) Offshore (>0.5 km).

TABLE 2.6. Mean FC counts by management regime.

Location	cfu/100 mL
MPA (1)	113.92±51.96
Non-MPA (2)	224.49±73.19

*=Mean±one standard error. Bold figures represent mean violations to current FC standards (200 cfu/100 mL).

TABLE 2.7. Summary results of one-way ANOVA of microbial counts between management regimes.

Parameter	**D.F. (within, between)**	**F**	***P* value***
Fecal coliforms	1,127	3.08	0.0817
Enterococci	1,127	7.05	**0.0090**

*=Bold figures represent statistically significant results ($p<0.0500$).

are impacting both geographic regions and that such pulses may elevate enterococci counts at *offshore* sites to levels similar to normal variation ranges at *inshore* sites. Pulses cause significant departures from normality in FC counts at *offshore* sites. This accounts for the geographically widespread nature of fecal pollution along the southwestern Puerto Rico shelf.

Fecal coliform patterns between management regimes.

FC data was pooled into two management regimes to test for spatial differences in FC counts between MPA and non-MPA waters. Tables 2.6 and 2.7 show no significant differences in mean FC counts between management regimes. However, mean FC counts at non-MPA sites violated the current standard. The box and whisker graph shown in Figure 2.8 indicates the presence of long upper whiskers suggesting that both, MPA and non-MPA sites, are frequently impacted by non-point source sewage pollution pulses and that mean FC counts show similar variability. This also accounts for the geographically widespread nature of fecal pollution along the southwestern Puerto Rico shelf.

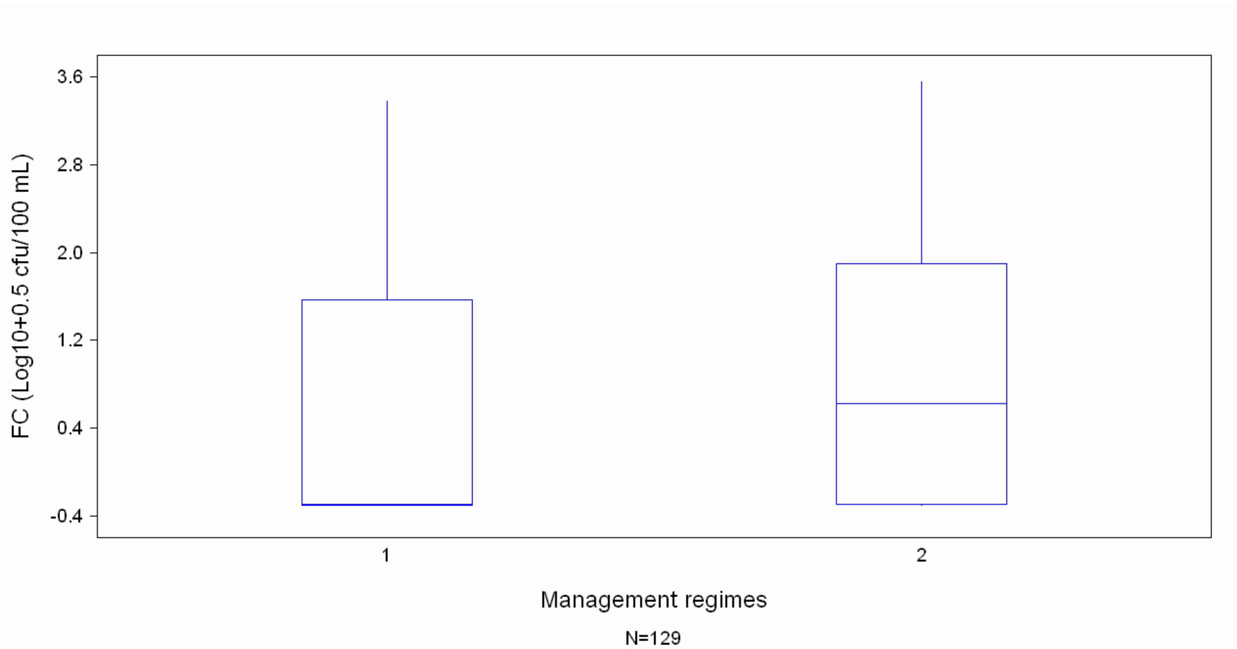

FIGURE 2.8. Box and whisker plot of FC counts (Log_{10}+0.5 cfu/100 mL) by management regime. Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Management regimes: (1) MPA; (2) Non-MPA.

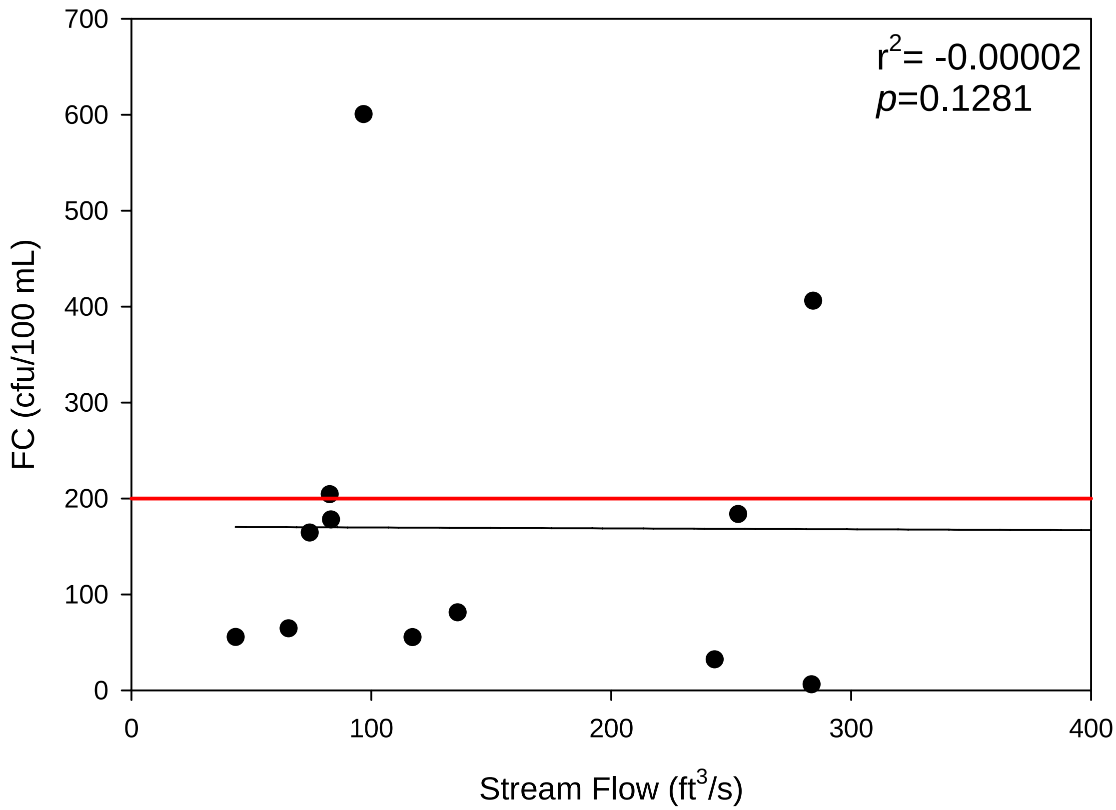

FIGURE 2.9. Linear regression analysis of FC counts as a function of Guanajibo River stream flow.

Fecal coliform patterns and the Guanajibo River stream flow.

The Guanajibo River plume may often impact extensive areas of the southwestern Puerto Rico platform following heavy rainfall and increased stream flow values. However, there is no evidence of how much relationship may exist between mean stream flow values across time and mean temporal FC counts. Regression analysis of FC counts as a function of Guanajibo River stream flow showed no significant correlation (r^2=-0.00002, p=0.1281) (Figure 2.9). When these counts were compared to average daily rainfall for this area over our study period, no correlation was found (r^2=-0.00002, p=0.1323), (Fig. 2.9). Monthly stream flow averages might not be an adequate indicator of impacts on microbial water quality because occasional daily stream flow pulses are masked within monthly averages.

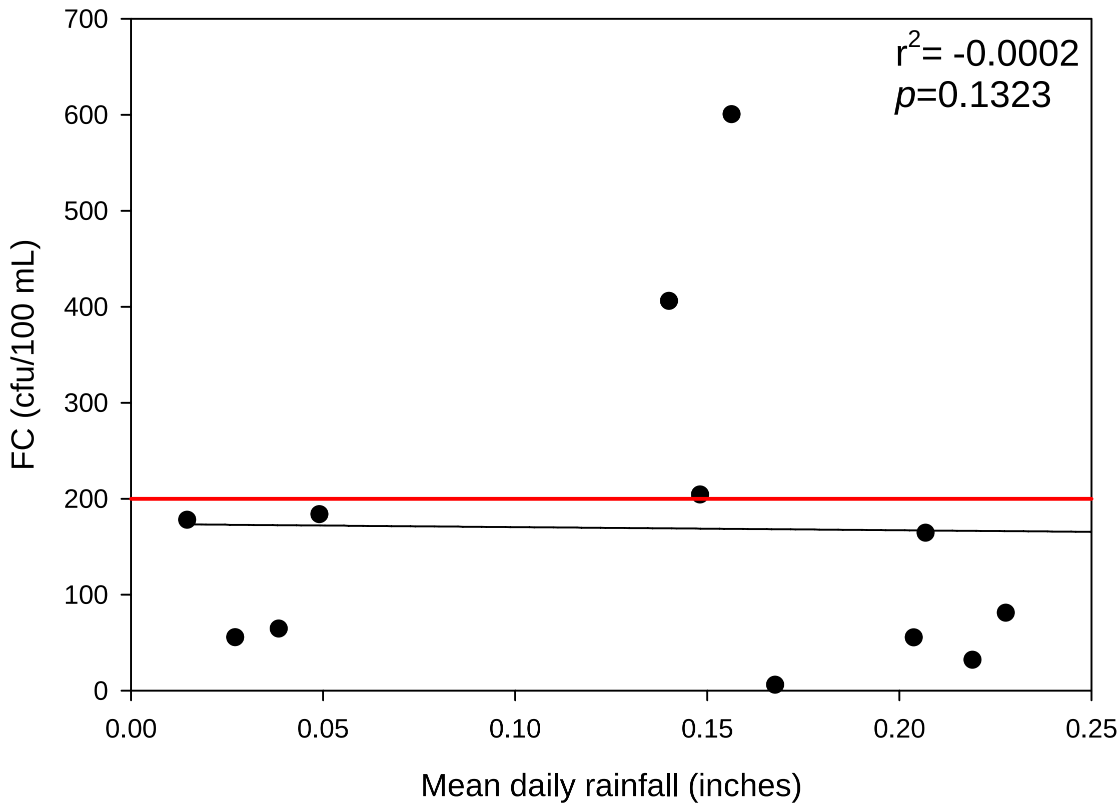

FIGURE 2.10. Linear regression analysis of FC counts as a function of mean daily rainfall measured in the Guanajibo River watershed.

Fecal coliform patterns and mean daily rainfall.

FC counts were also regressed as a function of mean daily rainfall to test for any temporal pattern (Figure 2.10). However, no significant relationship was found (r^2=-0.00002, p=0.1323). This suggests that during our study, mean daily rainfall (pooled by month) did not have any significant impact on FC counts. Monthly rainfall averages might not be an adequate indicator of impacts on microbial water quality because occasional daily pulses are masked within monthly averages.

Enterococci patterns among sites.

Enterococci data also showed that Guanajibo River estuary (bridge) (367±134 cfu/100mL), Guanajibo River mouth (195±53 cfu/100 mL), Puerto Real-pier (63±20cfu/100 mL), and Puerto Real-fishing village (37±28cfu/100 mL) showed significantly higher (p<0.0001) enterococci counts in comparison to other sites (Figure 2.11; Tables 2.2 and 2.8). Annual mean values at these four sites resulted in permanent violations of current SB water classification standards for enterococci set by EQB at 35 cfu/100 mL. Values from the Guanajibo River mouth felt just below the standard. Figure 2.12 shows a box and whisker plot of enterococci counts that also confirms the effects of non-point source sewage pollution pulses by the presence of long upper whiskers at most sites, and the particular presence of high outlier enterococci counts at Arrecife El Ron, Arrecife Fanduco, Punta Guanajibo, Punta Arenas and Cayo Ratones. Enterococci pulses at Arrecife El Ron confirms recurrent non-point source sewage pollution pulses covering a significant geographic area of the southwestern Puerto Rico shelf.

There were also a total of 21 violations to enterococci standards through time, accounting for 16% of the time (Table 2.9). However, Guanajibo River estuary (bridge) violated enterococci standards 100% of the time, while the Guanajibo River mouth showed violations 71% of the time. Puerto Real-pier site violated standards 33% of the time, Puerto Real-fishing village 17% of the time, while Punta Lamela-inner bay, Punta Carenero and Punta Guanajibo showed violations to enterococci standards only once during our survey.

TABLE 2.8. Mean enterococci counts by site.

Site	cfu/100 mL*
Arrecife El Ron	2.75+1.87
Punta Ostiones	1.30+0.71
Arrecife Fanduco	0.38+0.27
Punta Carenero	6.61+4.74
Punta Lamela-outer bay	5.74+2.19
Punta Lamela-inner bay	11.62+5.82
Puerto Real-fishing village	**37.22+27.77**
Puerto Real-pier	**62.75+20.13**
Punta Guanajibo	2.14+1.33
Punta Arenas	14.64+14.31
Cayo Ratones	0.04+0.04
Guanajibo River estuary (bridge)	**367.38+133.53**
Guanajibo River mouth	**194.52+52.9**

*=Mean±one standard error. Bold figures represent mean violations to current enterococci standards (35 cfu/100 mL).

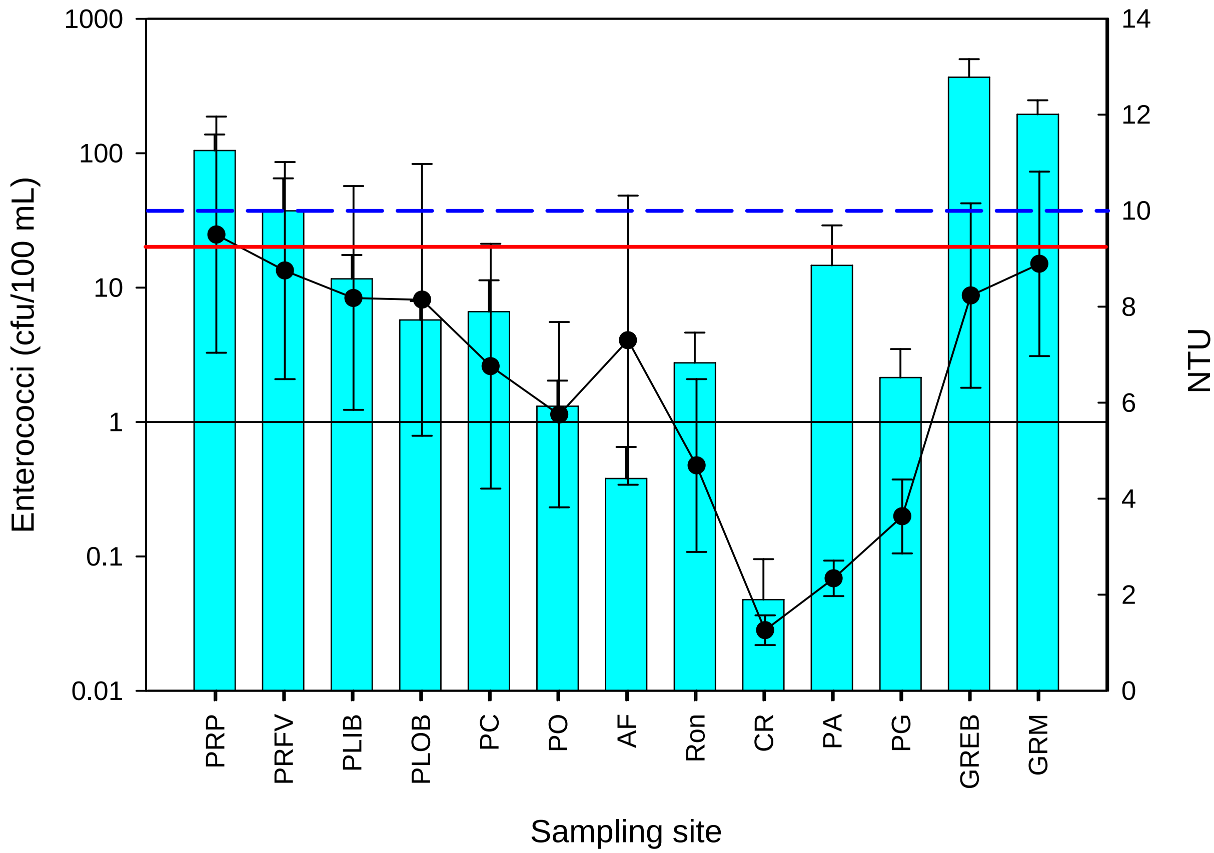

FIGURE 2.11. Enterococci counts (turquoise bars) as a function of water turbidity by site (mean±one standard error). Red solid line represents enterococci standard (35 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

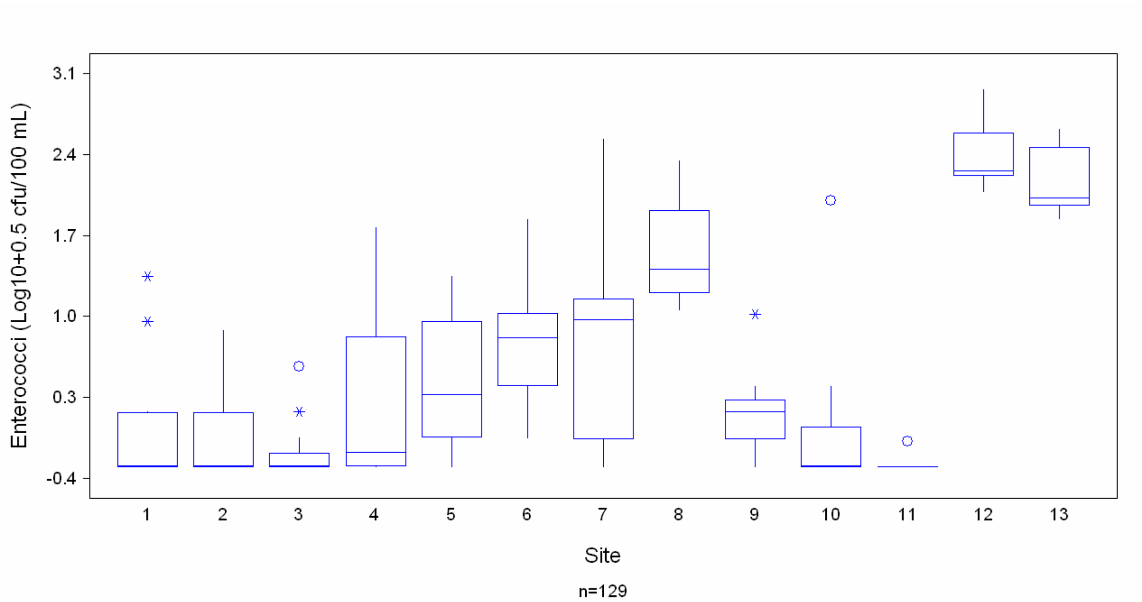

FIGURE 2.10 Box and whisker plot of enterococci (Log_{10}+0.5 cfu/100 mL) by site. Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). These outliers represent pulse events associated to non-point source runoff events or sediment resuspension associated to local oceanographic dynamics (described in Appendix A-1). Sites: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth.

TABLE 2.9. Microbiological water quality standard violations for enterococci for all sites sampled between June 2005 and June 2006.

Sample	Jun	Jul	Aug	Sept	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Ron	1	5	1	22	0	0	0	0	0	0	0	0
PO	0	5	0	1	6	1	0	0	0	1	0	0
AF	0	1	0	1	1	2	0	0	0	0	0	0
PC	39	0	0	5	5	0	0	4	1	0	1	0
PLIB	0	3	1	16	1	1	7	1	2	9	4	62
PLOB	0	3	10	7	14	1	0	0	1	4	1	1
PRFV	113	3	1	46	7	1	5	4	0	0	0	16
PRP	5	10	14	14	220	7	40	177	33	0	70	15
PA	**ns**	**ns**	**ns**	**ns**	**ns**	1	1	3	0	1	1	0
CR	**ns**	**ns**	**ns**	**ns**	**ns**	0	1	0	0	0	0	1
PG	**ns**	**ns**	**ns**	**ns**	**ns**	0	1	0	0	67	0	0
GRE-B	**ns**	**ns**	**ns**	**ns**	**ns**	120	110	60	53	40	860	907
GRM	**ns**	**ns**	**ns**	**ns**	**ns**	33	417	70	220	53	23	1253

NS= Site not sampled for that time period. Red color= violation to EQB class SB water quality classification enterococci standard (35 cfu/100 mL).

Higher mean turbidity values did not correspond always to high mean enterococci counts due to high variance in the data. This was the result of variable pulses of non-point source sewage pollution, pulses of turbid runoff from land-cleared areas, and rapid changing oceanographic dynamics. However, mean enterococci counts showed a moderate significant logarithmic increase as a function of water turbidity (r^2=-0.2294, p< 0.0001), particularly above 6.5 NTU (Figure 2.12). Turbidity data was pooled across time (Figure 2.13) and plotted against enterococci counts. There were continuous turbidity standard violations from August to November 2005, coinciding with the rainy season peak. Mean monthly enterococci counts violated enterococci standards 50% of the time, during the months of June 2005, from January to March 2006, May and June 2006, coinciding with lower turbidity values (Figure 2.13). Also, the months of November 2005, and April 2006 enterococci counts felt just short of standard violations. Enterococci counts showed a significant temporal decline (r^2=-0.2622, p=0.0005) with increasing NTU (Figure 2.14), indicating possible sediment interference in with plate

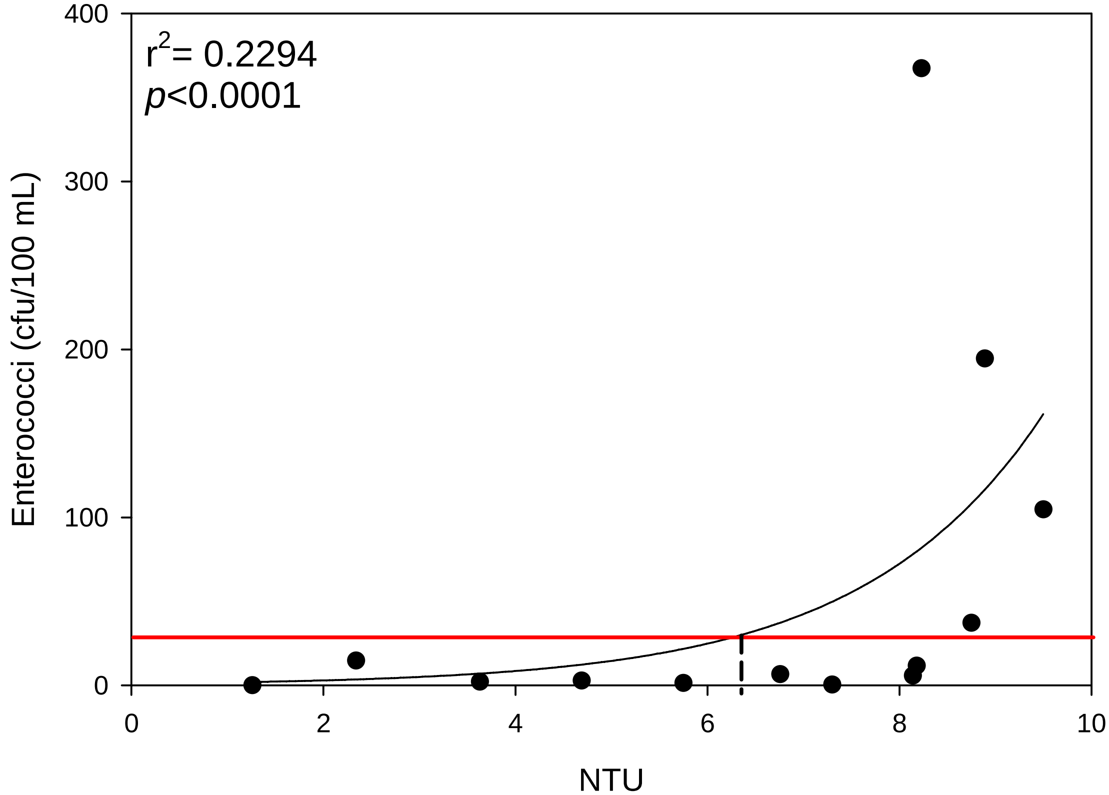

FIGURE 2.12 Logarithmic regression for enterococci counts and water turbidity by site. Red solid line represents enterococci standard (35 cfu/100 mL). Dashed line represents approximate turbidity level at which enterococci counts may exceed current standards.

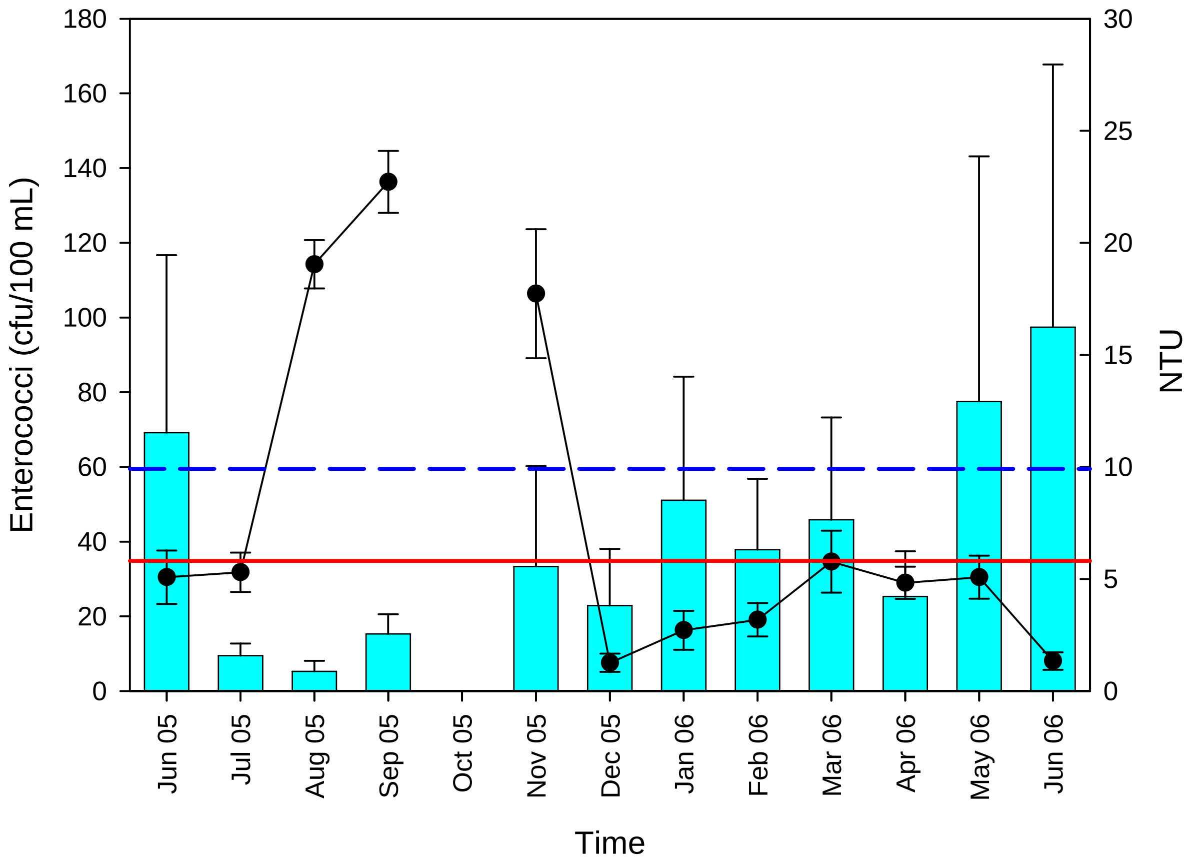

FIGURE 2.13. Enterococci counts (turquoise bars) as a function of water turbidity through time (mean±one standard error). Red solid line represents enterococci standard (35 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

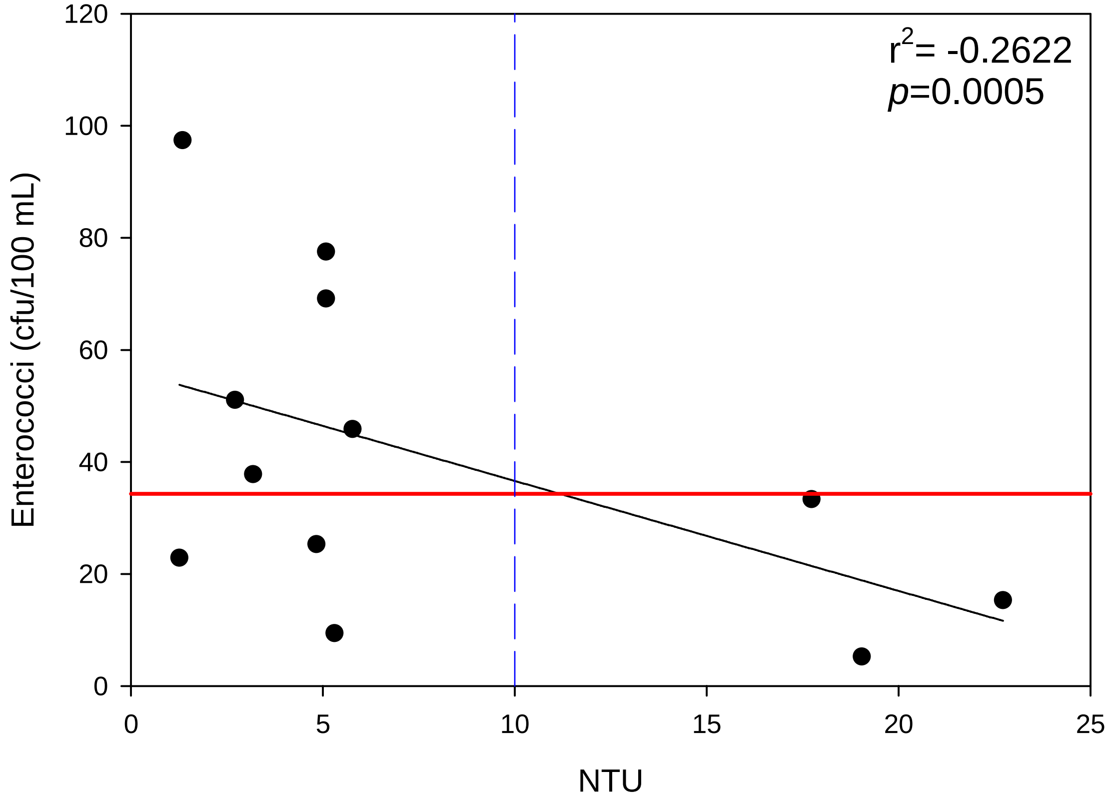

FIGURE 2.14 Logarithmic regression for enterococci counts and water turbidity by site. Red solid line represents enterococci standard (35 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

counts, but also high variability as a result of non-point source pollution pulse events and variability in oceanographic dynamics.

TABLE 2.9. Mean enterococci counts by geographic location.

Location	cfu/100 mL
Inshore (1)	**79.69±20.60**
Offshore (2)	3.80±1.88

*=Mean±one standard error. Bold figures represent mean violations to current enterococci standards (35 cfu/100 mL).

Enterococci patterns between geographic locations.

Enterococci data was pooled into two geographic location to test for spatial differences in enterococci counts between inshore (<0.5 km) and offshore (>0.5 km) waters. Tables 2.5 and 2.9 show that sites clustered within the *inshore* category had overall significantly higher (p<0.0001) mean enterococci counts that violated the current standard. The box and whisker graph shown in Figure 2.15 indicates that even though *offshore* sites had mean lower enterococci counts than inshore sites, they also presented significant high outlier counts which were remarkably similar to the normal enterococci count variability found at *inshore* sites. This suggests that non-point source sewage pollution pulses are impacting both geographic regions and that such pulses may elevate enterococci counts at *offshore* sites to levels similar to normal variation ranges at *inshore* sites. Pulses cause significant departures from normality in enterococci counts at *offshore* sites. This confirms for the geographically widespread nature of fecal pollution along the southwestern Puerto Rico shelf.

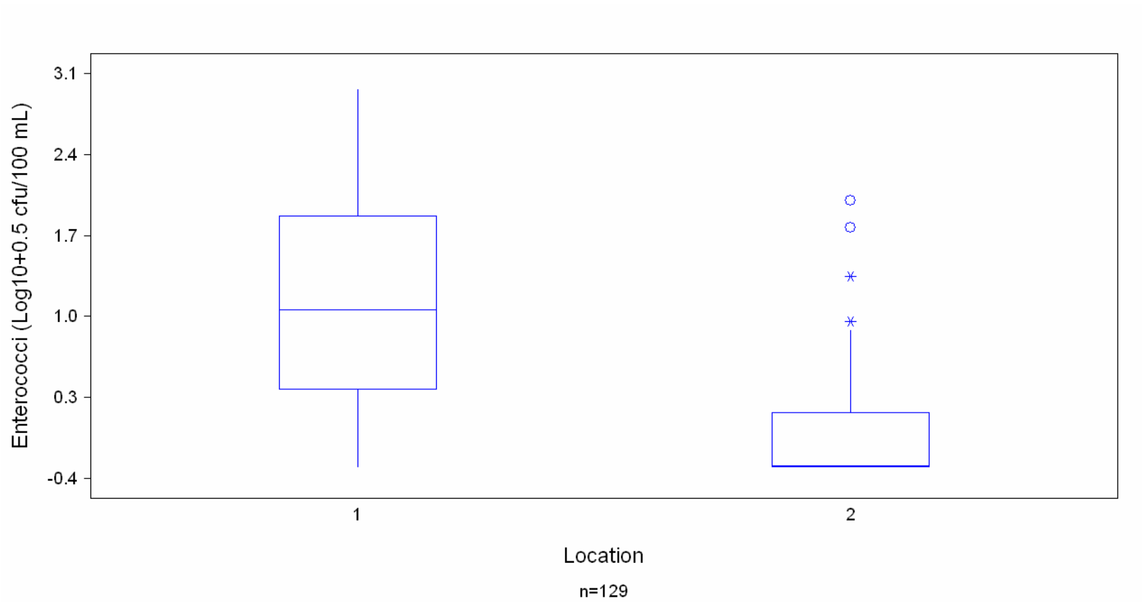

FIGURE 2.15 Box and whisker plot of enterococci (Log_{10}+0.5 cfu/100 mL) by location. Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Outliers documented within location 2 represent pulse events associated to non-point source runoff events or sediment resuspension associated to local oceanographic dynamics (described in Appendix A-1). Locations: (1) Inshore (<0.5 km); (2) Offshore (>0.5 km).

TABLE 2.10. Mean FC counts by management regime.

Location	cfu/100 mL
MPA (1)	15.82±5.54
Non-MPA (2)	**63.57±18.81**

*=Mean±one standard error. Bold figures represent mean violations to current enterococci standards (35 cfu/100 mL).

Enterococci patterns between management regimes.

Enteroocci data was pooled into two management regimes to test for spatial differences in enterococci counts between MPA and non-MPA waters. Tables 2.7 and 2.10 show a significant (p<0.0090) difference in mean enterococci counts between management regimes. However, mean enterococci counts at non-MPA sites also violated the current standard. The box and whisker graph shown in Figure 2.16 indicates the presence of long upper whiskers suggesting that both, MPA and non-MPA sites, are frequently impacted by non-point source sewage pollution pulses and that mean enterococci counts show a nearly similar variability. This also accounts for the geographically widespread nature of fecal pollution along the southwestern Puerto Rico shelf.

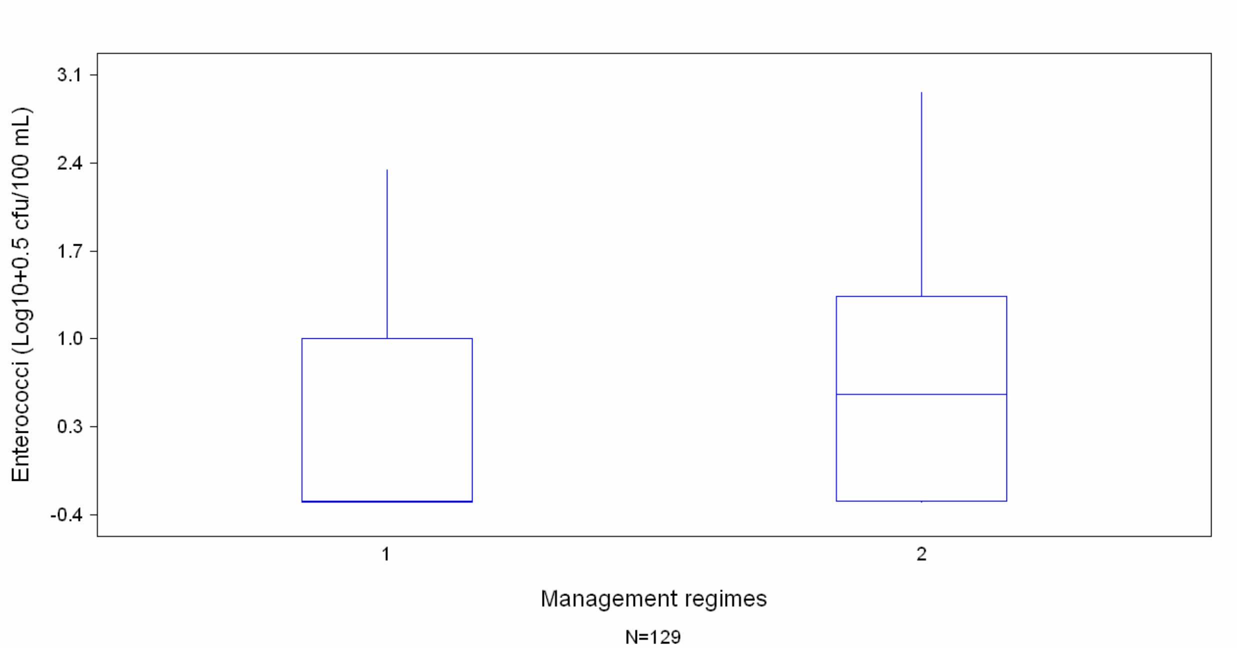

FIGURE 2.16. Box and whisker plot of enterococci counts (Log_{10}+0.5 cfu/100 mL) by management regime. Each box plot is composed of a box and two whiskers. The box encloses the middle half of the data. The box is bisected by a line at the value for the median. The vertical lines at the top and the bottom of the box are the whiskers, and they indicate the range of "typical" data values. Whiskers always end at the value of an actual data point and can't be longer than 1½ times the size of the box. Extreme values are displayed as "*" for possible outliers and "O" for probable outliers. Possible outliers are values that are outside the box boundaries by more than 1½ times the size of the box. Probable outliers are values that are outside the box boundaries by more than 3 times the size of the box (Analytical Software, 2003). Management regimes: (1) MPA; (2) Non-MPA.

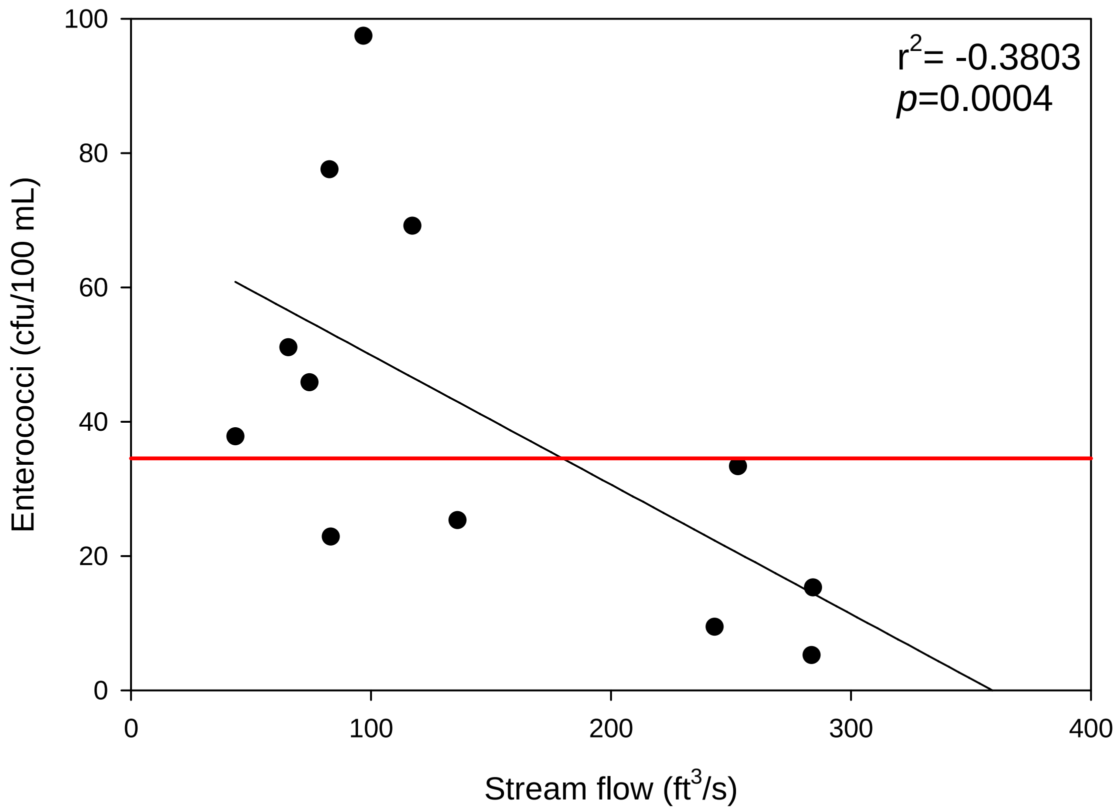

FIGURE 2.16 Linear regression analysis of enterococci counts as a function of Guanajibo River stream flow.

Enterococci patterns and the Guanajibo River stream flow.

Regression analysis of enterococci counts as a function of Guanajibo River stream flow showed a moderate significant correlation (r^2=-0.3803, p=0.0004), suggesting that increasing stream flow may result in increased water turbidity levels across some areas of the southwestern shelf (Figure 2.16). Increasing turbidity was already shown to significantly interfere with enterococci counts.

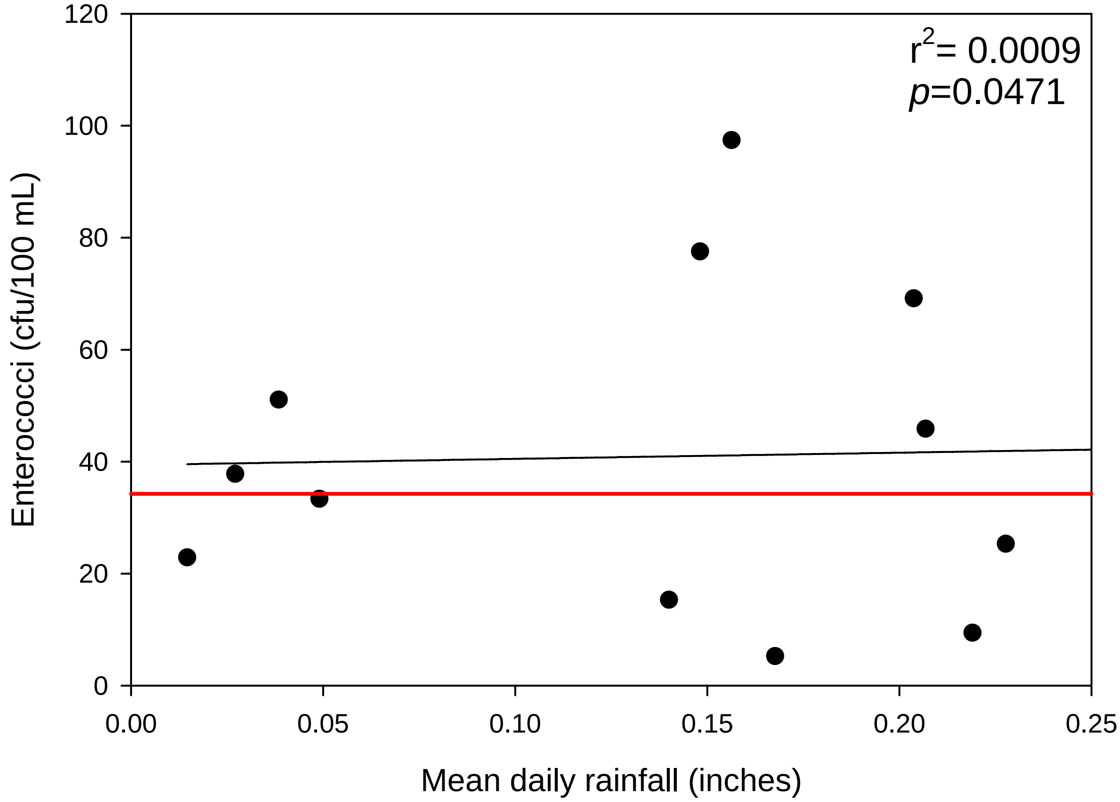

FIGURE 2.17 Linear regression analysis of enterococci counts as a function of mean daily rainfall measured in the Guanajibo River watershed.

Enterococci patterns and mean daily rainfall.

Enterococci counts were also regressed as a function of mean daily rainfall to test for any temporal pattern (Figure 2.17). There was a significant but very weak relationship between them (r^2=-0.0009, p=0.0471). This suggests that during our study, mean daily rainfall (pooled by month) did not have any significant impact on enterococci counts. Monthly rainfall averages might not be an adequate indicator of impacts on microbial water quality because occasional daily pulses are masked within monthly averages.

Discussion

This study showed that widespread geographic areas of the southwestern Puerto Rico platform are being frequently impacted by non-point source fecal pollution. Chronic high fecal indicator counts at some of the inshore sites revealed stronger influences of various non-point sewage pollution sources. In several sampling sites, mostly from inshore locations, fecal indicators detected were above the recognized standards. For beaches these are 200 cfu/100mL, for fecal coliforms and 35 cfu/100mL for enterococci (EQB, 2006). It is important to note that USEPA has delegated the EQB the recommendation to designate this coast as a SB body of water. These waters are mainly coastal and estuarine waters used for primary and secondary contact. In spite of that, most inshore SB waters along the southwestern Puerto Rico shelf showed a highly questionable water quality. Sites that were clustered into the *inshore* category (<0.5 km off the coast) clearly showed violations to the EQB fecal indicator standards when all the sampling periods were pooled. It is evident that these sites receive frequent impacts from fecal pollution sources. These are located closer to point- and non-point sources of fecal contamination. The fact that some of these sampling stations failed to comply with these standards is alarming, since these waters are intensively used for recreational purposes, as described later. They also support extensive coral reef and seagrass communities of paramount ecological importance.

The high variability of the results suggests that presence of indicator bacteria varied notably during short periods of times. Clearly, sites that went over the standards (i.e., Guanajibo River estuary, Guanajibo River mouth, and some areas at Puerto Real Bay) are not suitable for recreational purposes. It could be possible that fluctuations caused by the influence of non-point pollution sources and complex variable oceanographic conditions interfered with the presence of bacterial indicators. But bacterial counts were still high enough to register standard violations that should prevent their use for primary contact activities and fishing.

Sites located within local MPAs (Arrecifes Tourmaline Natural Reserve, Cayo Ratones and Adjacent Waters Natural Reserve, Finca Belvedere Natural Reserve marine extension) also showed unequivocal evidence of non-point source fecal pollution. MPAs support significant coral reefs and seagrass communities that provide important nursery, shelter, feeding and spawning grounds to a wide variety of fish and macroinvertebrate species of commercial importance. Thus, they support a large artisannal fishery. However, most coral reefs through the impacted region have shown dramatic long-term degradation due to constant non-point source pollution pulses (discussed in Appendix A-4). There were higher microbial counts at non-MPA sites, but these were largely influenced by the high outlier microbial counts registered at the Guanajibo River estuary (bridge) and at the Guanajibo River mouth. Regardless of the management regime, the presence of fecal pollution at coral reef and seagrass habitats suggests the need to establish region-wide integrated water quality management measures. All of these MPAs are still lacking a management plan. But the establishment of a standard MPA management plan will not be enough to prevent fecal pollution impacts and will require integrated approaches to manage activities at the coastal zone and the Guanajibo River watershed, just to mention the two most important aspects of managing water quality impacts.

Microbial concentrations were overall higher in inshore sampling stations when compared to offshore sites. However, they showed high temporal and spatial variability that seemed to be associated to variable oceanographic dynamics associated to: (1) tidal flow; (2) turbidity; 3) wind direction and velocity; (4) wind-driven circulation; (5) wave action; (6) northwestern swells; and (7) the behavior of the Guanajibo River plume. These factors were discussed in Appendix A-1. These fluctuations were suggested by the presence of higher concentrations of microbial indicators in samples collected during strong wind-driven circulation, ebbing tides followed by heavy rainfall runoff, following wind-driven sediment resuspension, and following impacts by the Guanajibo River plume.

Environmental parameters, such as salinity, can influence indicator bacteria survival rates (Boehm, et al, 2003; Anderson, et al., 2005). Only Guanajibo River estuary (bridge) and Guanajio River mouth showed significantly lower salinity through this study (Phasse 1 report). These two locations showed consistently the highest microbial indicators concentrations, suggesting chronic non-point source fecal contamination sources.

Offshore remote sites, such as Arrecife El Ron, as well as some of the inshore sites (i.e., Punta Ostiones, Punta Carenero, Arrecife Fanduco, Punta Lamela-inner bay, Puerto Real-pier) showed also peak microbial counts during the 2005 rainy season peak. This also coincided with peak turbidity values. However, it is interesting to point out that for some of the sites the highest values of microbial indicators were detected when water turbidity was lowest. This suggests that possibly microbial indicators can survive marine conditions and remain viable and culturable in suspended sediments. Desmarais et al. (2002) demonstrated that for *Escherichia coli* and enterococci, the addition of more sediment corresponded to a more rapid rate of re-growth in the presence of more sediment, and the number of bacteria showed little dependence on the initial population of microbes. The lack of relationship between FC counts and water turbidity may indicate that other environmental variables may affect FC survival, viability and culturability. When turbidity values were high, enterococci were not detected in considerable concentrations. These two parameters were negatively correlated, suggesting interference by suspended sediments.

It is evident that Guanajibo River and other streams from the region have influences in water quality of the south western shelf area of Puerto Rico. Mean peak value of Guanajibo River stream flow was registered in October 2005, but it was not possible to sample in this date due to adverse weather conditions. However, during moderate but significant stream flow peaks during the months of July, August, September and November 2005, turbidity values were the highest and FC counts were largely variables. During this period enterococci counts were variable but relatively low.

Conclusions

Coastal waters along the southwestern Puerto Rico shelf are polluted by non-point fecal sources of human and animal origin. Variable and geographically widespread sources may include (1) malfunctioning septic tanks, (2) illegal sewage discharges from private houses and businesses (i.e., restaurants), (3) malfunctioning and/or overloaded sewers, (4) cattle grazing grounds, (5) farms, (6) house boats, and (7) trailer grounds. Non-point source fecal pollution is impacting widespread coral reef and seagrass areas, even within three local MPAs. This suggest a need to design integrated management strategies to address marine, coastal-maritime zone, and watershed management needs in order to reduce fecal pollution impacts in the coast. Water quality management needs include identification of strategies to control most of the dispersed sources of fecal contamination. It is necessary to determine and take into account the Total Maximum Daily Loads (TMDLs) for the Guanajibo River, in order to implement regulations for discharges, since there were no potential sources or TMDLs reported to the USEPA (http://oaspub.epa.gov/tmdl/enviro.control?p_list_id=PRWR0262b_00&p_cycle=2004 accessed on August 30, 2006). In order to achieve this, surveys of sources of pollution (i.e. agricultural and industrial activities, non-PRASA water systems around the watershed, etc.) need to be performed and published. Such information may provide important tools to help prioritize land use.

There is also a strong need to implement management practices for Puerto Real Bay. Results obtained from these sampling stations suggest frequent episodes of human fecal pollution for sites near the shoreline in a timely manner. This is alarming, since these waters are used for recreational (i.e. non-swimming water-related sports), as well as for fishing purposes. These waters are also nursery and feeding grounds of commercially important species, as well as for dolphins, and the endangered Caribbean manatee (*Trichechus manatus*), hawksbill turtle (*Eretmochelys imbricata*), and brown pelican (*Pelecannus occidentalis occidentalis*) It is of importance to mention that the northern portion of Puerto Real Bay falls within the boundaries of the recently designated Finca Belvedere Natural Reserve marine extension (DRNA, 2002). This reserve harbor a nursery ground of a myriad of fish and macroivertebrate species of commercial significance. Thus, there is a need to develop a management plan for this, as well as for the other two natural reserves in the region. Fecal pollution and eutrophication impacts should be thoroughly documented at these sites in order to identify specific integrated management responses to reduce such impacts.

It is even of major concern that sites located as far as 10 Km offshore are occasionally impacted by fecal pollution and high turbidity pulses, mostly associated to the Guanajibo River plume. These sites support extensive coral reef and seagrass communities which are already showing signs of degradation. Frequent episodes of such conditions can result in a long-term decline of their ecological conditions. Such declines could further result in a major community phase shift, negatively affecting ecosystem functions, degrading essential fish habitats and losing the economic value of these ecosystems. The significance of such conditions was described in Appendix A-4.

Finally, there is a need to develop mathematical models to predict the possible fate of microbial pollutants under variable meteorological and oceanographic conditions. Modeling, in combination with calculated TMDLs, would provide useful tools to manage marine ecosystems and potentially reduce pollution effects. To achieve this, additional microbiological and physico-chemical water quality monitoring, as well as oceanographic dynamics characterization, are needed in order to parameterize the model. Such model should be tested with further long-term monitoring efforts, as well as applied to other similar nearby scenarios such as Boqueron Bay or other polluted embayments in Puerto Rico.

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Appendix A-3

Use of Microbial Source Tracking (MST) methods to determine non-point source fecal pollution along the southwestern Puerto Rico shelf.

Edwin A. Hernández-Delgado

A-3. Use of microbial source tracking methods (MST) to determine non-point source fecal pollution along the southwestern Puerto Rico shelf.

Introduction

Fecal contamination of coastal waters has become a serious concern among coastal communities in developing countries over the last years (Byamukama, et al., 2005). This issue raises doubts about water quality, as well as public health problems. The potential adverse impacts of wastewater could affect both the environment and human health (Lipp et al., 2002). In this context it is important to determine sources of fecal contamination of coastal waters in order to prevent diseases and assure water quality (Scott, at al., 2002), since many coastal areas are used for multiple activities, including fishing and recreation. Therefore, fecal microorganisms are ideally the most useful biologic indicators to assess water contamination, since they compose the major microbial flora of the human gastrointestinal tract (Carrillo, et al., 1985).

It has been recommended by the United States Environmental Protection Agency the use of *Escherichia coli*, a member of the fecal coliform group, as a microbial indicator for recreational freshwater bodies (USEPA, 2000). Conversely, it has been suggested that *E. coli* may not be a dependable indicator in tropical and subtropical environments, because it has the ability to replicate in polluted soils (Desmaris, et al., 2002, Solo-Gabriele, et al., 2000), as well as various tropical environments (Hazen and Toranzos, 1990; Hernández-Delgado, 1991).

Members of *Bacteroides* have also been suggested as fecal pollution indicators, since the most numerous members of the human colonic flora belong to this genus. *Bacteroides* represent nearly 1011 organisms per gram of feces (dry weight) (Finegold, 1983). They are Gram-negative, anaerobic bacilli or cocco-bacilli. Each species is morphologically distinct and most are encapsulated. In order to maintain their high numbers, the *Bacteroides* are evidently able to compete with other members of the flora, as well as transient organisms, for utilization of these resources. While members of the *Bacteroides* genus are very important in the human gastrointestinal normal flora, they also can be opportunistic pathogens, causing a variety of infections throughout the body (Sheehan, et al., 1989).

Recently, a series of novel Microbial Source Tracking (MST) methods have been developed to determine sources of fecal contamination. One of these methods consists in detecting host-specific molecular markers using the 16S rDNA gene of *Bacteroides* (Bernhard, et al., 2000a,b). This method is culture-independent and has shown to be useful in detecting sources of fecal contamination (Field, et al., 2003, Simpson, et al., 2003), as members of these genus show host-specificity (Dick, et al., 2005). Other microorganisms that could be potentially useful to determine fecal contamination using its 16S rDNA gene are members of the genus *Clostridium.* Matsuki et al. (2002) have also suggested the use of molecular markers from this genus.

This study was intended to test the applicability of MST methods in tropical coastal environments. We tested the null hypotheses of no significant difference in the frequency of positive bacterial primer signals among sites, between inshore and offshore locations, and between marine protected areas (MPAs) and non-MPA control sites. Also, we documented if there was any correlation between frequency of positive bacterial primer signals and concentration of indicator bacteria. A similar correlation was made with water turbidity.

Materials and Methods

Water Sample Collection

Sampling was conducted monthly from June 2005 to June 2006, with the exception of October 2005. Samples were collected at 13 sampling sites located off the Cabo Rojo coast in the western Puerto Rico shelf (Figure 3.1). These included the following: (1) Arrecife El Ron; (2) Punta Ostiones; (3) Arrecife Fanduco; (4) Punta Carenero; (5) Punta Lamela-outer bay; (6) Punta Lamela-inner bay; (7) Puerto Real-fishing village; (8) Puerto Real-pier; (9) Punta Guanajibo; (10) Punta Arenas; (11) Cayo Ratones; (12) Guanajibo River estuary (bridge); and (13) Guanajibo River mouth. Sampling sites were selected based on a geographical distance gradient from Puerto Real Bay towards offshore representative coral reef and seagrass habitats.Grab water samples were collected at each study site using autoclaved Nalgene 1 L plastic bottles (Nalgene Co.). Samples were collected at 30 cm below surface and immediately placed on ice until analyzed within 6 hr.

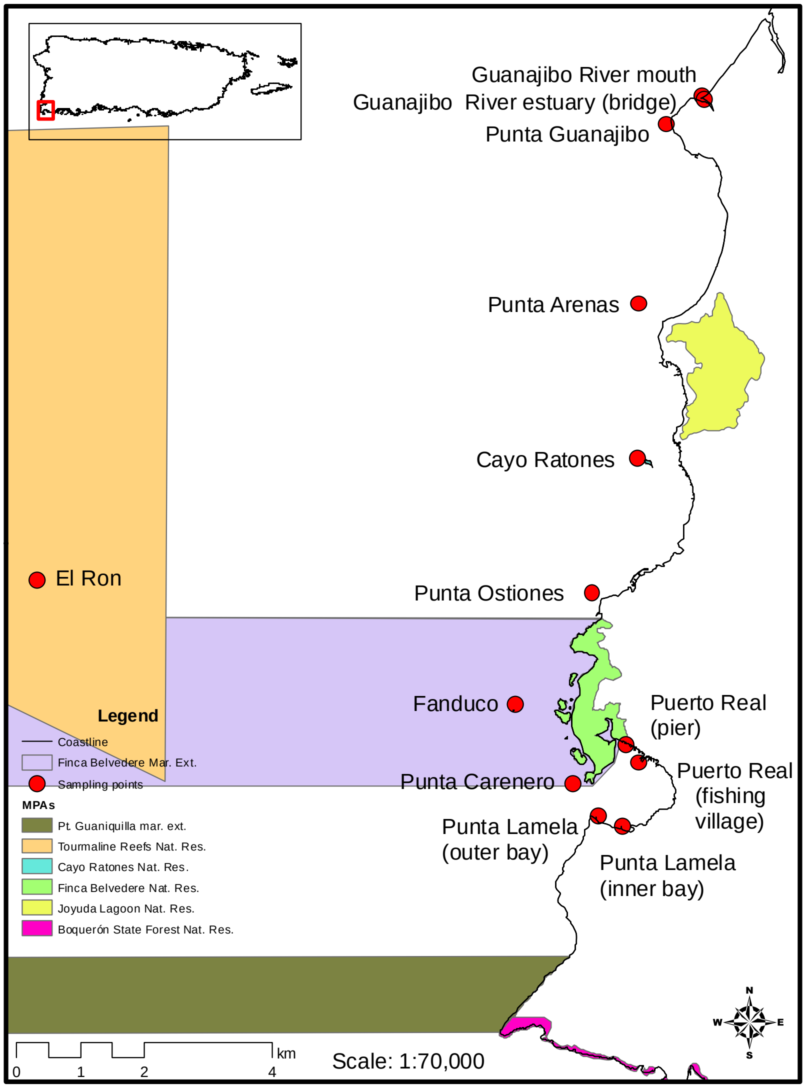

FIGURE 3.1. Water quality sampling sites and their relationship to the existing local PRDNER-managed natural protected area network.

Water physico-chemical variables.

In situ analysis of water physico-chemical parameters such as temperature, pH, salinity, conductivity, dissolved oxygen were collected in triplicates using portable instrumentation (Horiba Co.). Water turbidity was documented using a Orbeco 866 portable turbidimeter. Turbidity measurements were expressed in Nephelometric Turbidity Units (NTU). Physico-chemical variables were thoroughly discussed in Appendix A-1.

Fecal Coliform and Enterococci Counts

Samples were analyzed using the membrane filtration technique to quantify fecal coliforms and enterococci (APHA, 1992). Water samples were filtered onto cellulose acetate membranes, using MicroFunnel™ filter funnels (Pall, Co., Ann Arbor, MI). Volumes analyzed were generally 1mL, 10mL, and 100mL. Enterococci were cultured at 35ºC/48 h using *M Enterococcus* agar. Fecal coliforms were cultured at 44.5ºC/24 h using *mFC* agar. Confirmatory tests for both were done using *Azide Dextrose Broth* and *Lauryl Triptose Broth*, respectively. These tests were used to validate the presence of fecal microorganisms in the samples taken. Microbial indicators data was thoroughly discussed in Appendix A-2.

Molecular Microbial Source Tracking Methods

DNA extraction from water samples

A total of 100 ml of water were filtered using 0.2 µm polycarbonate membranes (Osmonics, Inc.). Once filtered, the membranes were stored in autoclaved 2ml centrifuge tubes, and kept at frozen at -80°C until ready to test (Bernhard, et al., 2000a). MoBio Soil DNA kit (MoBio Labs, Solana Beach, CA) was used to obtain genomic DNA (Simpson, et al., 2003).

Polymerase Chain Reaction with Bacteroides and Clostridia primers

DNA samples obtained from all the sites were amplified using *Bacteroides-Prevotella* primers (Bernhard, et al., 2000a,b), which amplify general (GB) and host-specific (HF134, HF183) 16S rDNA for this group. We also used an assay developed to amplify 16S rDNA for *Clostridia* (CP) group (Matsuki et al, 2002). Each 25µl PCR mixture contained 10X *Ex Taq* buffer, deoxynucleoside triphosphates (DNTPs) at a concentration of 2.5mM each, primers at a concentration of 25 pM each and 0.626U of *Ex Taq* (TaKaRa, Inc.). The thermal cycler programs were as follow: initial denaturing at 94°C for 2 min, 35 cycles at 94° for 1 min, 1 min for each annealing temperature for the primers, and 72°C for 1.5 min, followed by a final extension at 72°C for 7 minutes. Electrophoresis was performed by preparing 1% agarose gels stained with Gel Star (Cambrex, Inc.).

Clone Libraries and Sequencing Analysis.

Amplified products were purified with QIAQuick DNA purification kit (QUIAGEN, Inc., Valencia, CA). Cloning of amplified products was performed using TOPO TA Cloning reaction (Invitrogen, Inc.). Amplified clones were sent for sequencing at Cincinnati Children's Hospital (Cincinnati, OH). Sequences were analyzed using Sequencher 4.5 (Genes Code, Ann Harbor, MI, USA) and submitted to NCBI BLAST.

Hypothesis testing.

We tested the null hypothesis of no significant difference in the individual microbial markers +/- responses among sites. Temporal +/- responses were scored as 1 for positive hits and 0 for negative responses. Data was analyzed by means of a Kruskal-Wallis non-parametric one-way analysis of variance (ANOVA) using Statistix Software 8.0 (Analytical Software). *Site* was used as main variable and replicate samples through time as error term. We tested a second null hypothesis of no significant difference in microbial marker responses between geographical locations. Locations were subdivided into *Inshore* (<0.5 km) and *Offshore* (>0.5 km). Inshore sites included sites 5, 6, 7, 8, 9, 10, 12, and 13. Offshore sites included sites 1, 2, 3, 4, 10. Locations were arbitrarily selected based on distance of sampling site from known or suspected non-point source sewage pollution. Data were treated and tested as above. *Geographical location* was used as main variable and replicate samples through time as error term.

We also tested the null hypothesis of no significant effect of management regime (i.e., marine protected area, MPA) on the response of individual microbial markers. Sampling sites were grouped as follows: *Within MPA sites*: Arrecife El Ron, Punta Carenero, Arrecife Fanduco, Puerto Real-pier, and Cayo Ratones, with all remaining sites as *Control Outside MPA sites*. *Management* was used as main variable and replicate samples through time as error term. Regression analyses were performed to test relationships between individual microbial markers and indicator bacteria. A similar approach was used to test for any relationship with water turbidity. In addition, frequency of positive hits of molecular markers was analyzed against microbiological water quality violations of current standards to test efficiency of standard microbiological methods.

Results.

Molecular assays using general and host-specific primers were used to determine potential sources of fecal contamination. General *Bacteroides* (GB32), human-specific (HF134, HF183), and *Clostridia* (CP) markers were used to amplify DNA obtained from each sampling station throughout the study period (Table 3.1).

TABLE 3.1. PCR results (+/-) for all sites sampled between June 2005 and June 2006.

Sample	Jun	Jul	Aug	Sept	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Ron	-	-	-	C	-	-	C	C	-	-	-	-
PO	B/H	B/H	-	B/C	-	C	-	H/C	-	-	-	B/C
AF	-	-	-	-	-	C	-	-	-	-	-	-
PC	-	-	C	H/C	-		-	H/C	H	-	C	B/H
PLIB	B/H	B/H	C	B/H/C	H	B/H/C	B	H/C	B/C	B/H/C	B/C	C
PLOB	B/H	B/H	C	H/C	H/C	C	B	-	C	B/C	B	B/H
PRFV	B/H	B/H	-	B/H/C	C	-	B/C	B/H/C	C	B	-	B/C
PRP	B/H	B/H	B/H/C	H/C	C	B/C	B/H/C	B	B/C	B/C	-	H
PA	**ns**	**ns**	**ns**	**ns**	**ns**	-	-	-	B	B/C	C	B/H
CR	**ns**	**ns**	**ns**	**ns**	**ns**	-	-	-	-	H	-	B
PG	**ns**	**ns**	**ns**	**ns**	**ns**	H	-	-	B	B/C	B/C	B
GRE-B	**ns**	**ns**	**ns**	**ns**	**ns**	B	B/C	B/H/C	B/C	C	B/H/C	B
GRM	**ns**	**ns**	**ns**	**ns**	**ns**	B/H/C	B/H/C	B/C	B/C	B/C	B/C	B/H

B= General *Bacteroides* (GB32) primer; F= Human fecal (HF134); H= Human fecal (HF183) primer; C= Clostridia primer; NS= site not sampled for that time period. Red color= violation to EQB SB water quality classification FC standard (200 cfu/100 mL); Yellow color= violation to EQB SB water quality classification enterococci standard (35 cfu/100 mL); Green color= simultaneous violation to both standard.

Variation among sites.

The GB32 marker showed a significantly (p<0.0001) higher frequency of positive hits (100%) at the Guanajibo River mouth sampling site (Table 3.2, Figure 3.2). Positive signals for the molecular marker GB32 were not registered at Arrecife El Ron and Arrecife Fanduco. All other sites were not statistically different from each other. GB32 showed a moderate logarithmic

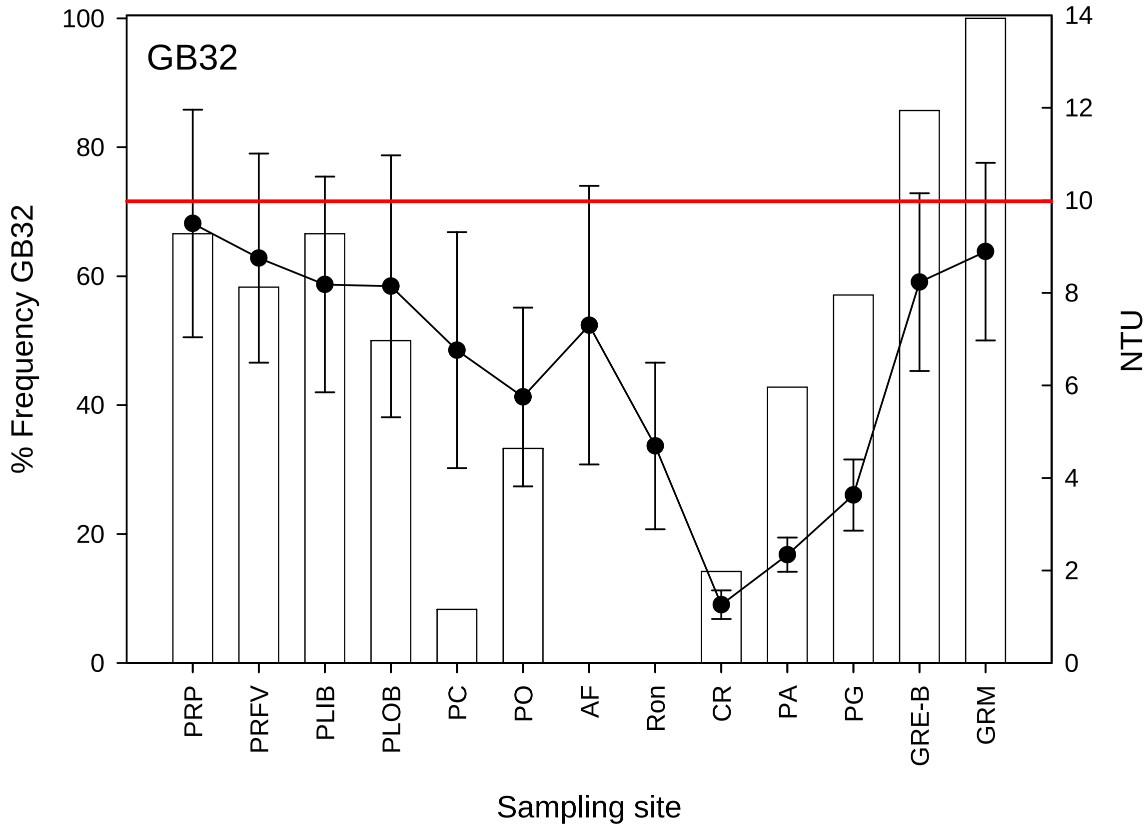

FIGURE 3.2. Marker GB32 signal frequency for each site (data pooled through time). Line shows mean±one standard error turbidity. Red line illustrates water turbidity standard (10 NTU) for SB classified waters, according to existing EQB water quality regulations. Mean values met current standard at all sites. However, violations occurred during pulse events at most sites.

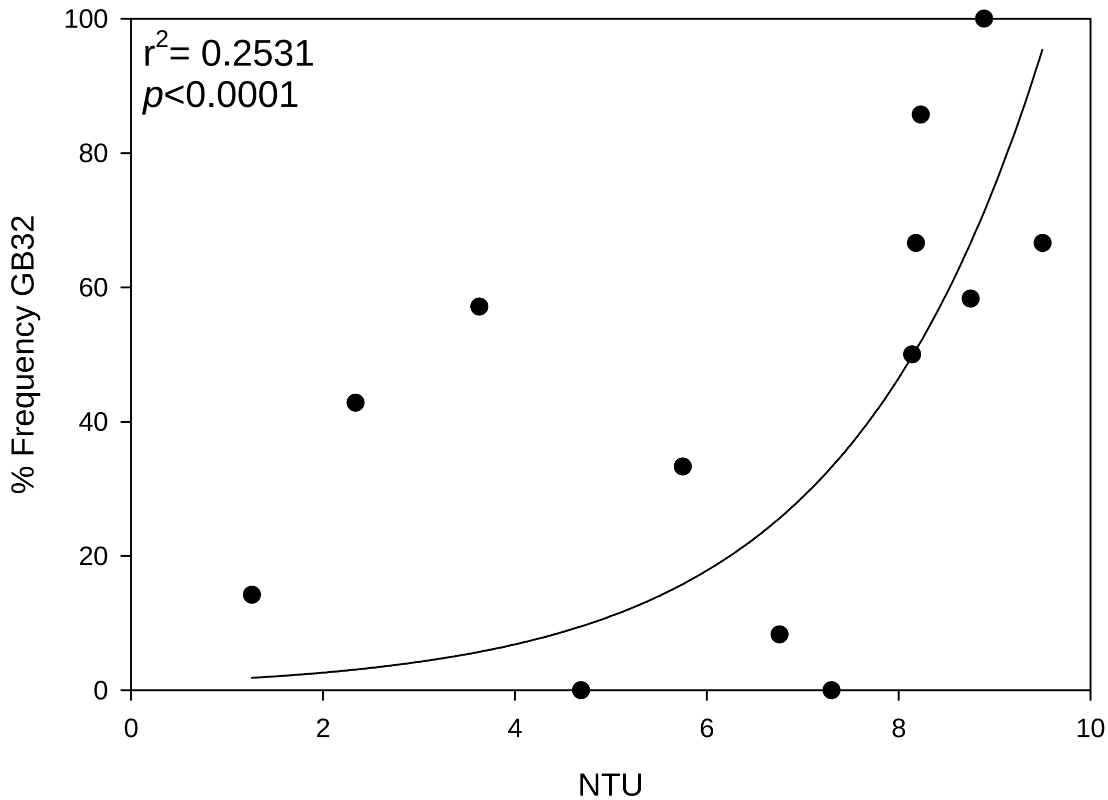

FIGURE 3.3. Logarithmic regression of frequency of positive signals for GB32 as a function of water turbidity.

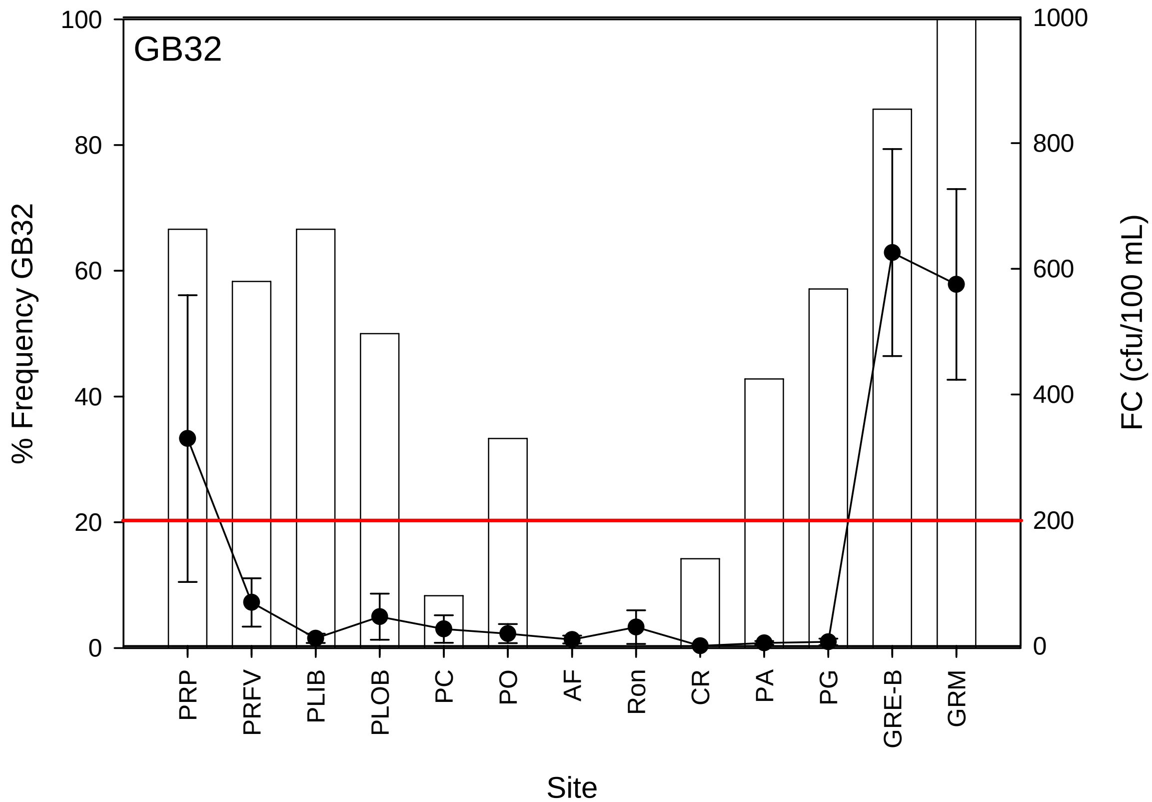

FIGURE 3.4. Marker GB32 signal frequency for each site (data pooled through time). Line shows mean±one standard error FC counts. Red line illustrates FC standard (200 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

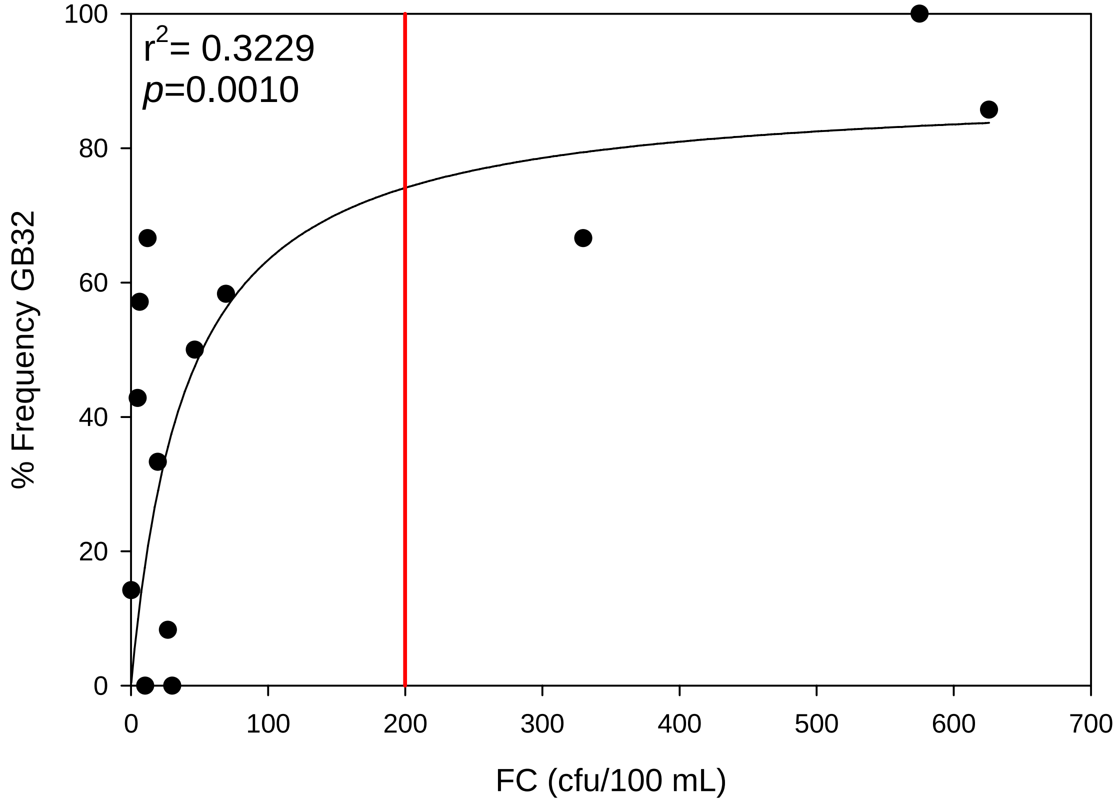

FIGURE 3.5. Logarithmic regression of frequency of positive signals for GB32 as a function of FC counts. Red line illustrates FC standard (200 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

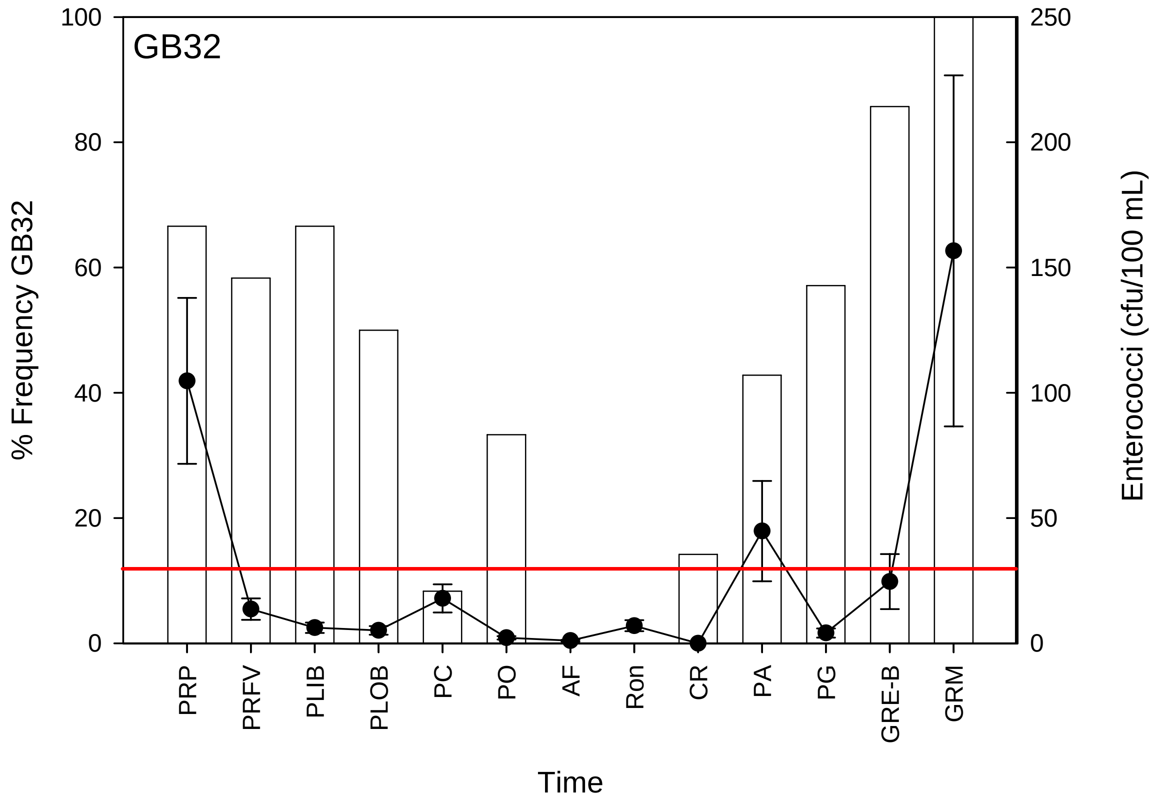

FIGURE 3.6. Marker GB32 signal frequency for each site (data pooled through time). Line shows mean±one standard error enterococci counts. Red line illustrates enterococci standard (35 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

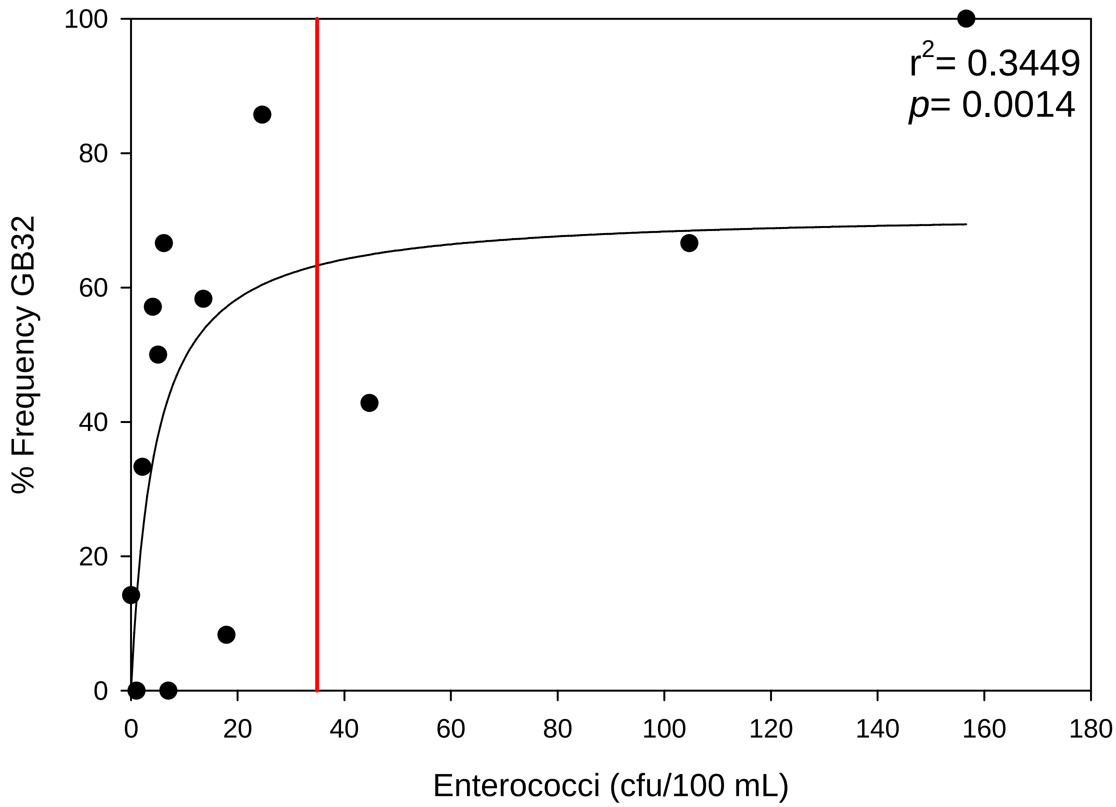

FIGURE 3.7. Logarithmic regression of frequency of positive signals for GB32 as a function of enterococci counts. Red line illustrates enterococci standard (35 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

relationship with increasing turbidity (r^2=0.2531, p<0.0001) (Figure 3.3). This suggests that the highest frequency of positive signals was documented from inshore sites under chronic high turbidity that receive frequent non-point source pollution pulses. Some of the inshore sites (i.e., Puerto Real-fishing village, Punta Lamela-inner bay, Punta Lamela-outer bay, Punta Arenas, and Punta Guanajibo), that also had low FC counts, had a considerable frequency (>35%) of positive GB32 responses (Figure 3.4). GB32 positive responses showed a significantly rapid increase with increasing FC counts (r^2=0.3229, p=0.0010) (Figure 3.5). A similar trend was documented when GB32 responses were plotted against enterococci counts (Figure 3.6). GB32 positive responses showed a significantly rapid increase with increasing enterococci counts (r^2=0.3449, p=0.0014) (Figure 3.7). This suggests that non-point source sewage pollution explained between 25 and 34% or the variation documented in GB32 responses. However, other factors such as oceanographic dynamics and methodological artifacts might have influenced these results.

TABLE 3.2. Summary results of Kruskal-Wallis one-way analysis of variance of primers presense/absence data among sites.

Parameter	**D.F. (within, between)**	**K-W statistic**	***P* value**
GB32	12,118	48.61	**<0.0001**
HF183	12,118	21.98	**0.0378**
CP	12,118	26.42	**0.0093**

Assays were performed to screen for human-specific *Bacteroides* (HF134 and HF183). The HF183 marker showed a significantly (*p*=0.0378) higher frequency of positive hits at Punta Lamela-inner bay (58%) and at Puerto Real-pier (50%), in comparison to other sites (Table 3.2, Figure 3.8). Arrecife El Ron and Arrecife Fanduco did not show any signal for HF183, but showed high turbidity levels. All other sites were not statistically different from each other. HF183 showed a moderately high logarithmic relationship with increasing turbidity (r^2=0.4693, p<0.0001) (Figure 3.9). This suggests that the highest frequency of positive signals for this marker was also registered from inshore sites under chronic high turbidity. Some of the sites throughout the study period had low fecal indicator counts but high frequency (>25%) of detecting this host-specific marker (Figure 3.10). HF183 showed a moderate significant relationship (r^2=0.2608, p=0.0007) to FC counts (Figure 3.11). The same response was observed when the results obtained with HF183 molecular marker were compared with enterococci counts. (Figure 3.12). HF183 showed a moderate significant relationship (r^2=0.2299, p=0.0011) to FC counts (Figure 3.11). The sites that showed this behavior were Puerto Real-fishing village, Punta Lamela-inner bay, Punta Lamela-outer bay, Punta Carenero, and Punta Ostiones. Interestingly, there was a lack of positive signals throughout the study period for the host-specific marker HF134. Our study showed that water turbidity explained 47% of the variation obsberved in HF183 responses. Further, non-point source sewage pollution explained less than 26% or the variation documented in HF183 responses. Similarly, other factors such as oceanographic dynamics and methodological artifacts might have influenced these results.

For the *Clostridia* (CP) PCR assay, frequency of positive results was significantly higher at Guanajibo River mouth and Guanajibo River estuary (Table 3.2; Figure 3.14). The only site that did not show signals for this molecular marker was CR. There were some signals present for the Clostridia primer for the sites Arrecife Fanduco (8.3%) and Arrecife El Ron (25%) even though these samples did not show amplification for any of the *Bacteroides* assays. If taken into account sites sampled throughout the whole year, Punta Lamela-inner bay presented the highest

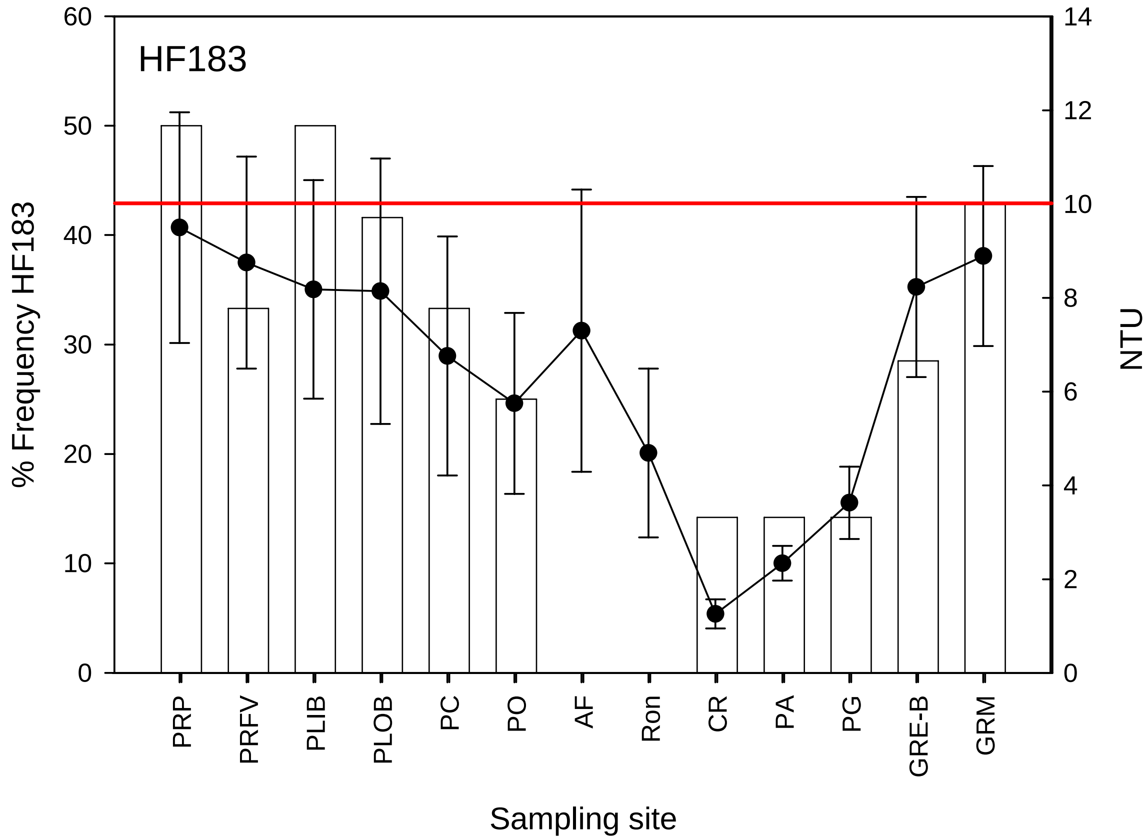

FIGURE 3.8. Marker HF183 signal frequency for each site (data pooled through time). Line shows mean±one standard error turbidty. Red line illustrates turbidity standard (10 NTU) for SB classified waters, according to existing EQB water quality regulations.

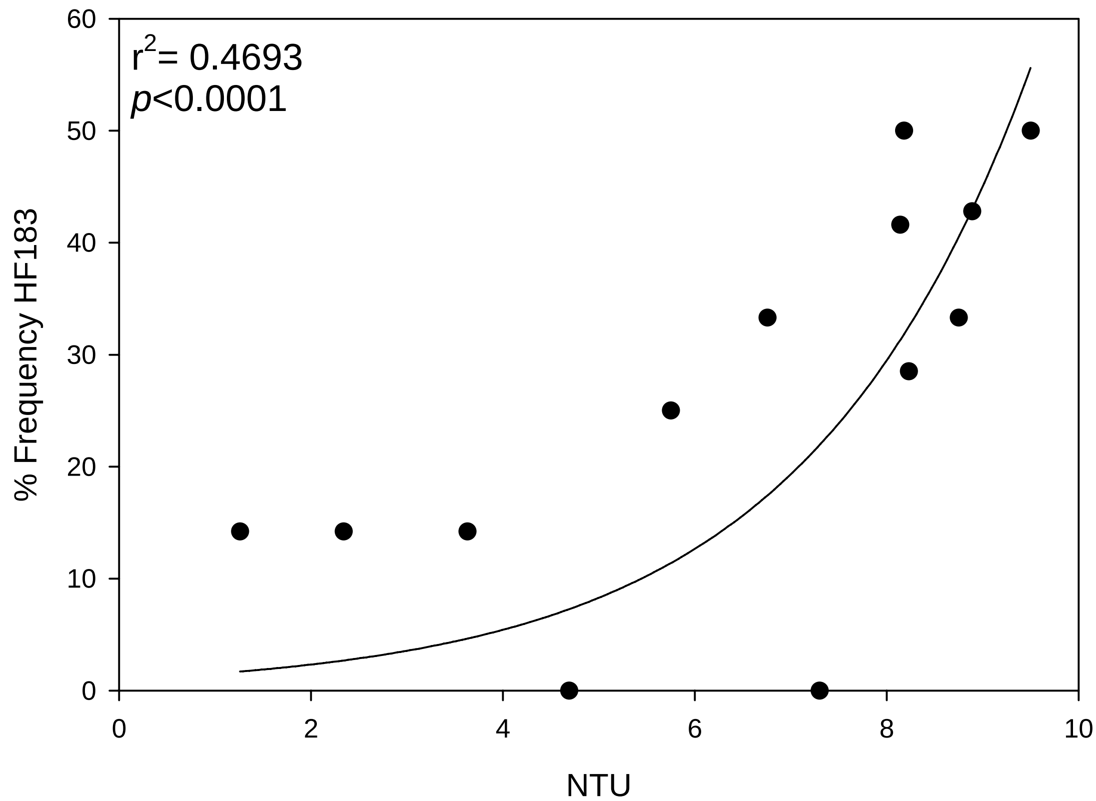

FIGURE 3.9. Logarithmic regression of frequency of positive signals for HF183 as a function of water turbidity.

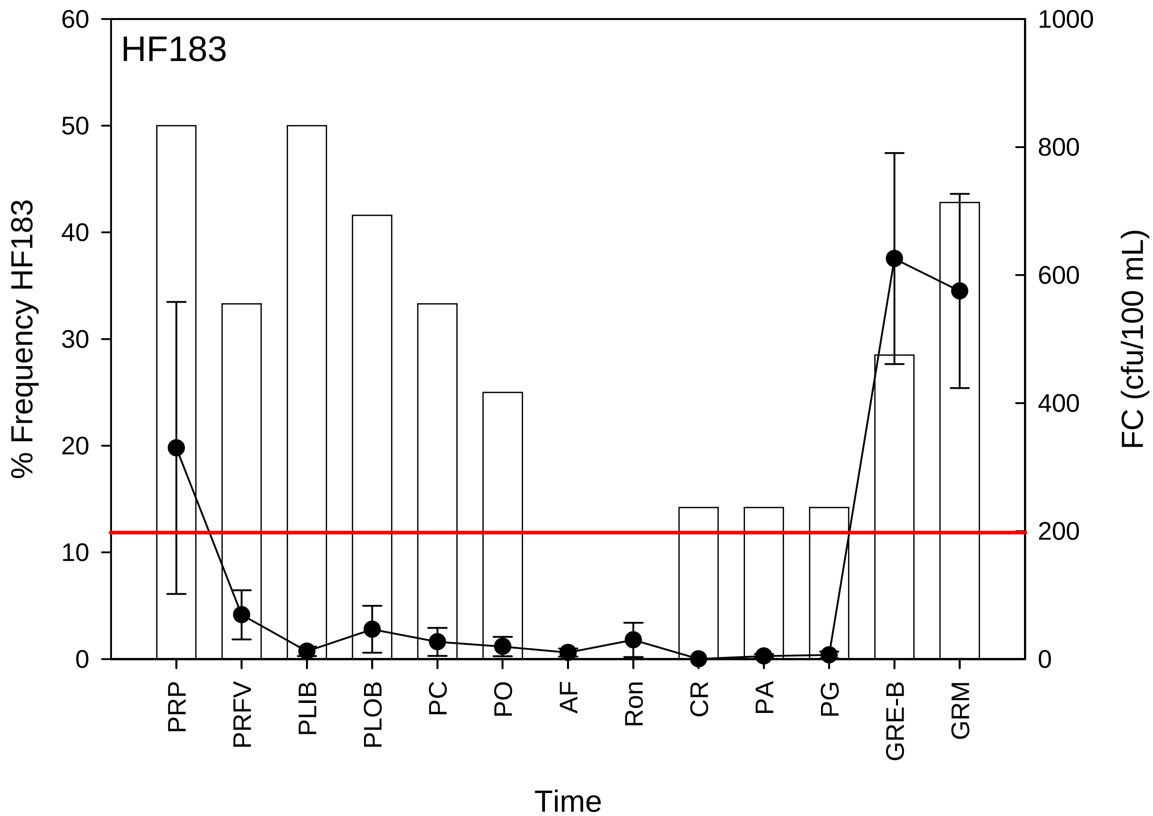

FIGURE 3.10. Marker HF183 signal frequency for each site (data pooled through time). Line shows mean±one standard error FC counts. Red line illustrates FC standard (200 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

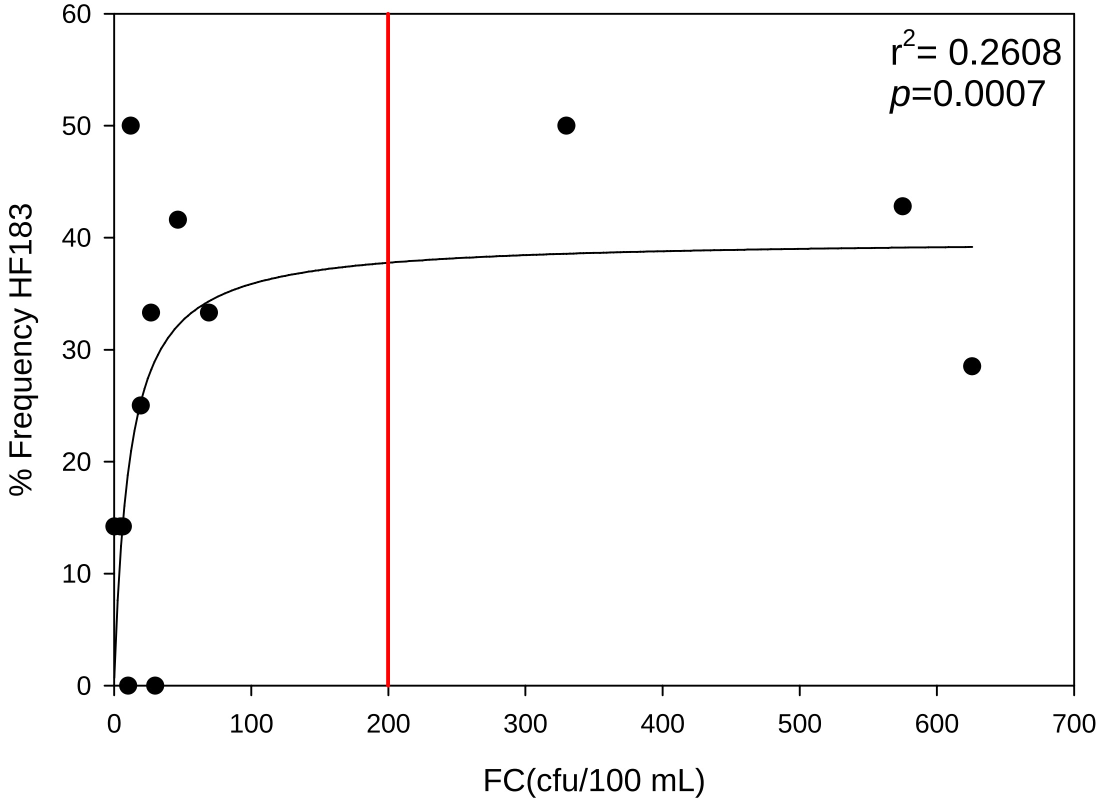

FIGURE 3.11. Exponential regression of frequency of positive signals for HF183 as a function of FC counts. Red line illustrates FC standard (200 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

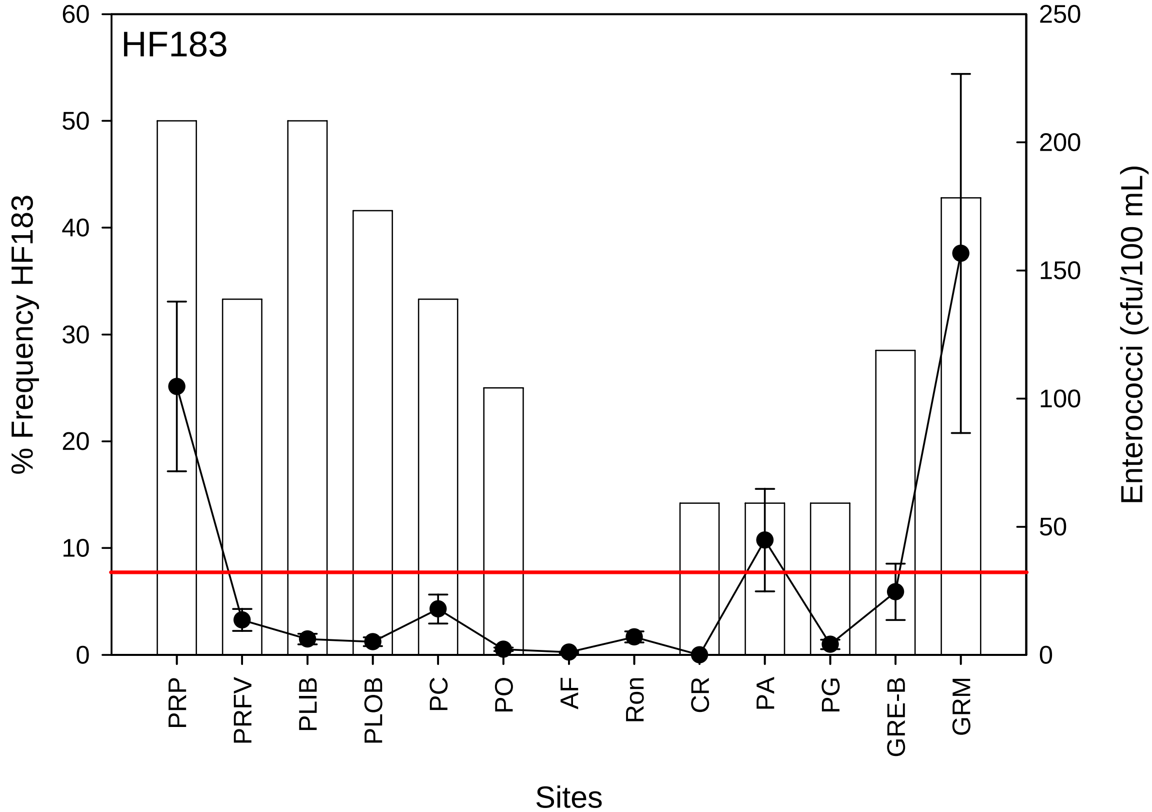

FIGURE 3.12. Marker HF183 signal frequency for each site (data pooled through time). Line shows mean±one standard error enterococci counts. Red line illustrates enterococci standard (35 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

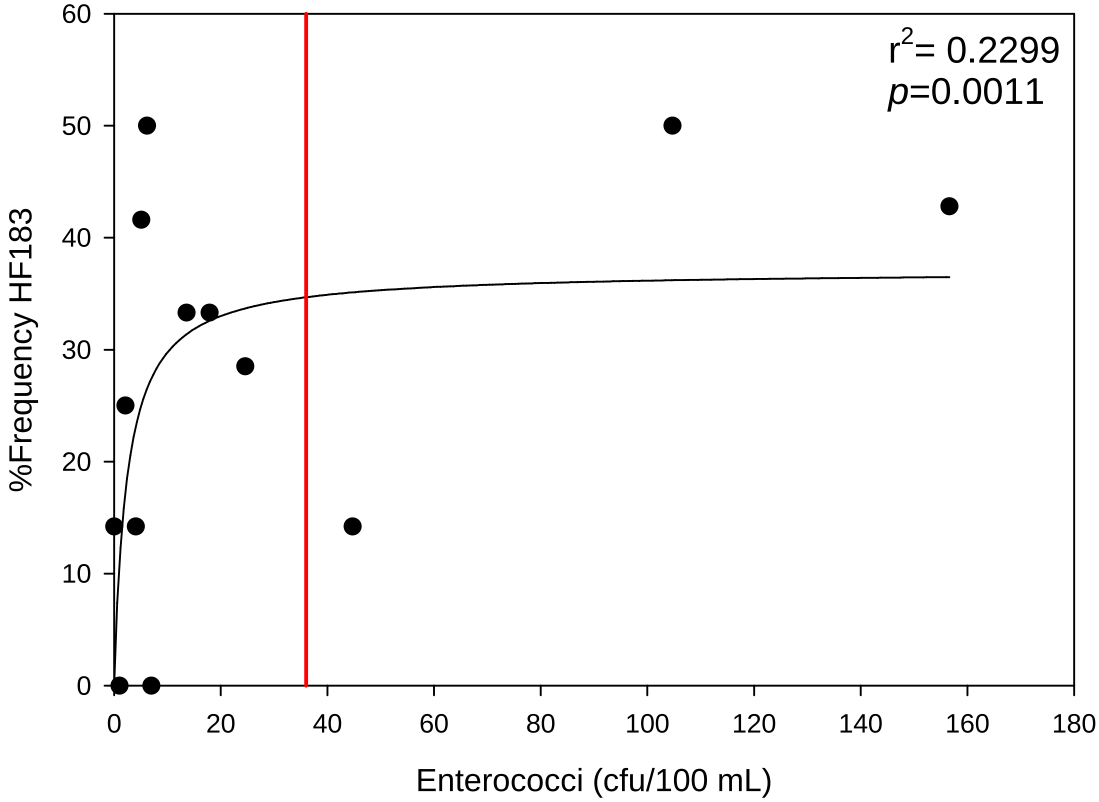

FIGURE 3.13. Exponential regression for the frequency for HF183 and enterococci counts for the sampling stations throughout the sampling period. Red line illustrates enterococci standard (35 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

ratio of positive signals (66.6%), followed by Punta Lamela-outer bay (58.3%). It was remarkable to observe that the ratios for positive signals for this assay were similar to the GB32 assay for some of the sites. As well as with the GB32 and HF183 assays, there was a moderate but significant relationship between CP frequency and water turbidity (r^2=0.3314; p<0.0001) (Figure 3.15). Also, there was a significant strong relationship between CP frequency and FC counts (r^2=0.5314; p<0.0001) (Figures 3.18 and 3.19). Some sites (i.e., Arrecife Fanduco and CR) showed high levels of turbidity, but low detection for the marker CP (Figs. 3.14 and 3.15).

Variation between inshore and offshore locations.

Sampling sites located farther than 0.5 km from the shoreline showed a significantly lower frequency of positive signals for the assays performed with GB32 (p<0.0001), HF183 (p=0.0006), and CP (p=0.0001), over the study period (Table 3.3). For instance, GB32 positive hits averaged 65.2±5.8% at inshore locations, but only 16.1±4.7% at offshore ones (Figure 3.20). GB32 positive hits averaged 65.2±5.8% at inshore locations, but only 16.1±4.7% at offshore ones. HF183 positive hits averaged 42.0±6.0% at inshore locations and only 14.5±4.5% at offshore ones. CP positive hits averaged 56.5±6.0% at inshore locations and only 22.6±5.4% at offshore ones.

TABLE 3.3. Summary results of Kruskal-Wallis one-way analysis of variance of primers presense/absence data between locations.

Parameter	**D.F. (within, between)**	**K-W statistic**	***P* value**
GB32	1,129	32.06	**<0.0001**
HF183	1,129	11.91	**0.0006**
CP	1,129	15.50	**0.0001**

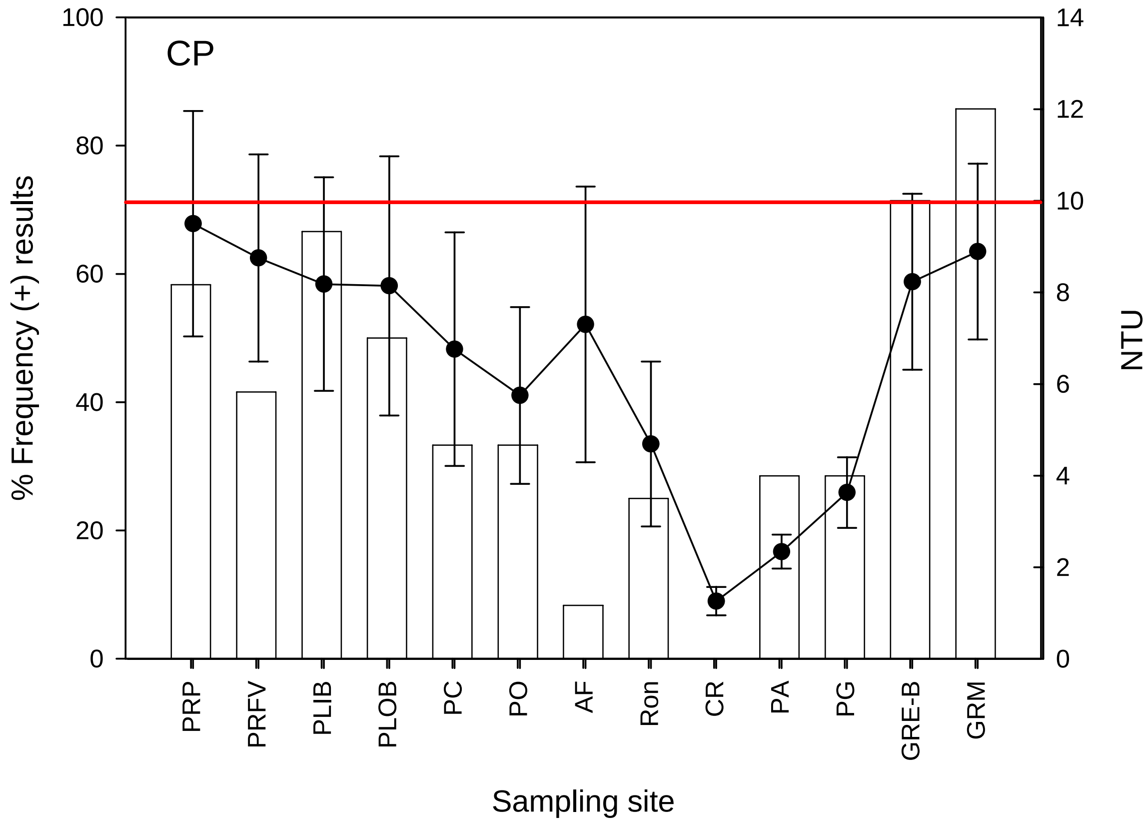

FIGURE 3.14. Marker CP signal frequency for each site (data pooled through time). Line shows mean±one standard error turbidity. Red line illustrates water turbidity standard (10 NTU) for SB classified waters, according to existing EQB water quality regulations.

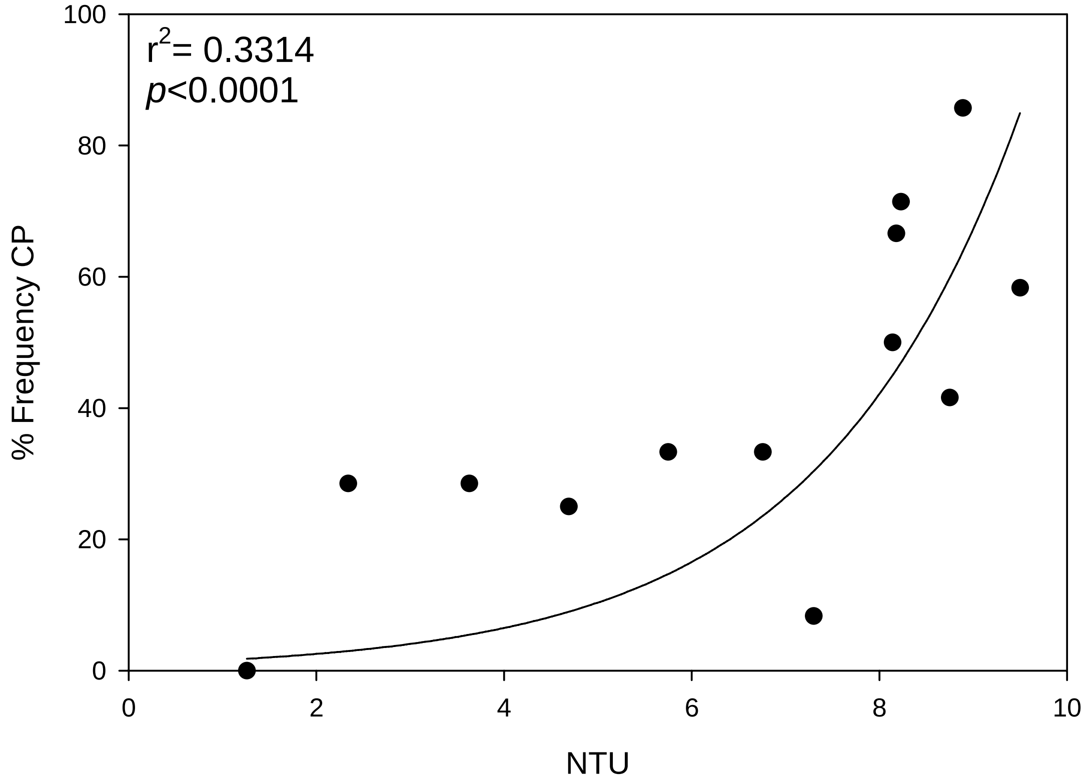

FIGURE 3.15. Logarithmic regression of frequency of positive signals for CP as a function of water turbidity.

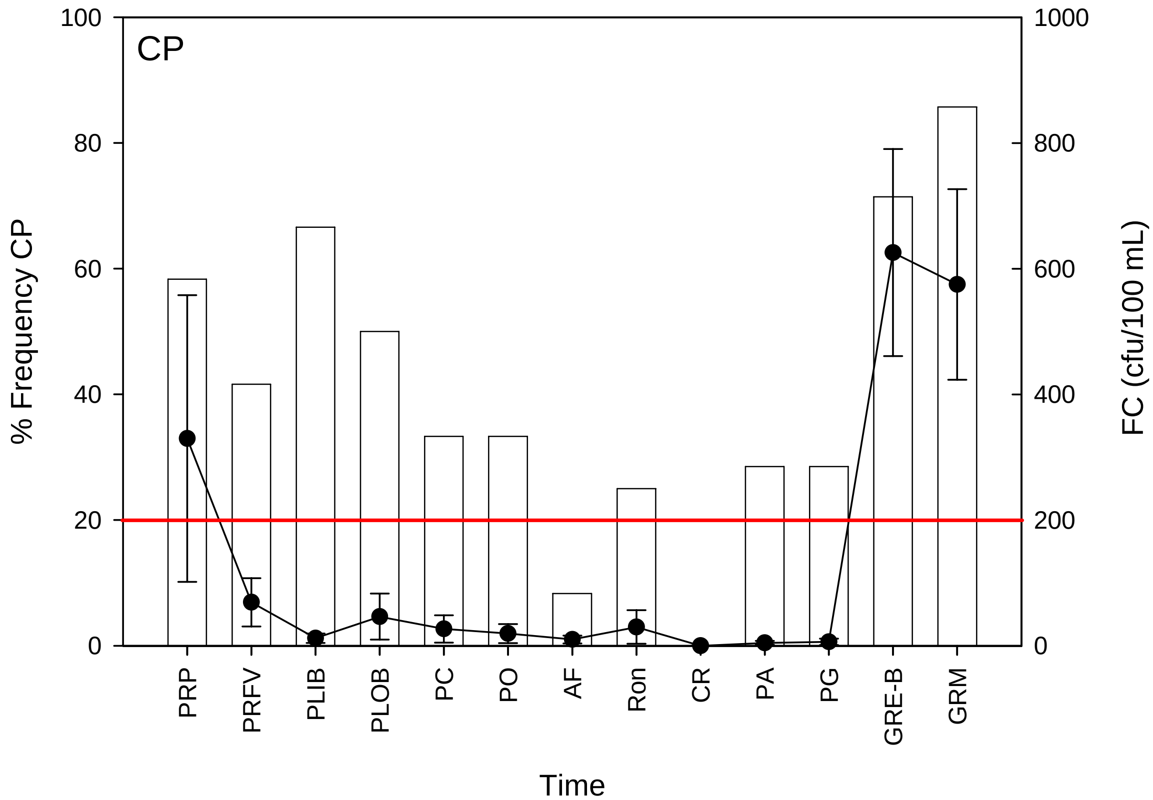

FIGURE 3.16. Marker CP signal frequency for each site (data pooled through time). Line shows mean±one standard error FC counts. Red line illustrates FC standard (200 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

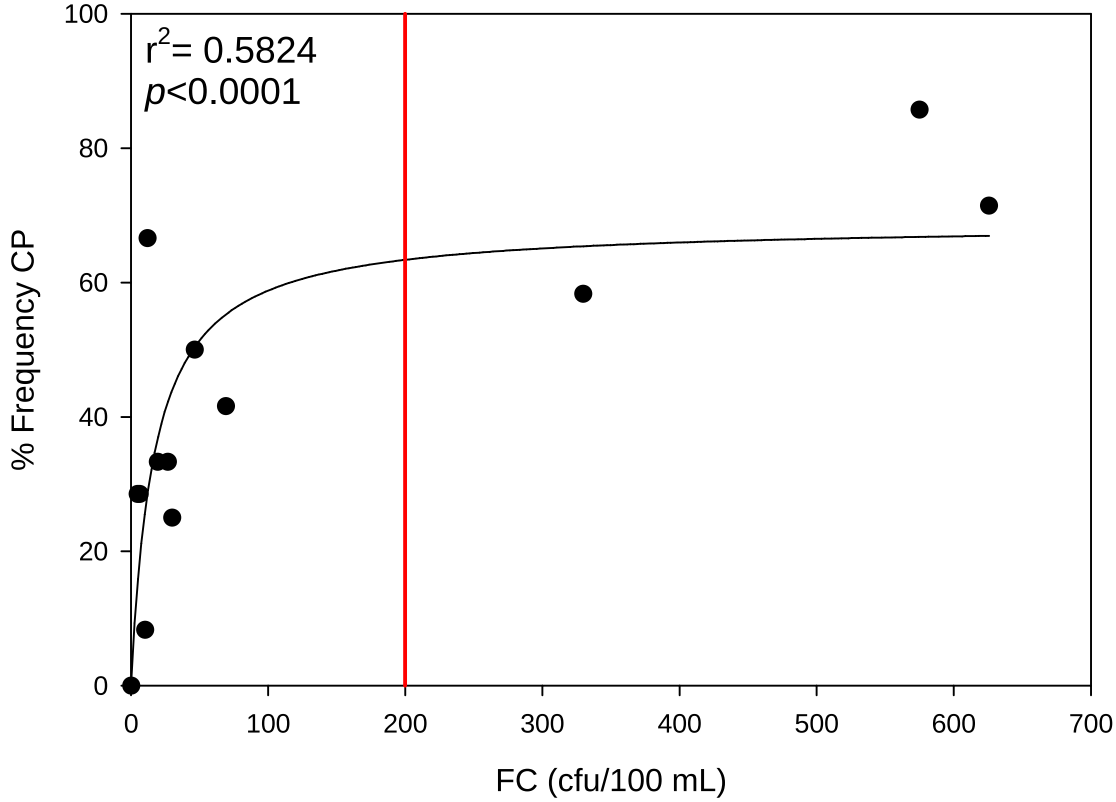

FIGURE 3.17. Exponential regression of frequency of positive signals for CP as a function of FC counts. Red line illustrates FC standard (200 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

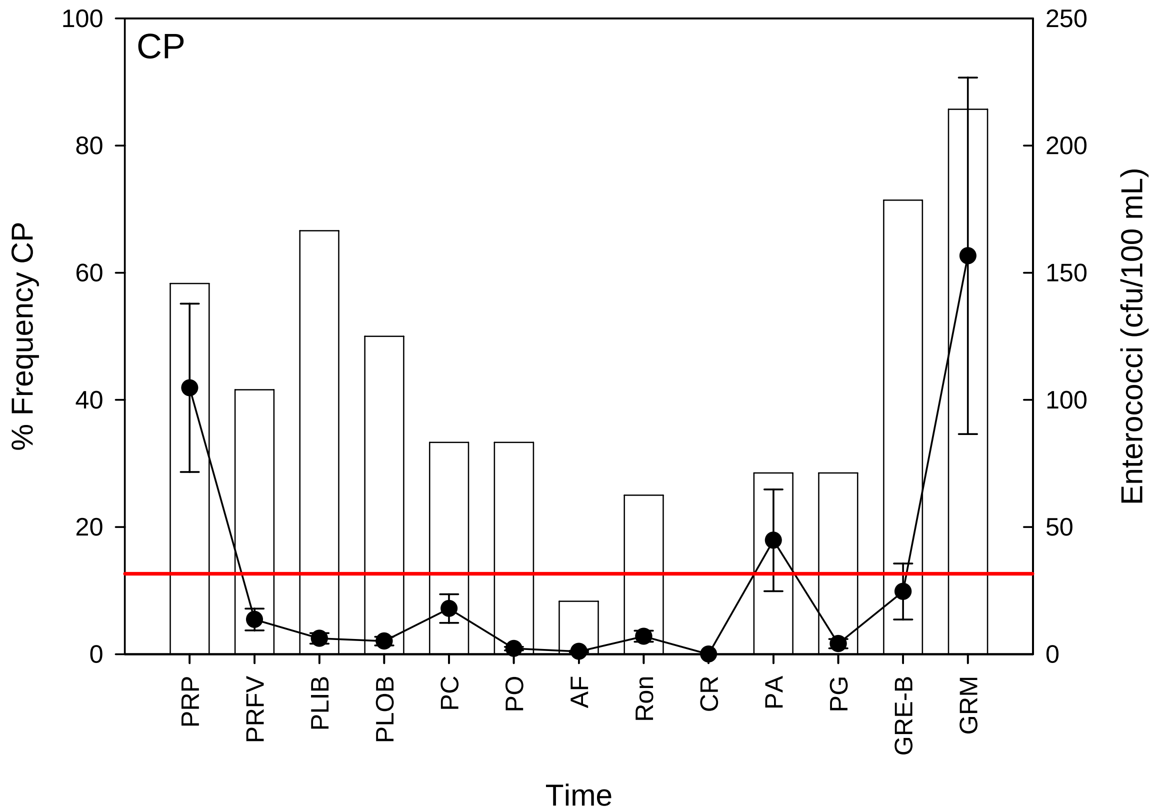

FIGURE 3.18. Marker CP signal frequency for each site (data pooled through time). Line shows mean±one standard error enterococci counts. Red line illustrates enterococci standard (35 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

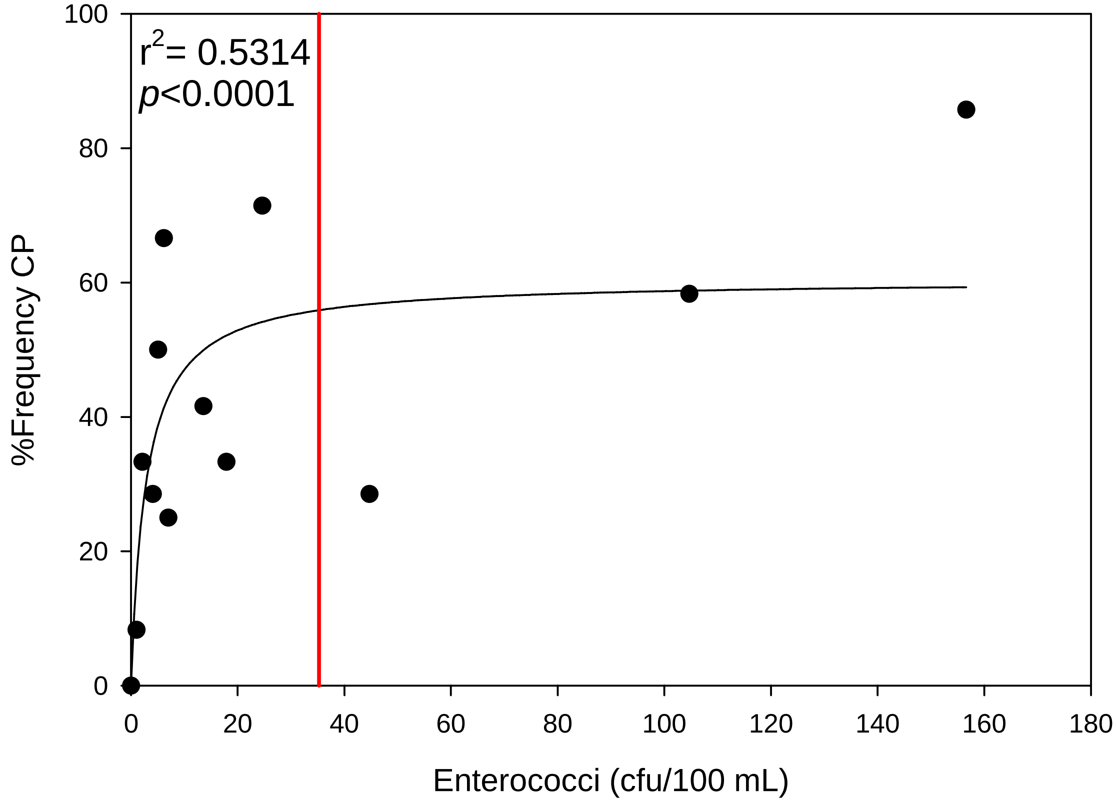

FIGURE 3.19. Exponential regression for the frequency for CP and enterococci counts for the sampling stations throughout the sampling period. Red line illustrates enterococci standard (35 cfu/100 mL) for SB classified waters, according to existing EQB water quality regulations.

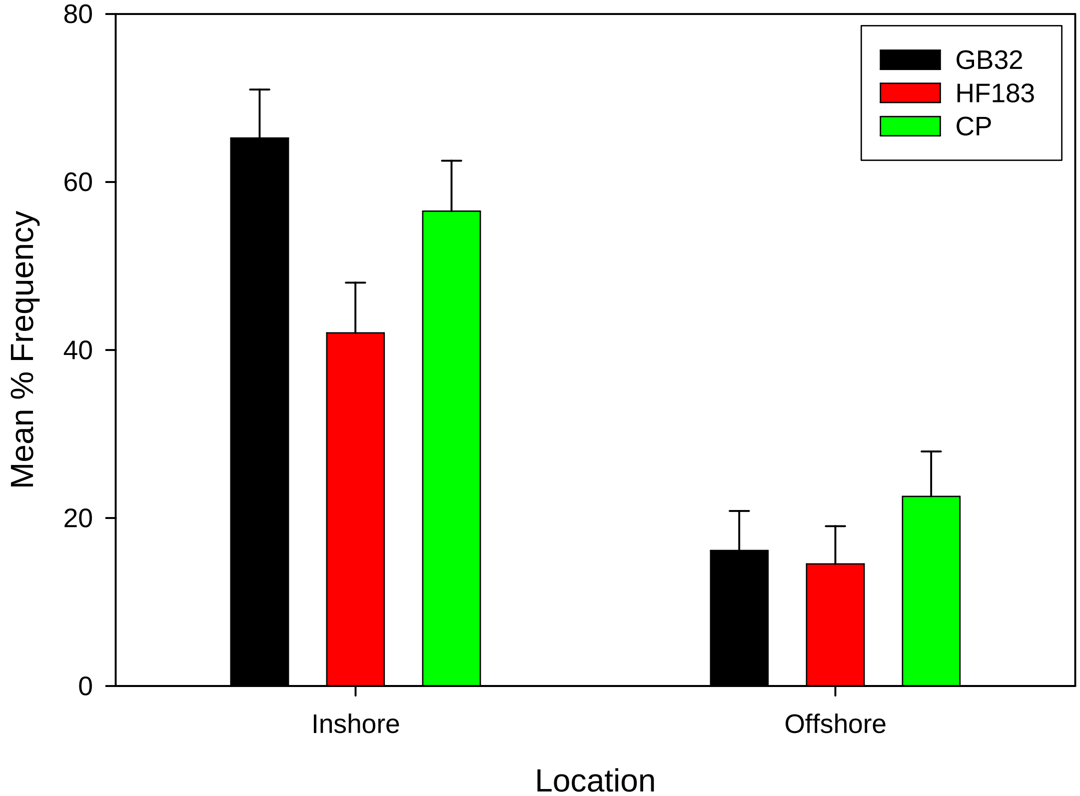

FIGURE 3.20. Mean % frequency of positive signals for molecular markers GB32, HF183, and CP between inshore and offshore locations.

***Variation between management regimes*.**

GB32 positive hits averaged 23.6±5.8% within MPA sites, and 55.3±5.7% at non-MPA control sites (Figure 3.20), a difference that felt just short of significance (Table 3.4). HF183 positive hits averaged 20.0±5.4% within MPAs, and 35.5±5.5% at non-MPA control sites. CP positive hits averaged 30.9±6.3% within MPAs, and 47.4±5.8% at control non-MPA sites.

TABLE 3.4. Summary results of Kruskal-Wallis one-way analysis of variance of primers presense/absence data between management regimes.

Parameter	**D.F. (within, between)**	**K-W statistic**	***P* value**
GB32	1,129	3.60	0.0577
HF183	1,129	0.36	0.5473
CP	1,129	0.28	0.5997

***Positive signals in waters meeting current microbiological standards*.**

Table 3.1 shows that 16% of the FC counts violated current Environmental Quality Board (EQB, 2003) SB water classification (200 cfu/100 mL). However, with the exception of the month of August 2005, there was at least one sampling site during 11 of the 12 sampled months (92%) that violated FC count standard (8-38% of the sites in violation per month). Enterococci standard (35 cfu/100mL) was violated in 17% of the samples, but at least one site violated the standard 10 out of the 12 months (83%), with 13-23% of the sites in violation per month. GB32 showed positive hits 71% of the time there were FC count violations and 82% when there were enterococci count violations (Figure 3.22). HF183 showed positive hits 48% of the time there were FC count violations and 36% when there were enterococci count violations. CP showed positive hits 67% of the time there were FC count violations and 64% when there were enterococci count violations. Overall, positive marker signals (pooled data) were obtained during 95% of the FC

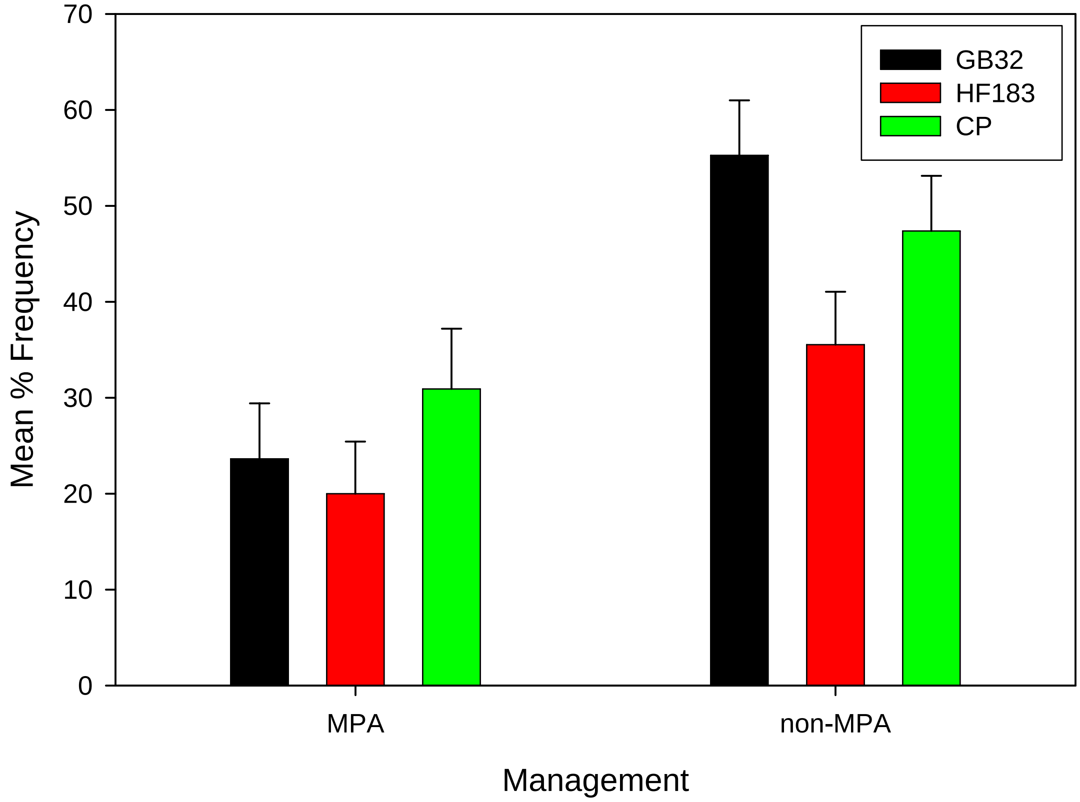

FIGURE 3.21. Mean % frequency of positive signals for molecular markers GB32, HF183, and CP between management regimes.

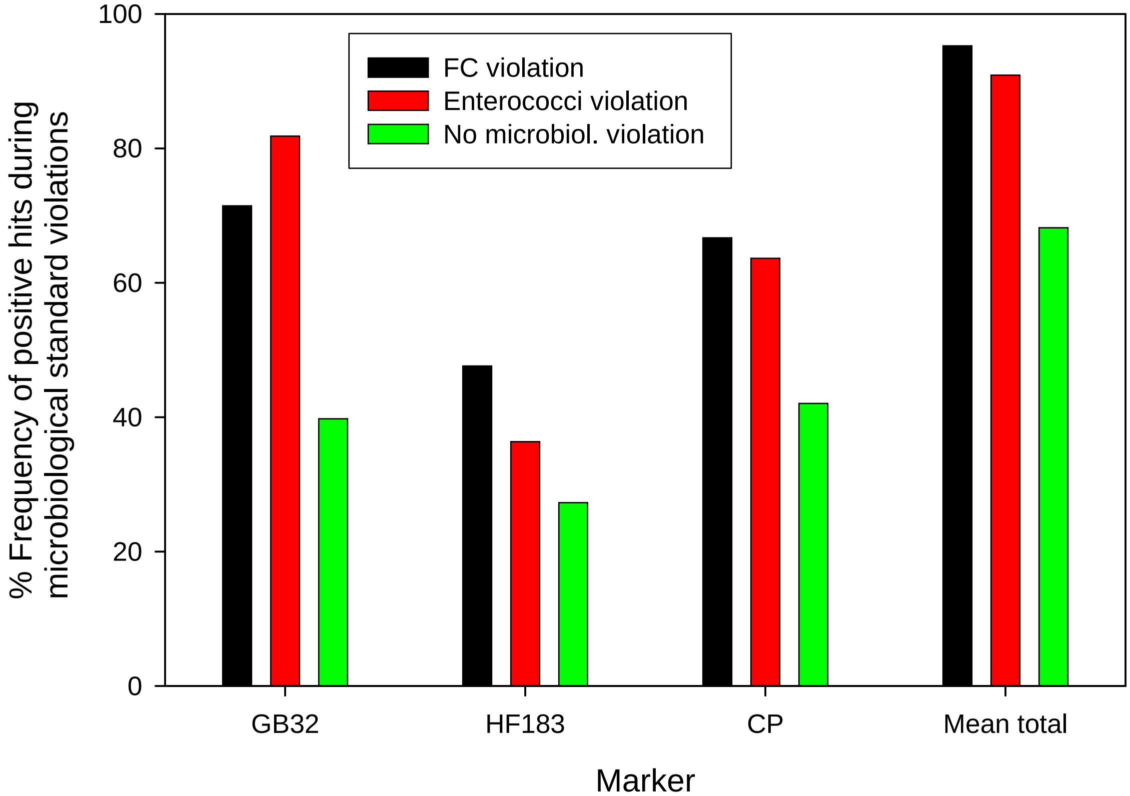

FIGURE 3.22. Frequency of positive signals for molecular markers GB32, HF183, and CP during microbiological standard violations.

violations and during 91% of the enterococci violations. These results are very significant since they suggest that most of the time molecular markers gave positive responses immediately following non-point source sewage pollution pulse events. Alternatively, it may also suggest that markers gave positive responses immediately following sediment resuspension associated to variations in oceanographic dynamics. Sediments are known to be potential reservoirs of microbes. Therefore, if anoxic mud and sand bottoms are frequently impacted by non-point source sewage discharges, they can probably retain potentially large concentrations of pathogenic microorganisms. Oceanographic variables such as tidal cycles, wind-driven surface currents, wave action, and anthropogenic activities such as recreational navigation, dredging and trampling over shallow soft bottoms may resuspend pathogens, as well as indicator microorganisms, from polluted sediments.

However, what is really alarming is that GB32 was documented 39% of the time under no microbiological standard violation (Figure 3.22). Similarly, HF183 was registered 27% of the time, and CP 42% of the time there were no violations. Overall (pooled data), markers were registered 68% of the time there were no violations. This clearly suggests that even coastal waters meeting current microbiological quality standards showed frequent positive signals of fecal pollution using MST methods. Further, we showed that MST methods can be extremely sensitive and useful detecting low-level, remote, non-point source sewage pollution in tropical marine waters. This could be of paramount value to detect impacts of remote sewage pollution on coral reef and seagrass communities.

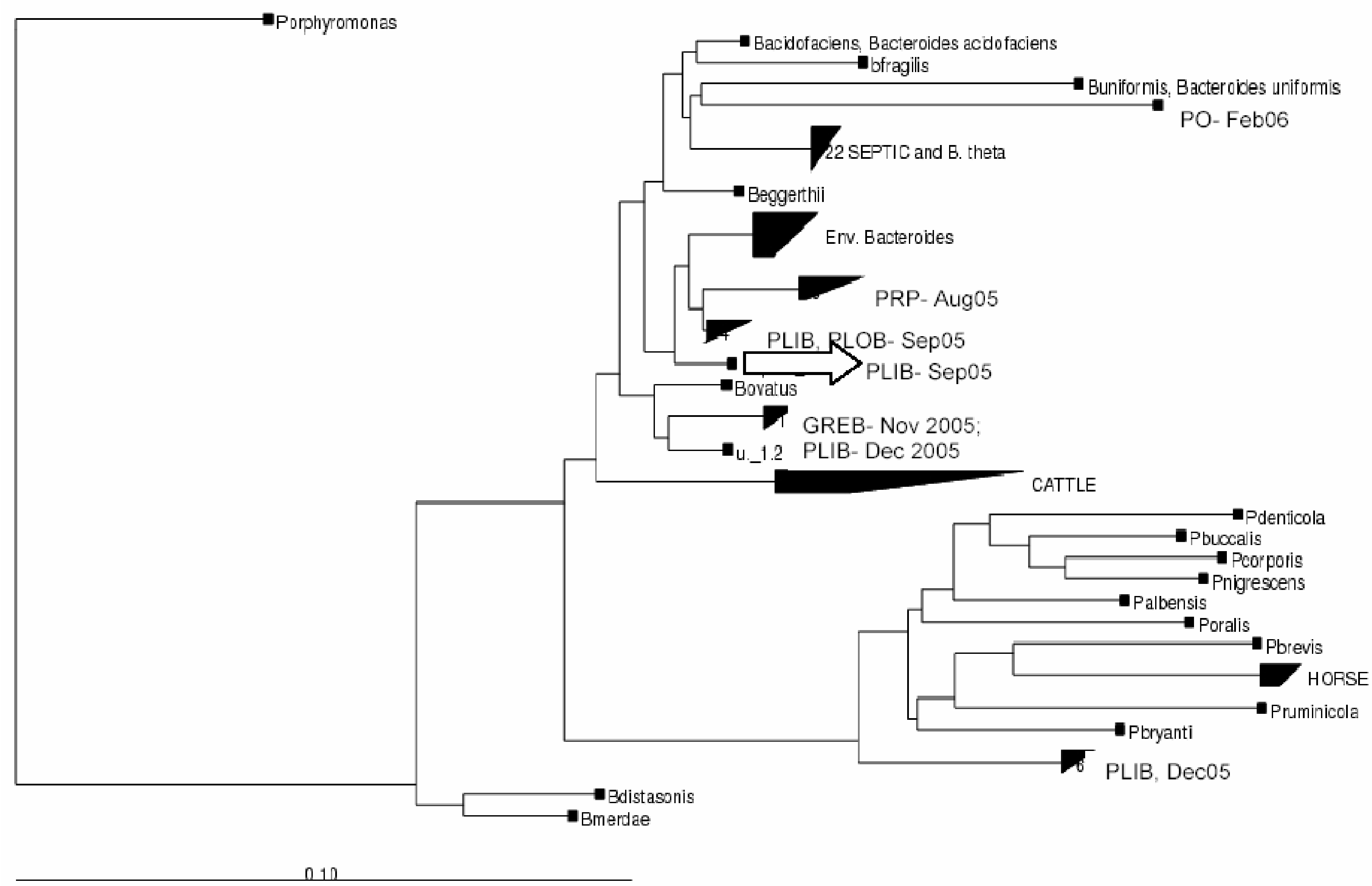

FIGURE 3.23 . Phylogenetic tree obtained with PCR products from HF183 assays. The tree starts with *Porphyromonas* as an outlier ancestor group. The horizontal line below represents the degree of divergence for the sequences. PO= Punta Ostiones; PRP= Puerto Real-pier; PLIB= Punta Lamela-inner bay; PLOB=Punta Lamela-outer bay; GREB= Guanajibo River estuary (bridge).

Phylogenetic analysis of microbial rDNA sequences.

Phylogenetic tree analysis of amplified rDNA sequences is one of the most powerful genetic tools to identify specific microorganism strains, and can be used to infer sources of specific fecal-borne microorganisms. Figure 3.23 shows a phylogenetic tree obtained with selected rDNA sequences amplified with the human fecal host-specific *Bacteroides* molecular marker HF183. This tree shows that PCR products obtained from Punta Ostiones in February 2005 are similar to those from 16S rDNA from *Bacteroides uniformis*, a species found in the human gut. Sequences analyzed from samples collected in August 2005 from Puerto Real-pier (within Belvedere Natural Reserve), Punta Lamela-inner bay and from Punta Lamela-outer bay, both within the Puerto Real Bay area, show that there was a strong similarity among them. They were also similar to some *Bacteroides* strains found in a previous study in sediments, further suggesting the possible role of anoxic mud as a reservoir of fecal-borne microorganisms. Sequences found in November 2005 from Guanajibo River estuary (bridge) were fairly similar to those from *B. ovatus* 16S rDNA, which is found in the gut of cattle. Cattle grazing is abundant along a significant portion of the Guanajibo River banks and alluvial plains, even in its estuarine zone (Hernández-Delgado, pers. obs.). Further, cattle drink water from the Guanajibo River, and hundreds of animals cross the river several times in daily basis to access grazing grounds. Lastly, sequences obtained from Punta Lamela-inner bay in December 2005 appear to be similar to *Porphyromonas bryantii*, which is also found in the ruminant gut. It is interesting to note that these sequences corresponded to the sites and sampling periods that had the highest turbidity values.

Discussion

This study showed that non-point source fecal pollution detected using MST methods was present across a very large portion of the southwestern Puerto Rico shelf, affecting extensive coral reef and seagrass communities. However, there was a definite gradient of non-point source sewage pollution with increasing distance from the shoreline that was largely influenced by a combination of non-point source sewage pulses and by local oceanographic dynamics (discussed in Appendix A-1). Oceanographic variables included reversals in wind direction, shifting wind velocity, wind-driven ocean surface currents, tidal flushing of inshore polluted waters, influence of occasional northwestern swells bringing clean off-shelf water, and the combination of rainfall, stream flow and shifting behavior of the Guanajibo River plume. Oceanograohic influences have been previously documented to impact microbiological water quality. Toranzos and Hernández-Delgado (1992) documented higher microbial indicator counts in Puerto Rico shores following sediment resuspension during increasing wind-driven wave action. More recently, Ufnar et al. (in press) informed higher microbial counts in the Gulf of Mexico following wind shifts and increasing wave action.

Positive signals of the general *Bacteroides* (GB32), HF183 and CP molecular markers varied throughout the sampling sites over the study time. Inshore and estuarine sites showed significantly higher frequencies for each of the markers. The particular presence of anaerobic *Bacteroides* molecular markers in the tropical marine environment suggest that these might be reflecting recent non-point source sewage pulses probably coming from variable sources such as human settlements, cattle grazing areas, farms, and malfunctioning septic tanks, sewers and treatment facilities, among others. *Bacteroides* species show low survival rates once they encounter aerobic conditions. These markers were successfully used in a previous study in temperate coastal and estuarine habitats to elucidate temporal and spatial dynamics of fecal pollution from different sources (Shanks et al., 2006).

Sites that showed the highest frequency of positive signals for the primers GB32 and CP were Guanajibo River estuary (bridge) and Guanajibo River mouth. These sites receive a variety of direct anthropogenic impacts in comparison to the other sampled sites. Results obtained from these primers suggest that there were also definite recent events of non-point source fecal contamination from human and animal sources. These results were consistent with those from Shanks et al. (2006), which found a frequency of positive signals suggesting human and ruminant fecal pollution under temperate estuarine conditions. It has to be taken into account that primers used in this study can amplify rDNA from *Bacteroides* and *Clostridia*, which are found as normal gut flora for various animals (Matskuki et al., 2002). Based on these results, there is a need to identify specific fecal pollution sources in this area using source-specific methods in order to improve sensitivity and precision of identification of pollution sources. Moreover, application of GIS-based methods, in combination with MST analysis, may allow us in the future identify, track and map non-point pollution sources. This will be a paramount tool to establish concrete management measures to reduce impacts of non-point source pollution.

There was a lack of signals detected for Arrecife El Ron and Arrecife Fanduco for the GB32 and HF183 assays. This is consistent with the fact that there were low counts for FC (<270 cfu/100 mL), as well as enterococci (<22 cfu/100 mL). In contrast, signals were detected with the CP assay, but to a lesser context compared to the rest of the sites. Evidence from previous assays (reviewed by Hazen and Toranzos, 1990) support the fact that *Clostridia* members can survive in soil, and can be present as a result of resuspension in the water column due to rainfall. This can also indicate that there were indeed some fecal contamination episodes, since this group can be found in feces. We suggest that distance of Arrecife El Ron from known local pollution sources and local oceanographic dynamics at each site influenced these results. Arrecife El Ron receives more clean waters from outer shelf areas during each tidal cycle. However, it can be occasionally impacted by the Guanajibo River plume following heavy rainfall, stream flow and north-south movement of the river plume. Arrecife Fanduco, in spite of being located closer to the coastline, receives also strong flushing from outer shelf waters during each tidal cycle. However, it also receives occasional polluted waters from flushing tides coming from Puerto Real Bay. Thus both sites may show high variability of results.

Puerto Real-pier and Punta Lamela-inner bay had the highest percentage of positive signals throughout the study period for the primer HF183, a molecular marker that amplifies a 16S rDNA gene in *Bacteroides* specifically located in the human gastrointestinal tract. These sites receive discharges from septic tanks located close to sea level in the Puerto Real community, as well as illegal sewage discharges from private houses and commercial establishments (i.e. restaurants). These results are also consistent with Shanks et al. (2006) which found higher frequencies of human fecal pollution impacts in areas known to receive human point- and non-point fecal pollution. The high frequency of signals for this human-specific primer within Puerto Real Bay suggests a major threat to human health and to the ecological integrity of the bay due to consistent non-point source sewage impacts mainly of human origin. Further, this suggests that

Finca Belvedere Natural Reserve is being directly impacted by constant non-point source human fecal pollution. Further, constant boating activities along the bay over mud-dominated bottoms suggest constant sediment resuspension. This may result in constant resuspension of turbid waters, nutrients and microbial pathogens, further contributing to a chronic state of low water transparency, hypertrophic conditions, and a high risk of human exposure to microbial pathogens. These conditions may cause long-term negative impacts on local benthic communities that remain to be documented, but which most likely have resulted in a net long-term decline in their ecological conditions and functions.

Many inconsistencies were, however, encountered for these markers. No GB32 and HF183 markers were detected at some offshore sites such as Arrecife El Ron and Arrecife Fanduco, even under high turbidity pulses, although CP marker was occasionally detected. These results indicate that these sites are not impacted by chronic heavy fecal pollution episodes, or that the fecal pollution might dilute due to currents before it arrives to those sites. However, sewage-polluted Guanajibo River plume pulses are known to occasionally impact these sites following heavy rainfall and stream flow, and dramatically reducing water transparency (Hernández-Delgado, pers. obs.). Therefore, we suggest that river plume pulses also represent temporal non-point fecal pollution inputs to these systems. The inconsistency as for the turbidity could be a result of complex oceanographic dynamics, further suggesting that the combined effect of non-point source sewage pollution and variations in oceanographic dynamics need to be addressed and mathematically modeled to understand these interactions.

There were significant inconsistencies between microbial indicator counts and *Bacteroidales* fecal markers. For instance, in spice of the fact that yearly mean microbial indicator counts met current standards in 10 out of 13 sampled sites (77%), GB32 and HF183 markers were detected in 11 of them (85%).CP marker was registered in 12 of them (92%). Salinity has been suggested to negatively affect the survival rates of fecal coliforms in coastal waters (Anderson et al., 1979) and sediments (Anderson et al., 2005). It is possible that salinity might have resulted in some kind of sublethal stress in microbes making them turn into a viable but maybe not culturable state. Thus, standard plate count methods might have actually underestimated their real concentrations. Shanks et al. (2006) also found very large spatial inconsistencies in temperate environments, including presence of molecular markers in waters with low *Escherichia coli* counts, suggesting that temperatures, as well as environmental persistence and growth might have influenced these results.

FC and enterococci concentrations showed large fluctuations that were largely independent of rainfall, turbidity or known distance from pollution. Several sites such as Puerto Real-fishing village, Punta Lamela-inner bay, Punta Lamela-outer bay, Punta Ostiones, Punta Arenas, and Punta Guanajibo showed low FC and enterococci counts, but had high frequencies of signals for GB32. Low indicator counts could have resulted from a salinity osmotic shock, or as a result of the dilution of fecal pollution due to variable patterns of wind-driven ocean circulation and tidal flushing. Microbes could have accessed coastal waters from pollution sources or from soils through runoff (Desmarais et al, 2002). However, regardless of the low FC and enterococci counts in these sampling sites, the high frequency of positive marker results, suggests unequivocal contributions from fecal sources primarily by human impact, even in waters meeting microbiological water quality standards. However, we must remind that PCR-based assays could in theory detect dead or non-culturable cells (since it amplifies DNA found in the environmental sample), in contrast to indicator bacteria assays that depend on living, viable and culturable cells. In this context, more information is needed in order to use *Bacteroides* source-specific markers to estimate human health risks of fecal contamination in tropical waters.

Field, et al. (2003), suggested the development of host-specific primers based on geographical microbial analysis of fecal microbiota and successfully tested human-specific primers, such as HF134 and HF183 in fecal samples from subjects living in temperate environments. However, HF134 did not amplify rDNA from environmental samples during our study. These results are consistent with a previous study conducted in southeastern Puerto Rico where HF183 produced positive results, but HF134 failed to amplify marine samples (M. Bonkosky, unpub. data). HF183 was useful indicating human fecal pollution of coastal habitats. However, there is a possibility that geographic factors might have masked its effectiveness and reliability as an indicator in the tropical marine environment, thus leading to a significant underestimation of the real fecal pollution level. Therefore, there is a need to develop host-specific molecular markers for tropical geographical areas. This should lead to improved precision and sensitivity in the detection of remote fecal pollution in the tropics.

Markers were also tested to account for the effectiveness of management practices within marine protected areas (MPA) along the southwestern Puerto Rico shelf. Based on the results for the host-specific molecular marker, management practices at sampling sites located within the boundaries of Finca Belvedere Natural Reserve (Puerto Real Bay), Cayo Ratones and Adjacent

Waters Natural Reserve (Joyuda Bay), and at Tourmaline Natural Reserve, off Cabo Rojo and Mayagüez, do not prevent fecal pollution from non-point sources. It is interesting to point out that only one of the sequences obtained was similar to human-specific *Bacteroides.* The fact that the rest or the sequences analyzed were similar to ruminant-specific *Bacteroides* can indicate that there could be potentially a very high ruminant fecal contamination problem, with a very high dispersal rate through the Guanajibo River plume, and that human-specific molecular markers need to be developed for tropical environments.

Conclusions.

Non-point source fecal pollution was registered along vast areas of the southwestern Puerto Rico platform. This is an alarming threat for public health, since HF183 MST marker revealed fecal pollution coming from a human source. In this context, it is important to determine non-point sources of fecal pollution in order to prevent diseases, especially within MPAs used for fishing and recreational purposes. Moreover, we found unequivocal alarming evidence of non-point source human fecal pollution within MPA systems that support significant coral reef, seagrass and mangrove communities. These areas function as nursery, shelter, feeding and/or spawning grounds for a wide variety of fish and macroinvertebrate species of commercial significance. Chronic sewage pollution and eutrophication, in addition to other anthropogenic causes of stress (i.e., turbidity, sedimentation, overfishing), could have significantly contributed to the overall environmental degradation and ecological decline of these communities.

PRDNER and NOAA should provide much more attention and efforts into studying non-point source fecal pollution impacts on coral reef habitats by implementing MST-based methods, in combination with GIS-based approaches. This will allow identify and map the distribution of direct fecal pollution impacts in coral reefs and seagrasses, as well as in representative edible fish and macroinvertebrate species. Further, it may allow classify those impacts as human, ruminants, etc., thus facilitating the identification of management measures. High sensitivity of MST-based methods could provide unequivocal evidence of sewage exposure of individual coral colonies, seagrass shoots, fish, invertebrates, etc., thus providing a quantitative basis of pollution at highly variable spatial scales. This would provide us a state-of-the-art opportunity to quantify spatial and temporal patterns of sewage impacts, as well as testing different hypothesis-driven questions. Further, it could provide us a mechanism to document the interaction of sewage impacts with variables such as coral disease prevalence, mortality, growth rates, tissue regeneration ability, etc.

Even though there were some inconsistencies throughout the sampling stations, MST methodologies proved to be of potential use to account for sources of fecal contamination along with the existing conventional plate count methods. However, it is still necessary to consider the development of molecular markers that could be specific to tropical waters, as the fecal microbiota could change in various geographical areas due to wildlife (migratory or native), as well as variable diet habits of the human and animal populations encountered in that area.

MST techniques have been used by several agencies, including the U.S. Environmental Protection Agency (USEPA) to discriminate between host-specific sources to determine fecal contamination. Recently, *Microbial Source Tracking Guide Document* was published by the USEPA (2005). The guide's purpose was to provide scientists, engineers, and environmental managers with a comprehensive, interpretive analysis of the current and relevant information related to MST. This document included several watershed case studies across the United States showing various methodologies to describe sources of fecal pollution, and how can the results be interpreted when performing watershed management practices as well as prevention of potential contamination episodes as to prevent waterborne diseases. It would be useful to explore the use of MST methodologies to test several hypotheses regarding the spatial and temporal variation patterns of non-point source fecal pollution from different sources in the tropics, and its relationship to different oceanographic variables.

Considering the human health risk that fecal pollution represents, we strongly recommend the PR Environmental Quality Board (EQB) to apply this kind of methods to identify the potential sources of fecal contamination, as to identify the areas that are not appropriate for recreational uses. It would also be useful to prioritize areas to cleanup and to monitor in a frequent basis. Further, MST methods could be useful to review and validate current regulations of water microbiological quality for determined areas because they can be more specific and sensitive than standard microbial indicators in terms of the sources of fecal contamination and bring results more rapidly than the conventional methods of culture media. MST approaches can also provide more precise and sensitive results in the tropics than standard microbial count methods. However, as mentioned earlier, there is a need to develop specific methodologies for each geographical area.

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Appendix A-4

Actual status of shallow-water coral reef assemblages across a non-point source sewage stress gradient along the southwestern Puerto Rico shelf.

Edwin A. Hernández-Delgado

A-4. Actual status of shallow-water coral reef assemblages across a non-point source sewage stress gradient along the southwestern Puerto Rico shelf.

Background.

Marine sewage pollution is a major cause of concern in tropical countries, particularly near coral reefs and reef-associated communities. Major negative effects have been associated to eutrophication and turbidity effects (Pastorok and Bilyard, 1985; Cloern, 2001). These can typically result in a combination of system-specific responses, as well as cascading direct and indirect effects that could result in major long-term phase shifts in coastal community structure These can trigger phase shifts in community composition from coral and algal turf dominance towards fleshy macroalgal and non reef-building taxa dominance (Figures 4.1 and 4.2). It has been suggested that such phase shifts could be irreversible in long-term temporal scales (Knowlton, 1992; Hughes, 1994). Sewage-associated eutrophication effects could also be often confounded with other factors such as sedimentation and overfishing (Szmant, 2002), and could result in accelerated reef decline trends due to a combination of synergistic effects (Meesters et al., 1998)

Sewage has been previously implicated in coral reef degradation in Puerto Rico (Goenaga and Boulon, 1992; Hernández-Delgado, 2000, 2005; Morelock et al., 2002; Weil et al., 2002; García et al. 2003;). Corals are the principal reef builders and coral survival rates (McKenna et al., 2001), as well as reef-building activity (i.e., skeletal extension rates), are highly susceptible to sewage impacts, although effects seem to be species-specific (Tomascik and Sander, 1985;

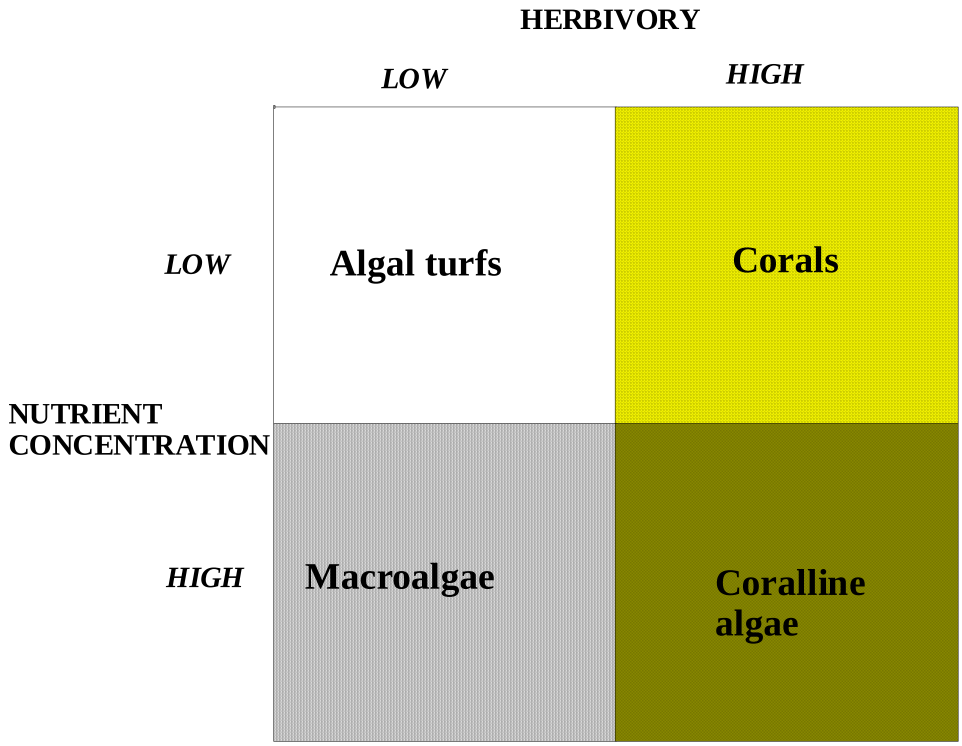

FIGURE 4.1. Relative benthic community dominance model proposed by Littler and Littler (1984). This model is based on the four most significant photosynthetic functional groups in coral reefs (coral, algal turf, coralline algae and macroalgae). In this model nutrients (bottom-up cascade effects) and herbivory-associated disturbance (top-down cascade effects) interact to determine benthic community structure. Corals and algal turfs can dominate under low nutrient conditions depending whether herbivory levels are high or low, respectively. A dominance phase shift can occur towards fleshy macroalgae with increasing nutrient concentrations and declining herbivory. Based on this model, it is suggested that chronic sewage pollution can drive coral reef benthic communities towards macroalgal and non reef-building taxa dominance.

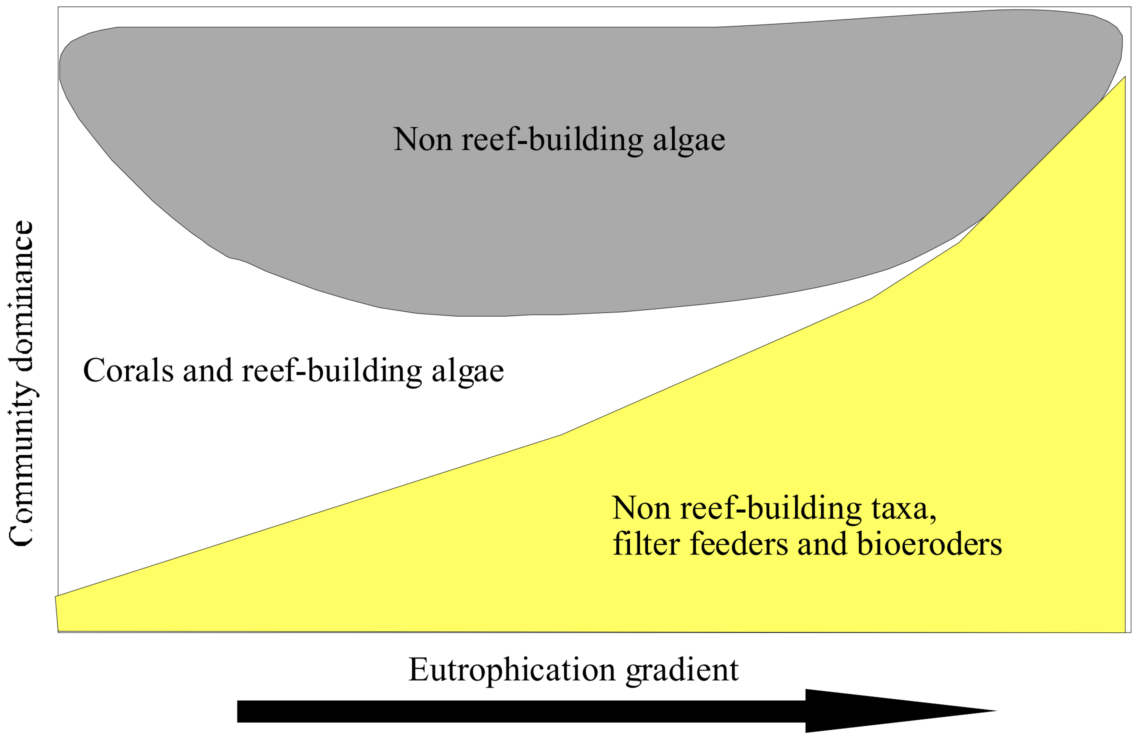

FIGURE 4.2. Eutrophication model proposed by Birkeland (1997). This model predicts that under low nutrient pulses and oligotrophic conditions, corals and reef-building algae co-exist with a limited abundance of non reef-building algae and filter feeders. Dominance of the latter two groups can increase along an eutrophication gradient. Particularly, filter feeders are highly dominant under the most eutrophic conditions. Based on this model, it is suggested that chronic sewage pollution can drive coral reef benthic communities towards macroalgal and non reef-building taxa dominance.

Spencer-Davies, 1990). Further, Kaczmarsky et al. (2005) documented a relationship between the prevalence of Black Band Disease and White Plague-Type II in coral colonies exposed to sewage in St. Croix, USVI. Similar conditions have been documented off San Juan and Carolina, northern PR, in coral reefs located close to non-point sewage pollution sources (Hernández-Delgado, unpub. data). Sewage can also have profound negative effects on the community structure of seagrasses (Bryars and Neverauskas, 2004). They can also result in frequent pulses of increasing biological oxygen demand and declining dissolved oxygen concentration events in coastal waters (Desa et al., 2005). In the long-term, the combination of non-point sewage-, nutrient-, and sediment-polluted runoff pulses can be harmful to coral reef communities triggering permanent and possibly irreversible phase shifts in community structure towards non reef-building taxa dominance.

Sewage impacts can further produce a major decline in the socio-economic value of coral reefs and associated communities due to the loss of ecological services (i.e., coastal protection, sinkhole of greenhouse gases, food-protein production, source of bio-active compounds), reef aesthetics (i.e., SCUBA, snorkeling, educational excursions), and the permanent loss of fisher communities livelihoods. Declining reefs may also represent a permanent phase shift in community livelihoods and a loss of cultural heritage. In spite of that, there is very limited information regarding the relationship of non-point source sewage pollution and the status of coral reef benthic communities. Such documentation is particularly absent in Puerto Rico.

This study was aimed at documenting what is the actual status of shallow-water coral reef assemblages along a non-point source sewage stress gradient in the southwestern Puerto Rico shelf. Only Elkhorn coral, *Acropora palmata*, historically-dominated shallow-water (<5 m depths) coral reef assemblages were sampled in this study. This species constitutes one of the most significant reef-builders in the Atlantic (Bruckner, 2002), but their populations have declined by an estimated 97% during recent decades through the region, including Puerto Rico, due to a combination of natural and anthropogenic factors (Hernández-Delgado, 2000; Weil et al., 2002). Elkhorn coral was designated in May 2006 as a threatened species under Endangered Species Act 4d rule. It constitutes a mono-specific coral functional group responsible for building the reef apron classic *palmata* zone that absorbs wave action energy and functions as a nursery ground for a myriad of reef fish species (Bellwood et al., 2004). Because *A. palmata* constitutes a mono-specific coral functional group, as a group it has an extremely low functional redundancy (*sensu* Bellwood et al, 2003). Therefore, any stressing factors (i.e., non-point source sewage) that can potentially result in a net decline in this species could have profound long-term irreversible consequences in the structure of shallow coral reef benthic communities and in the distribution of many commercially-important reef species. It is paramount to document what are the current conditions of shallow *palmata* assemblages through the region, including Puerto Rico. Moreover, there is also a need to assess non-point source sewage impacts in the distribution of this species.

Finally, this project is aimed at exploring what relationships exist between the status and distribution of corals, and the structure of reef communities, with mean microbial indicator concentrations, and mean values of water quality parameters. This approach will allow us to test the null hypothesis of no relationship between environmental gradients and coral reef community structure.

Methodology.

Coral reef study sites.

Studies were conducted at 8 coral reefs located along a non-point source sewage pollution gradient along the southwestern Puerto Rico shelf (FIGURE 4.3). Sampling was limited to shallow reef assemblages (<5 m). The first null hypothesis we tested was that there was no significant *site* effect of the non-point source sewage gradient in coral reef benthic community structure. Lack of *site* effects would imply that probably other large scale factors beyond water quality gradient impacts might have impacted coral reefs along the shelf. The second null hypothesis we tested was that there was no significant *geographic* effect of the non-point source sewage gradient in coral reef benthic community structure. Lack of *geographic* effects would imply that sewage impacts may not discriminate between sites regardless of their geographic location. Therefore, sites were arranged into two different *geographic* regions (*inshore*, *offshore*). *Inshore* reefs (<0.5 km) included Arrecife Fanduco, Punta Ostiones, Cayo Ratones and Punta Arenas. *Offshore* sites (>0.5 km) included Arrecife El Ron, Cayo del Medio, Corona del Norte and El Negro.

The third null hypothesis we challenged was that there was no significant *management* effect of the non-point source sewage gradient in coral reef benthic community structure. Lack of *management* effects would mean that sewage impacts may not discriminate between marine protected areas (MPAs) and non-MPA control sites. Study sites were arranged within two *management* categories (*MPA*, *non-MPA controls*). *MPA* sites included Arrecife El Ron, Cayo del Medio, Corona del Norte, Cayo Ratones and Arrecife Fanduco. The first three lie within the

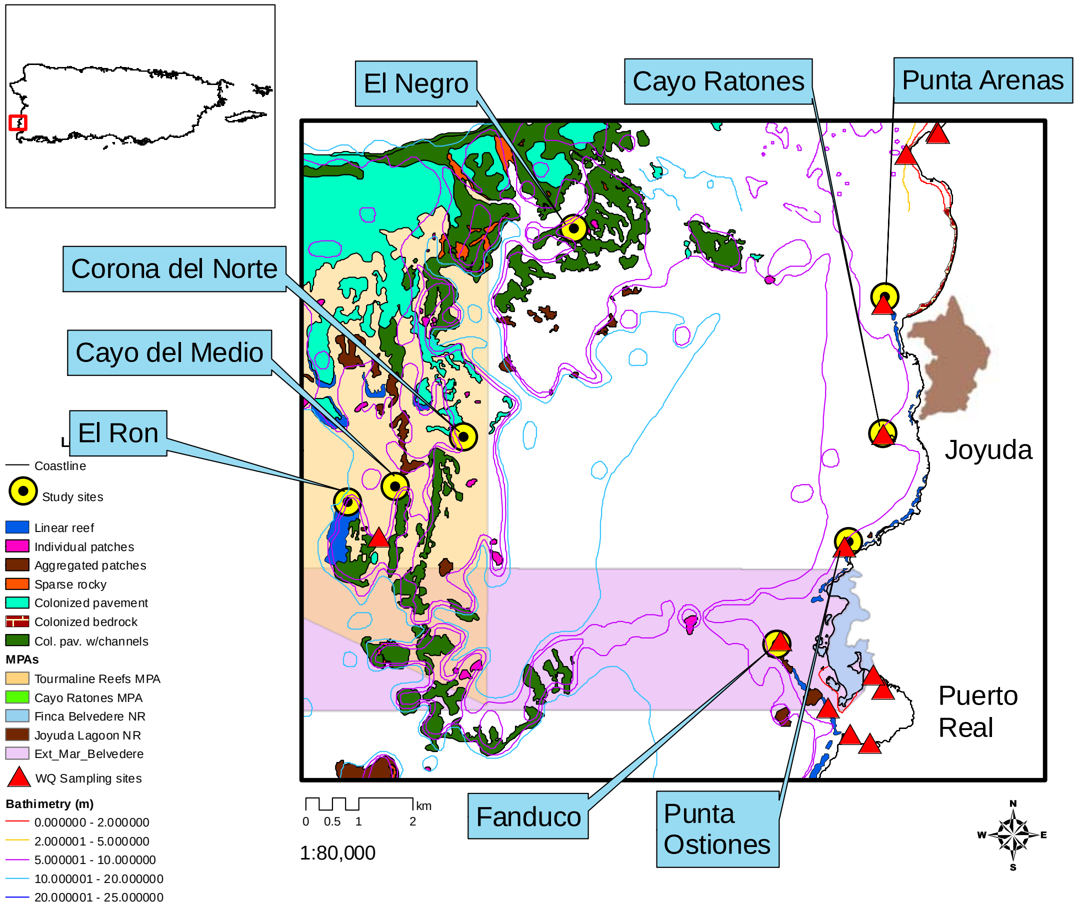

FIGURE 4.3. Coral reef study sites. A total of 8 sites were selected along a non-point source sewage stress gradient across a portion of the western Puerto Rico shelf. Sites were arranged into two different *geographic* regions (*inshore*, *offshore*). *Inshore* reefs included Arrecife Fanduco, Punta Ostiones, Cayo Ratones and Punta Arenas. *Offshore* sites included Arrecife El Ron, Cayo del Medio, Corona del Norte and El Negro. These sites were further arranged within two *management* treatments (*MPA*, *non-MPA controls*). *MPA* sites included Arrecife El Ron, Cayo del Medio, Corona del Norte, Cayo Ratones and Arrecife Fanduco. *Non-MPA control* sites included El Negro, Punta Ostiones, and Punta Arenas.

Tourmaline Natural Reserve. Cayo Ratones is located within the Cayo Ratones and Adjacent Waters Natural Reserve, while Arrecife Fanduco is located within the Finca Belvedere Natural Reserve marine extension. There are managed by the P.R. Department of Natural and Environmental Resources (DNER). *Non-MPA* control sites included El Negro, Punta Ostiones, and Punta Arenas.

Coral reef characterization studies.

A combination of linear transects and haphazard photo-quadrats were used during data collection. Briefly, four to six replicate 10 m-long transects were haphazardously sampled at each site. Five replicate non-overlapping 1 m^2 quadrats were haphazardously photographed at 1, 3, 5, 7 and 9 m along each transect using a high-resolution UW digital camera. Images were corrected for brightness and contrast, and analyzed using CPCe 3.4 software (Coral Point Count with Excel extensions) developed by the National Coral Reef Institute at Nova Southern University, FL. Twenty replicate randomly-generated counting points were projected over each image and used to quantify the proportion of benthic categories (FIGURE 4.4). Data included % cover by major benthic components such as coral (scleractinian, hydrocoral and octocoral), fleshy macroalgae, algal turf, crustose coralline algae (CCA), *Halimeda* spp., other erect coralline algae (ECA), sponge, zoanthid, pavement, rubble and sand. Hard coral (scleractinian + hydrocoral) data was also used to calculate coral species diversity index (H'n) (Shannon and Weaver, 1948) and evenness (J'n) (Pielou, 1966).

Univariate statistics.

Community parameter data were analyzed by means of one-way analysis of variance (ANOVA) to test *site* effects. *Site* was the main variable, with mean data from replicate transects as error term. A one-way ANOVA was also used to test *geographic* effects. *Geographic location* (inshore, offshore) was the main variable, with mean data from replicate transects as error term. A one-way ANOVA was also used to test *management* effects. *Management level* (MPA, non-MPA control) was the main variable, with mean data from replicate transects as error term. Significantly different sites, geographic locations and management regimes were identified using a Tukey test for comparisons of means. Pearson correlation analysis was performed to test relationships between benthic community parameters and water microbiological and physical quality parameters that could indicate possible sewage impacts. Data on coral species richness and colony abundance was √-transformed, while data on the proportion of % benthic components cover was *arcsin*-√-transformed to reduce variance (Zar, 1984).

Multivariate statistics.

Community matrices were compiled and imported into PRIMER 6.0 ecological statistics software package (Clarke and Warwick, 2001) for multivariate analysis. Mean data from each site, geographic location, and management level were classified with hierarchical clustering using the Bray-Curtis group average linkage method (Bray and Curtis, 1957) and then ordinated using a non-metric multidimensional scaling plot (Clarke and Warwick, 2001). Spatial variation patterns among sites, between geographic locations, and between management levels were tested using PRIMER's multivariate equivalent of a one-way ANOVA called a one-way ANOSIM (analysis of similarities). Interaction effects between geographic location and management level

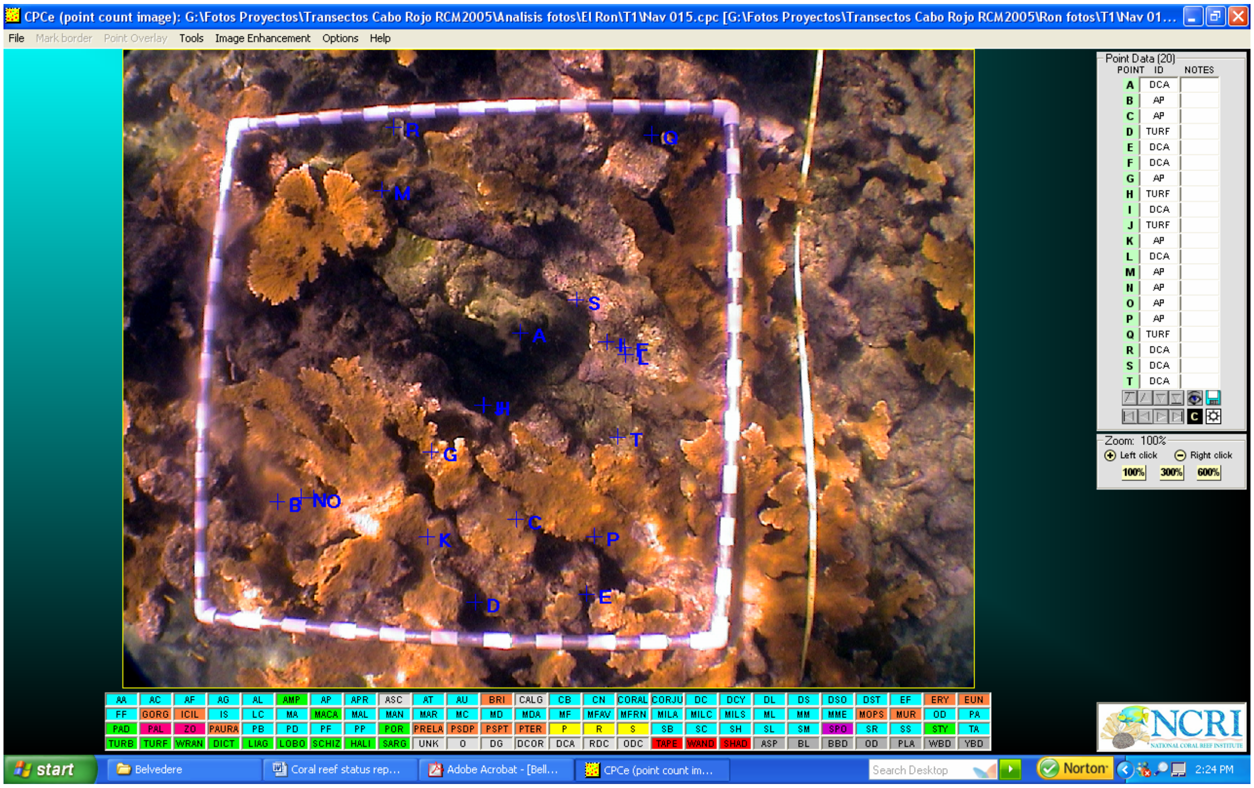

FIGURE 4.4. Computer screen image of a selected example quadrat sampled at Arrecife El Ron. CPCe software can generate a set of random points projected over the digital image within a selected area (1 m^2 quadrat). Benthic categories from the table in the lower part of the screen are selected and placed in a table that is linked to an Excel spreadsheet. Data is used to calculated different benthic parameters (i.e., % coral cover, % algal cover, species diversity index, etc.).

TABLE 4.1. Summary results of one-way ANOVA analysis of coral reef community parameters among sites.

Parameter	**D.F. (within, between)**	**F statistic**	***P* value**
Species richness	7,33	3.87	**0.0035**
Colony abundance	7,33	11.4	**<0.0001**
% Coral	7,33	5.33	**0.0004**
% *Acropora palmata*	7,33	7.59	**<0.0001**
H'n	7,33	0.91	0.5108
J'n	7,33	1.85	0.1098
% Octocoral	7,33	10.9	**<0.0001**
% Sponges	7,33	3.12	**0.0123**
% Zoanthids	7,33	27.6	**<0.0001**
% Macroalgae	7,33	14.2	**<0.0001**
% CCA	7,33	19.6	**<0.0001**
% Cyanobacteria	7,33	14.5	**<0.0001**
% SPR	7,33	5.57	**0.0003**

were tested using a two-way crossed ANOSIM. Key taxa responsible for spatial variation in community structure between groups, sites, geographic locations and management levels were determined using the SIMPER routine.

Results.

Variation of community parameters among sites.

Mean coral species richness per transect was significantly higher (p=0.0035) at Corona del Norte with 6.00±0.45 species (Table 4.1; Figure 4.5). Significantly lower coral species richness occurred at reefs subjected to hurricane disturbance and macroalgal overgrowth due to Guanajibo River influences (i.e., Arrecife El Ron), and at inshore reefs subjected to frequent pulses of non-point source pollution (i.e., Cayo Ratones, Arrecife Fanduco). Colony abundance showed a

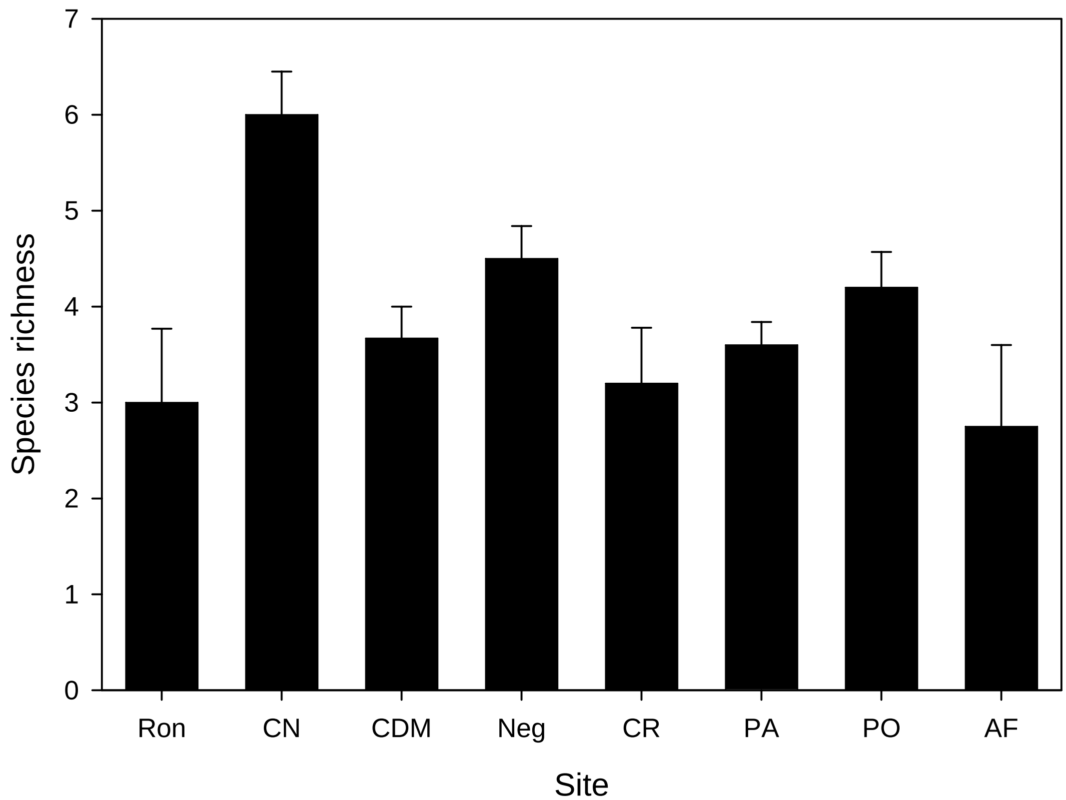

FIGURE 4.5. Hard coral species richness.

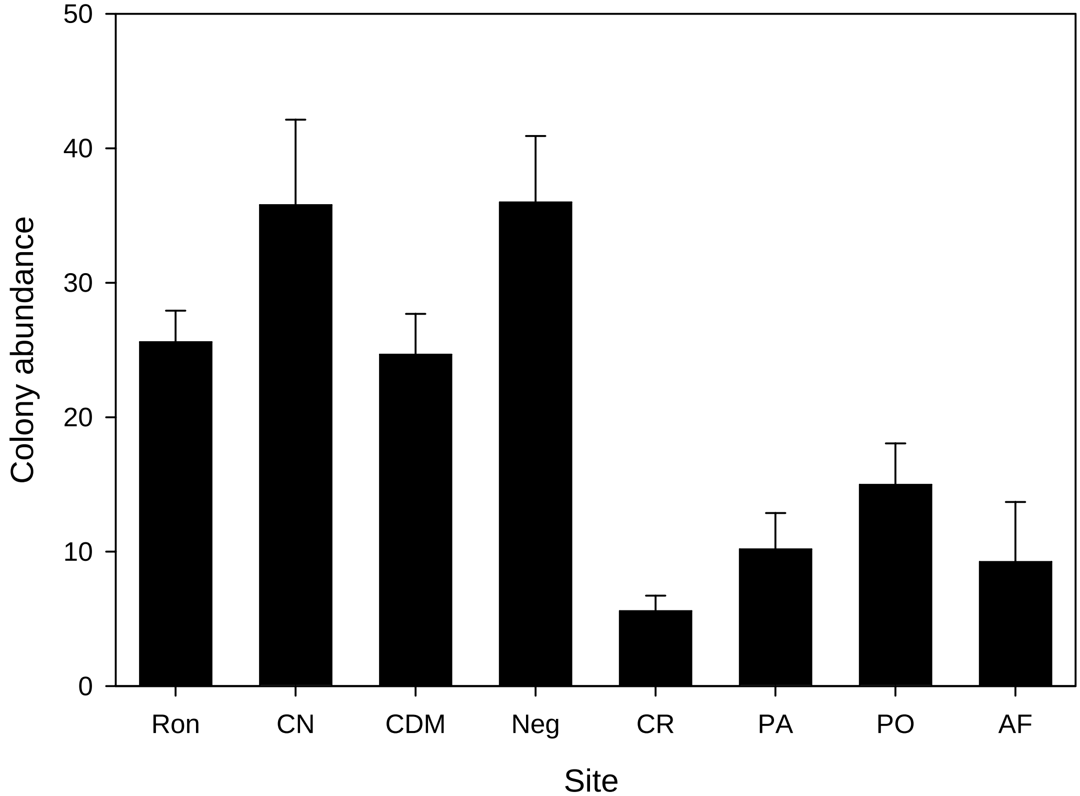

FIGURE 4.6. Hard coral colony abundance.

highly significant ($p<0.0001$) gradient that followed non-point source pollution influences, with a mean higher abundance at Arrecife El Negro (35.8±6.3 colonies), and a lower abundance, with 5.6±1.1 colonies (Figure 4.6). Percent hard coral cover of shallow-water reef assemblages also followed a significant trend ($p=0.0004$) towards increasing values with increasing distance from non-point source pollution (Figure 4.7), with the highest value at Arrecife El Ron (35.3±5.9%), and the lowest value at Arrecife Fanduco (3.8±1.3%). Percent cover of threatened Elkhorn coral, *Acropora palmata*, followed a similar trend with a highly significant ($p<0.0001$) abundance at offshore remote sites far from chronic non-point source pollution (Figure 4.8). The highest % cover for this species was registered at Arrecife El Ron, with 30.9±6.4%, followed at distance ¡ Arrecife El Negro, with 8.7±3.5%. Living elkhorn corals were completely absent from Punta Arena, Punta Ostiones and Arrecife Fanduco. These locations are subjected to chronic pulses of non-point pollution and high turbidity.

A total of 15 hard coral (scleractinians + hydrocorals) species were documented from all pooled transects across sites, including 13 scleractinians and 2 hydrocorals. The maximum cumulative species richness was 12 at Corona del Norte, followed by 9 at Corona del Medio and Arrecife El Negro, 8 at Cayo Ratones and Punta Ostiones, 7 at Arrecife El Ron and Arrecife Fanduco, and 6 at Punta Arenas. Most sites do not show substantial differences in cumulative species richness. However, species composition at each site was markedly different, particularly when offshore sites were compared to inshore sites. Dominant hard corals by % cover in all offshore coral reefs were *Acropora palmata*, distantly followed by *Porites astreoides* (Figure 4.9). Dominance at inshore reefs was variable, with *Millepora alcicornis* followed by *Montastraea cavernosa* at

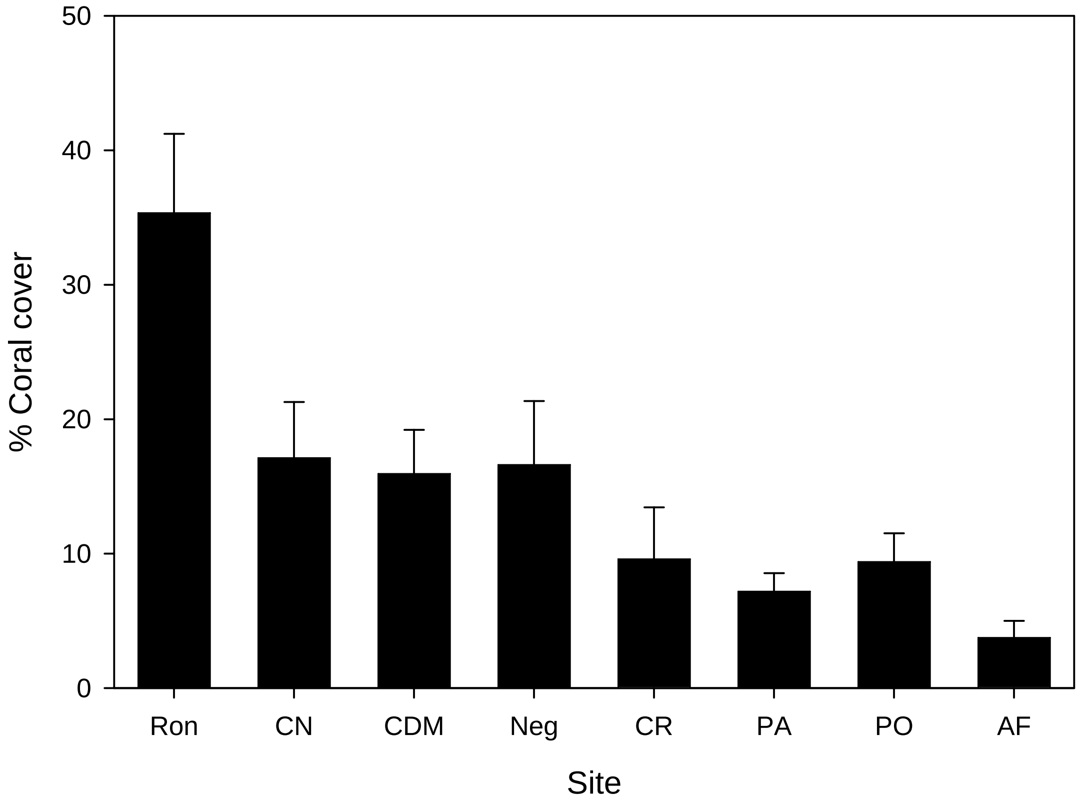

FIGURE 4.7. Percent hard coral cover.

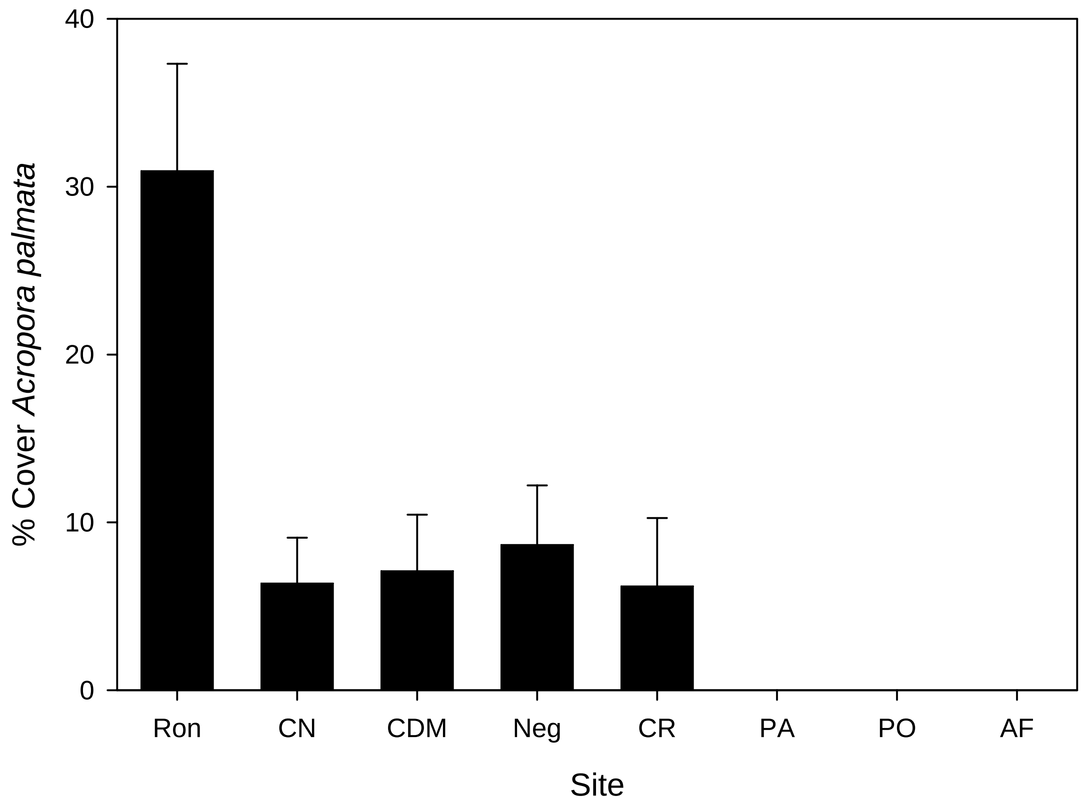

FIGURE 4.8.   Percent Elkhorn coral, *Acropora palmata*, cover.

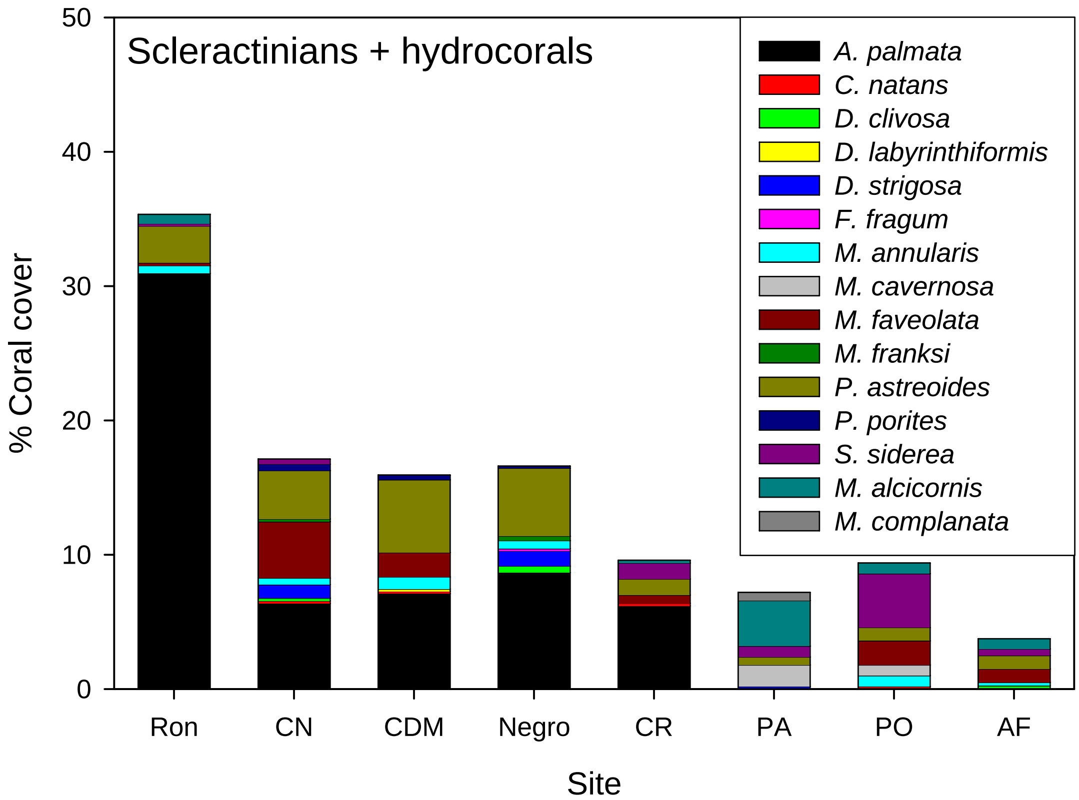

FIGURE 4.9. Percent relative cover of hard coral species.

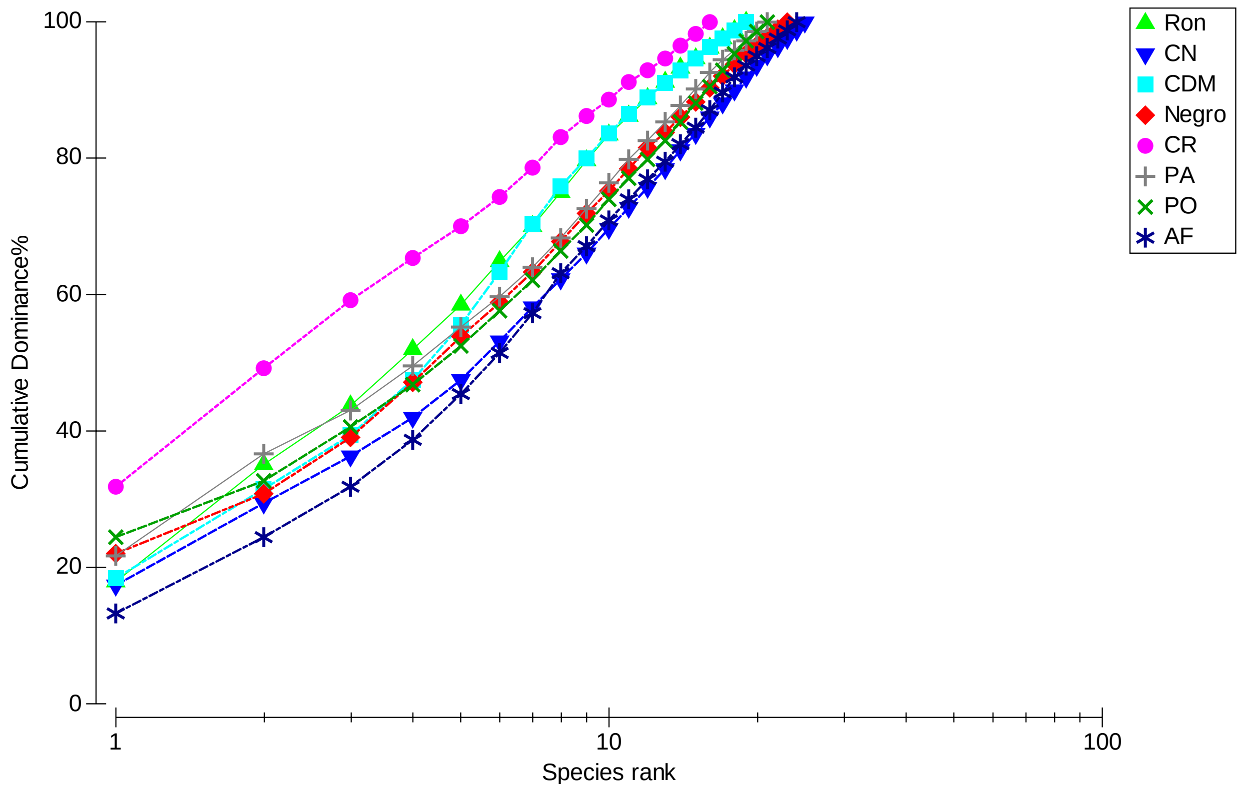

FIGURE 4.10. Dominance plots based on percent relative cover of hard coral species.

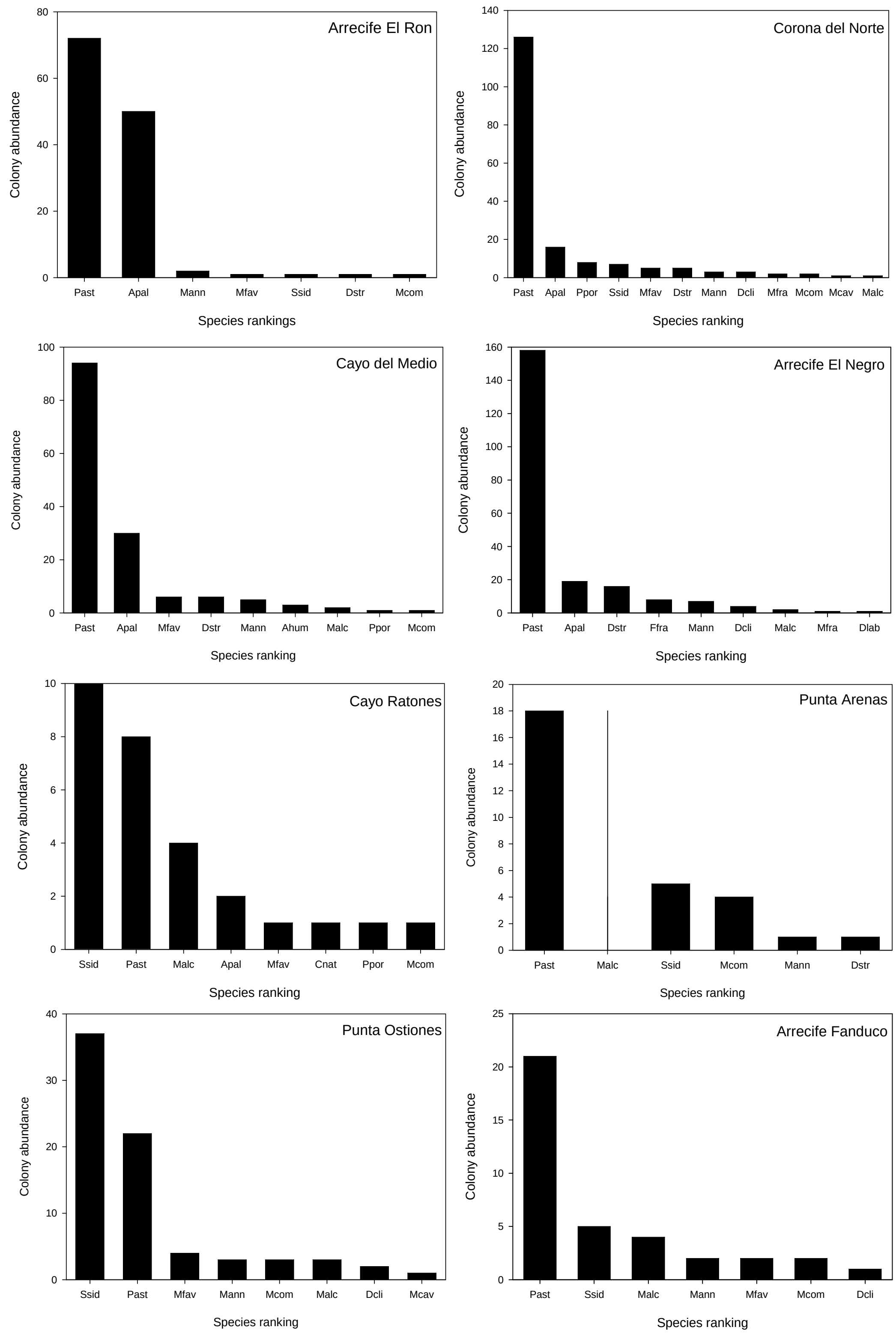

FIGURE 4.10. Hard coral species colony abundance rankings.

Punta Arenas, *Siderastrea siderea* followed by *Montastraea faveolata* at Punta Ostiones, and *M. faveolata* followed by *P. astreoides* at Arrecife Fanduco. The cumulative dominance plot of coral species rankings (Figure 4.10) show that only a few coral species at Cayo Ratones account for most of the cumulative % coral cover in contrast to most of the other sites. Punta Ostiones and Punta Arenas show a similar behavior, but less pronounced. Also, dominance exerted by *Acropora palmata* at Arrecife El Ron produced a similar effect. Dominance at inshore sites can be the result of chronic environmental degradation (i.e., Cayo Ratones) from permanent turbidity and frequent non-point source pollution. In the other hand, dominance by *A. palmata* at offshore sites (i.e., Arrecife El Ron) can result from lower anthropogenic stress pressure.

It is important when assessing coral species dominance to document also numerical dominance since there could be ephemeral, small-sized coral species that could be numerically abundant but showing very low % cover values. In the other hand, some species might exhibit very low abundance, but very large colony sizes, thus accounting for much of the % cover. Sensitivity to environmental stress could be highly variable, showing dramatic differences in species-specific responses. Therefore, it is important to account % cover and numerical dominance in order to understand potential community responses to disturbance. Dominance was determined as a function of colony abundance (Figure 4.10). This resulted in a complete different picture. *Porites astreoides* was numerically dominant at all four offshore sites, distantly followed by *A. palmata*. It was also the most abundant species at Arrecife Fanduco, a very shallow reef. This species undergoes lunar recruitment cycles and is dominant under shallow high energy conditions (Glynn, 1973; Hernández-Delgado, 2005). Highly turbid sites (i.e., Cayo Ratones, Punta

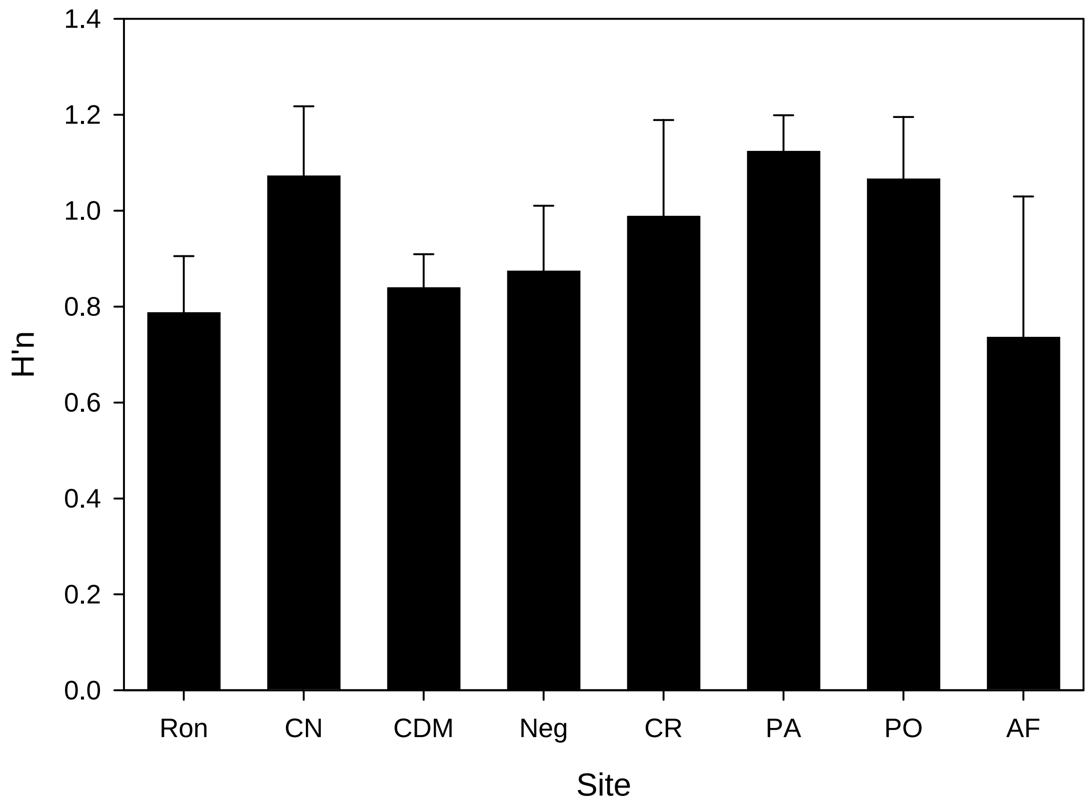

FIGURE 4.11. Hard coral species diversity index (H’n).

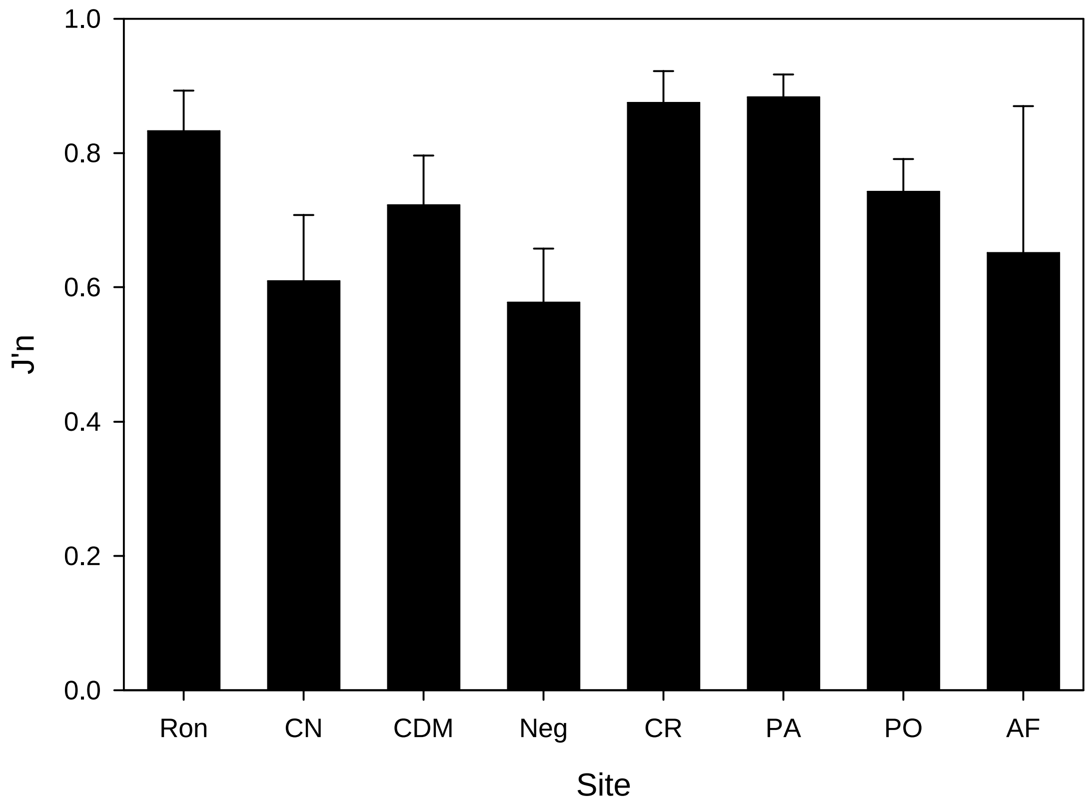

FIGURE 4.12. Hard coral species evenness (J'n).

Ostiones) showed dominance by sediment-tolerant *Siderastrea siderea*. *Millepora alcicornis* was co-dominant with *P. astreoides* at Punta Arenas. Hard coral species diversity index (H'n) and evenness (J'n) showed no significant trends among sites (Figures 4.11 and 4.12, respectively). Although dominance by *P. astreoides* and *A. palmate* at Arrecife El Ron, Cayo del Medio and Arrecife El Negro, as well as dominance by *P. astreoides* at Arrecife Fanduco resulted in slightly lower H'n and J'n values, this difference was not significant at the scale sampled.

Percent octocoral cover resulted significantly higher (p<0.0001) at Arrecife Fanduco (52.0±13.5%) and Punta Ostiones (18.2±6.6%), and significantly lower at Cayo del Medio (1.1±0.004%) and Arrecife El Negro (1.1±0.01%) (Figure 4.13). Further, there were major differences in species composition and ranking of octorals per site (Figure 4.14). A total of 11 octocoral species were registered from all pooled surveyed sites. Octocorals species richness was also predominantly abundant at inshore turbid sites. Arrecife Fanduco showed 10 species, followed by 7 at Punta Ostiones, 6 at Punta Arenas, 4 at Cayo Ratones, Corona del Norte, and Arrecife El Negro, 3 at Cayo del Medio, and 2 at Arrecife El Ron. *Pseudopterogorgia* spp. was the most dominant octocoral group at most sites, particularly, at Arrecife Fanduco (Figure 4.14). It was followed by *Plexaura* spp., then *Muricea* spp., *Erythropodium caribbaeorum*, and *Eunicea* spp. to a lower extent. *Gorgonia* spp. was more abundant at offshore sites.

Sponges were significantly (p=0.0123) more abundant at Cayo del Medio, with 6.7±2.3% (Figure 4.15). No sponges were documented at Cayo Ratones, and a significantly low % sponge cover was measured at Arrecife Fanduco (0.25±0.25%). Zoanthids were significantly (p<0.0001)

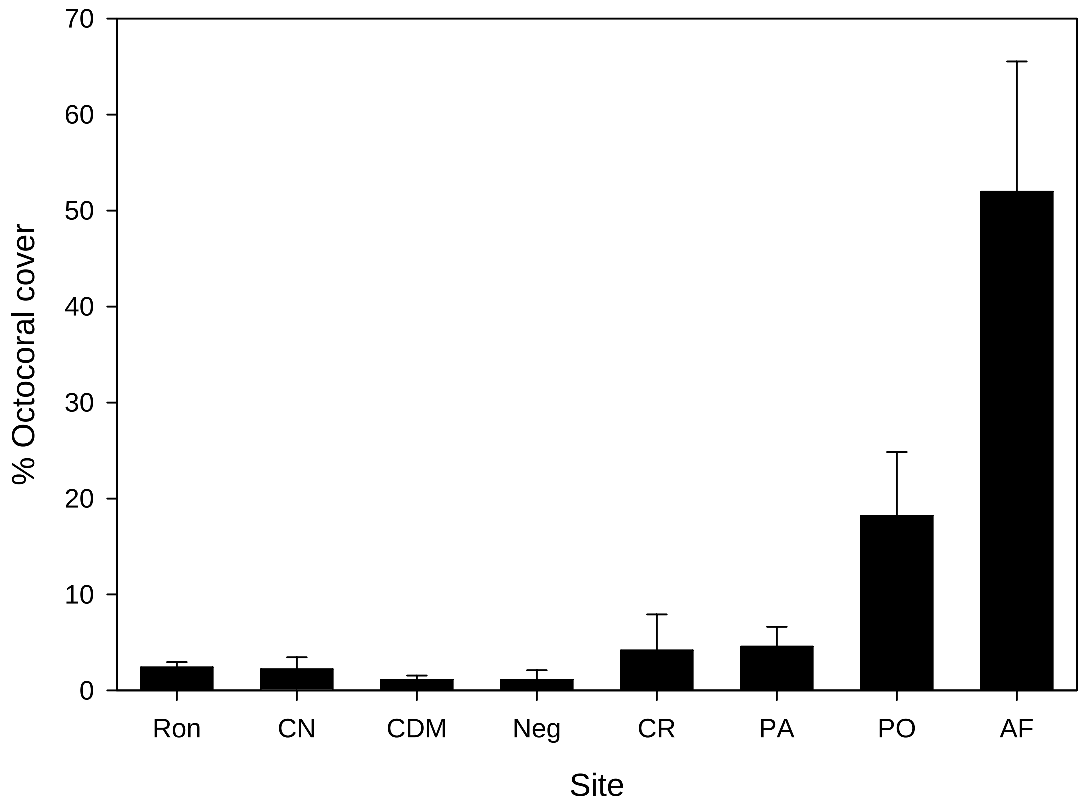

FIGURE 4.13. Percent octocoral cover.

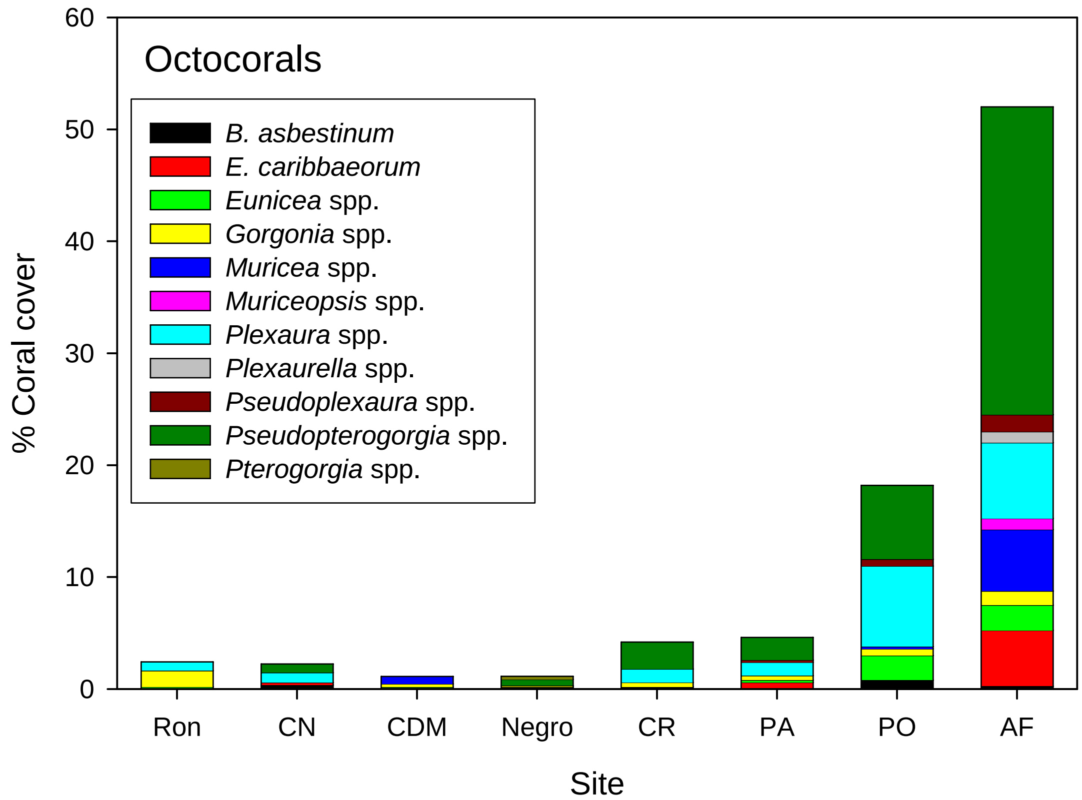

FIGURE 4.14. Percent relative cover of octocoral species.

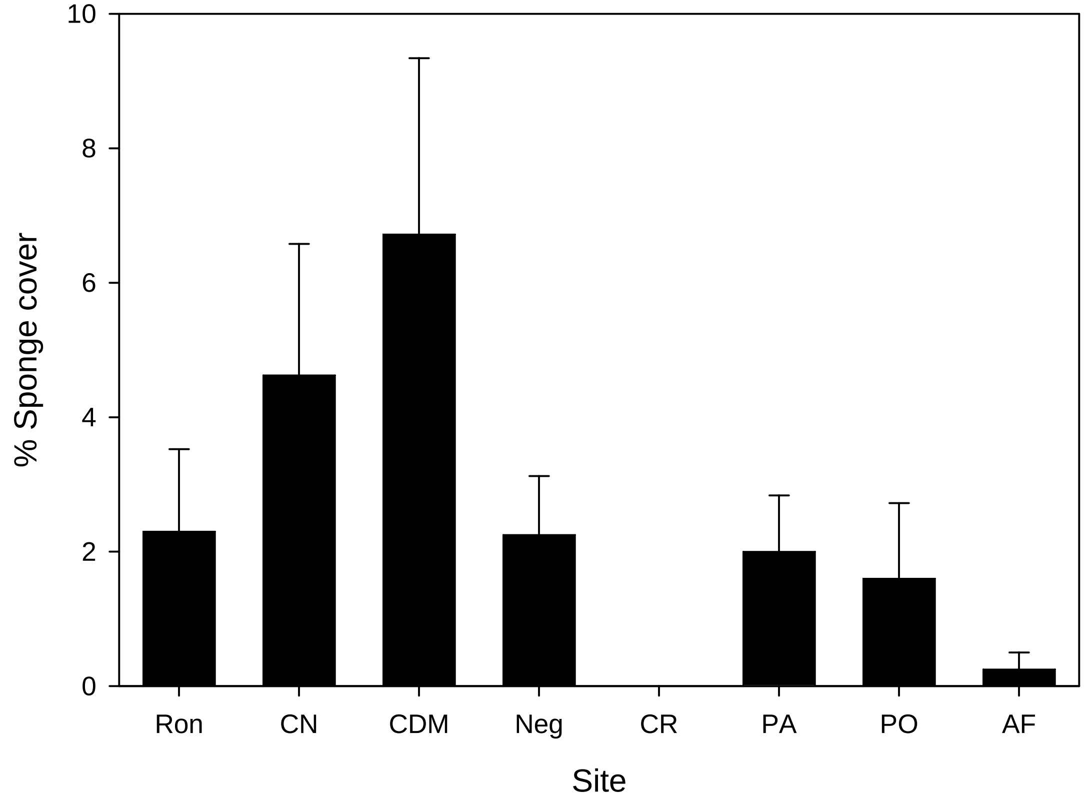

FIGURE 4.15. Percent sponge cover.

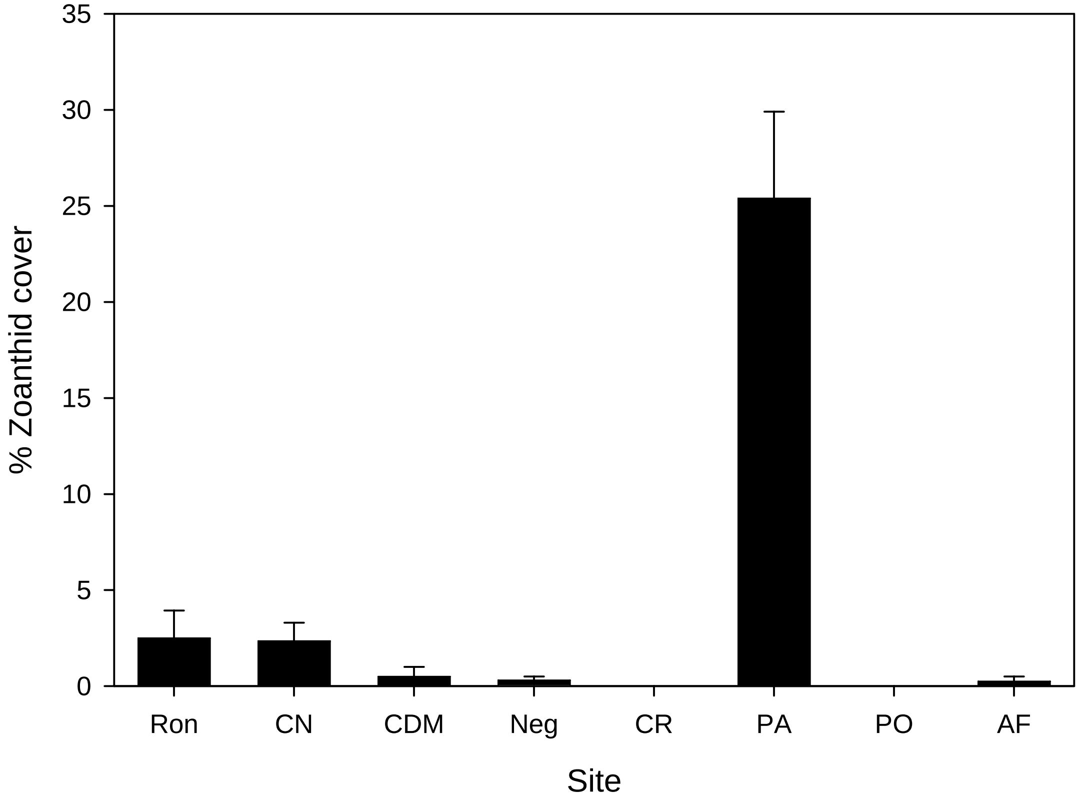

FIGURE 4.16. Percent zoanthid cover.

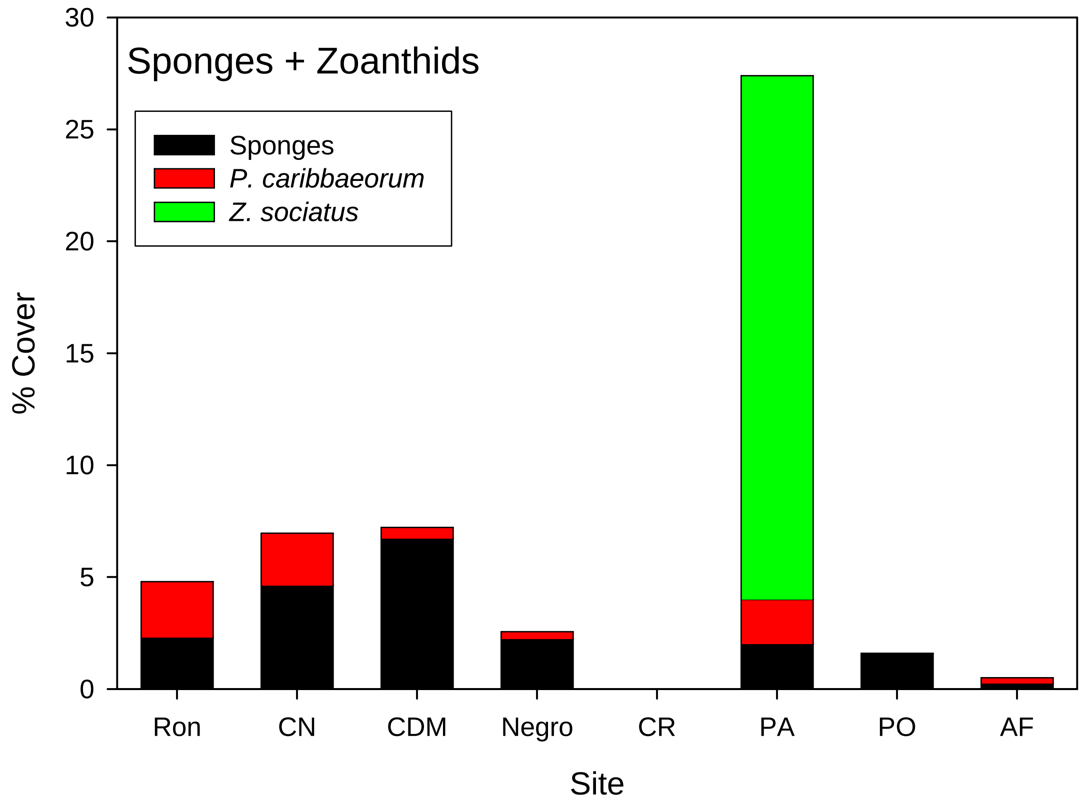

FIGURE 4.17. Percent relative cover of sponges and zoanthid species.

more abundant at Punta Arenas (25.4±4.5%), in comparison to other sites (Figure 4.16). *Palythoa caribbaeorum* showed a wider geographic distribution, but at lower benthic dominance. However, *Zoanthus sociatus* was significantly dominant at Punta Arenas. This species is also fairly abundant in other fringing marginal reefs under turbid waters in eastern Puerto Rico (Hernández-Delgado, 2005).

Percent macroalgal cover showed a highly significant (p<0.0001) increase under turbid and hypertrophic conditions at Cayo Ratones (84.4±3.6%) and at Punta Ostiones (66.6±7.8%), in comparison to offshore sites such as Arrecife El Negro (17.2±3.3%), and Arrecife El Ron (11.9±0.8%) (Figure 4.18). It is important to mention that offshore reefs such as Corona del Norte (23.6±12.2%) and Cayo del Medio (32.8±7.1%) exhibited moderate abundance of macroalgae, possibly as a combination of occasional impacts by Guanajibo River plume and serial overfishing that has partially depleted large herbivore fish guilds (Hernández-Delgado, pers. obs.). A dramatically opposite pattern was documented in crustose coralline algae (CCA). Percent CCA cover was significantly higher (p<0.0001) at offshore remote sites, with higher mean values at Arrecife El Negro (57.7±4.2), followed by Corona del Norte (45.9±4.1%) (Figure 4.19). Mean lower values coincided with dominance by macroalgae and other non reef-building taxa and were registered at Punta Ostiones (2.8±1.2%) and at Cayo Ratones (1.8±1.4%). Percent cysanobacterial cover resulted significantly (p<0.0001) at Arrecife El Negro in comparison to other sites (Figure 4.20), and may suggest potential eutrophication pulses associated to the Guanajibo River plume and tidal flushing from Mayagüez Bay. An analysis of algal dominance by functional group % cover (Figure 4.21) revealed that CCA was largely dominant under low turbid offshore conditions. Erect calcareous algae *Halimeda* spp. was more abundant also at

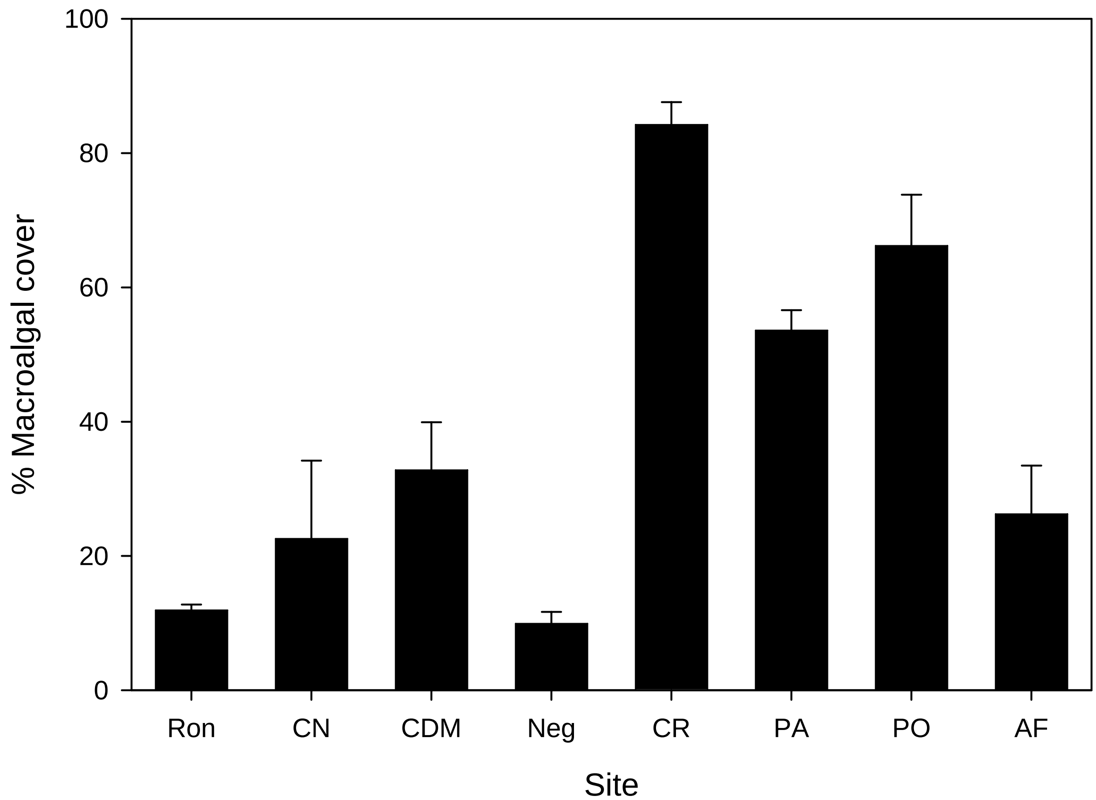

FIGURE 4.18. Percent macroalgal cover.

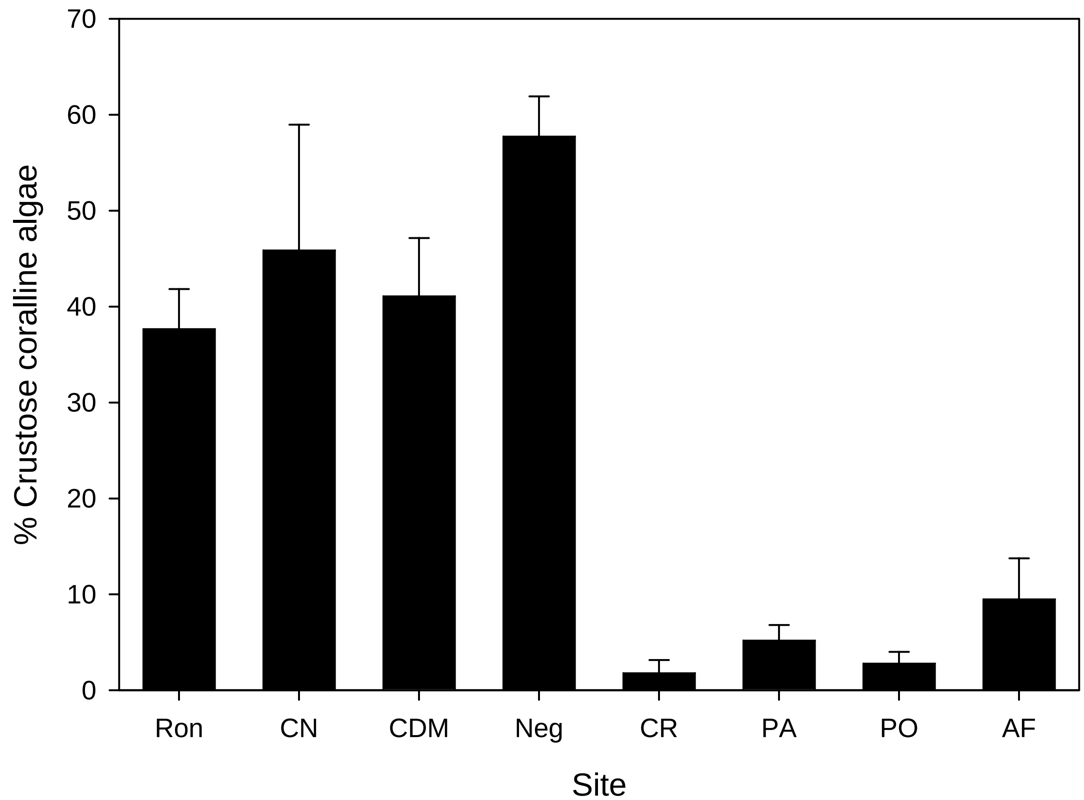

FIGURE 4.19. Percent crustose coralline algal (CCA) cover.

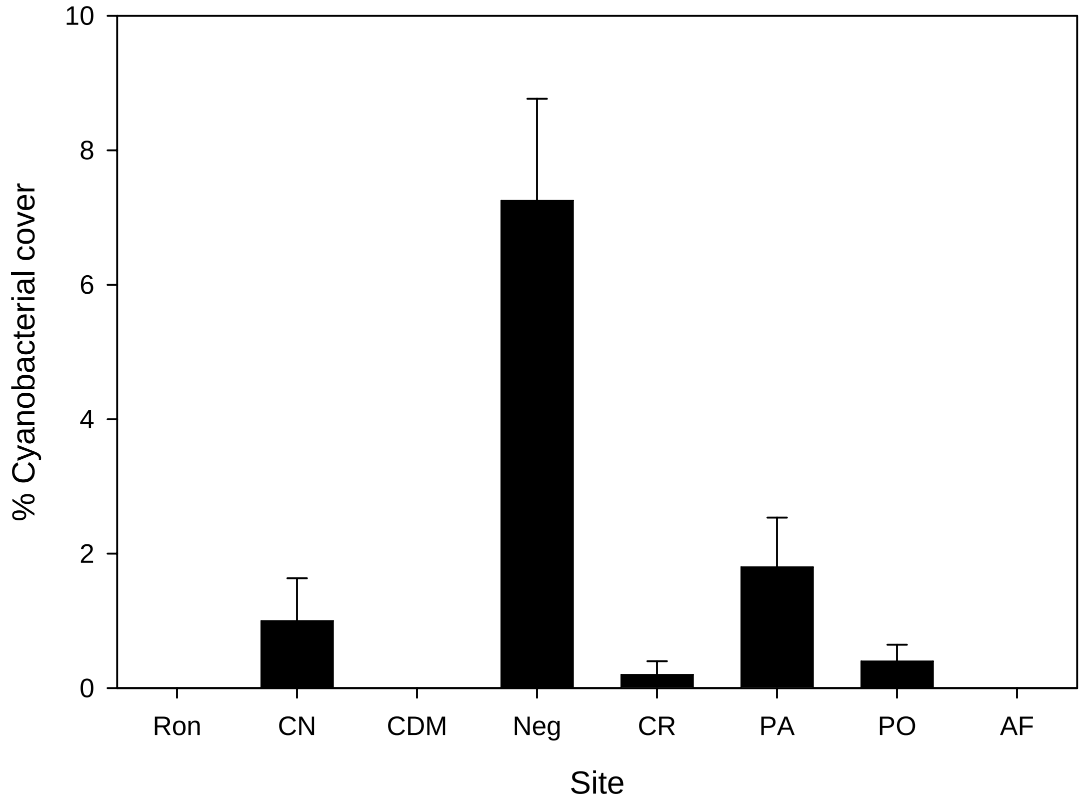

FIGURE 4.20. Percent cyanobacterial cover.

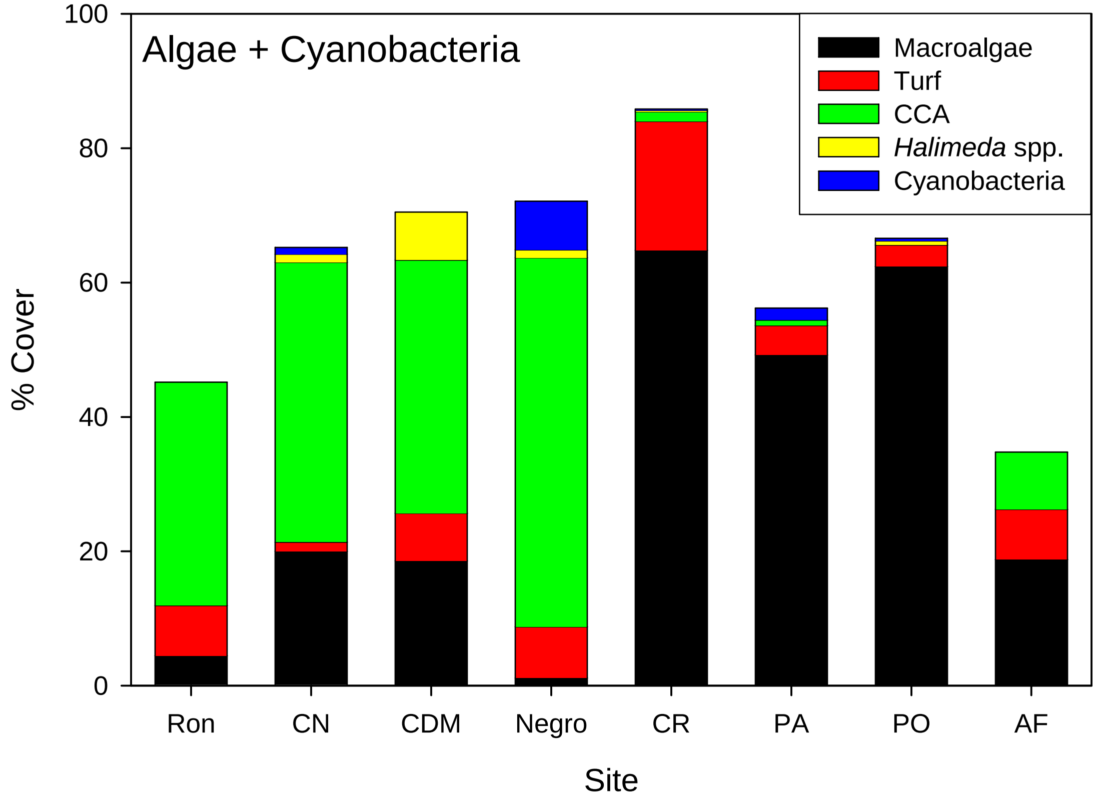

FIGURE 4.21. Percent relative cover of algal functional groups and cyanobacteria.

offshore sites. However, there was a significant phase shift favoring macroalgal dominance under chronic highly turbid and hypertrophic conditions of most inshore reefs. Further, cyanobacteria were more abundant in locations that are known to receive periodic pulses from polluted waters from the Guanajibo River plume and other non-point sources (ej. Arrecife El Negro, Punta Arenas and Punta Ostiones.

Percent cover of non-living benthos, including a pool of sand, pavement and rubble (SPR) showed a significant (p=0.0003) trend towards higher mean values at offshore sites, such as El Ron (7.8±2.0%). Lowest values were measured at Cayo Ratones and Punta Arenas, with 0.2±0.2%, each one. This coincided with dominance and rapid substrate pre-occupation by macroalgae at inshore turbid and hypertrophic sites. SPR categories were analyzed and open pavement was the dominant feature of SPR at offshore sites, as well as in Arrecife Fanduco (Figure 4.23). Rubble deposits were also an exclusive feature of offshore reefs which are more exposed to hurricane impacts and winter storm swells. Sand pockets were common at most sites, but particularly abundant at Arrecife Fanduco. Recently dead corals consistently accounted for 3-4% of benthic percent cover of non-living categories at offshore sites, and fluctuated from 1 to 5% at inshore reefs. Recent coral mortality was largely attributed to the year 2005 Caribbean-wide mass coral bleaching event that was followed by extensive coral mortalities until at least mid-summer of 2006 (Hernández-Delgado et al., in preparation). Actually, 50% of the sampled sites still showed bleached coral colonies, including a frequency of 6% at Arrecife Fanduco, 8% at Corona del Norte, 15% at Cayo Ratones, and 17% at Punta Ostiones (Figure 4.24). Higher frequencies were documented at the most turbid sites. In addition, approximately 5% of the coral colonies at Punta Ostiones and Arrecife Fanduco were infected by white plague-like

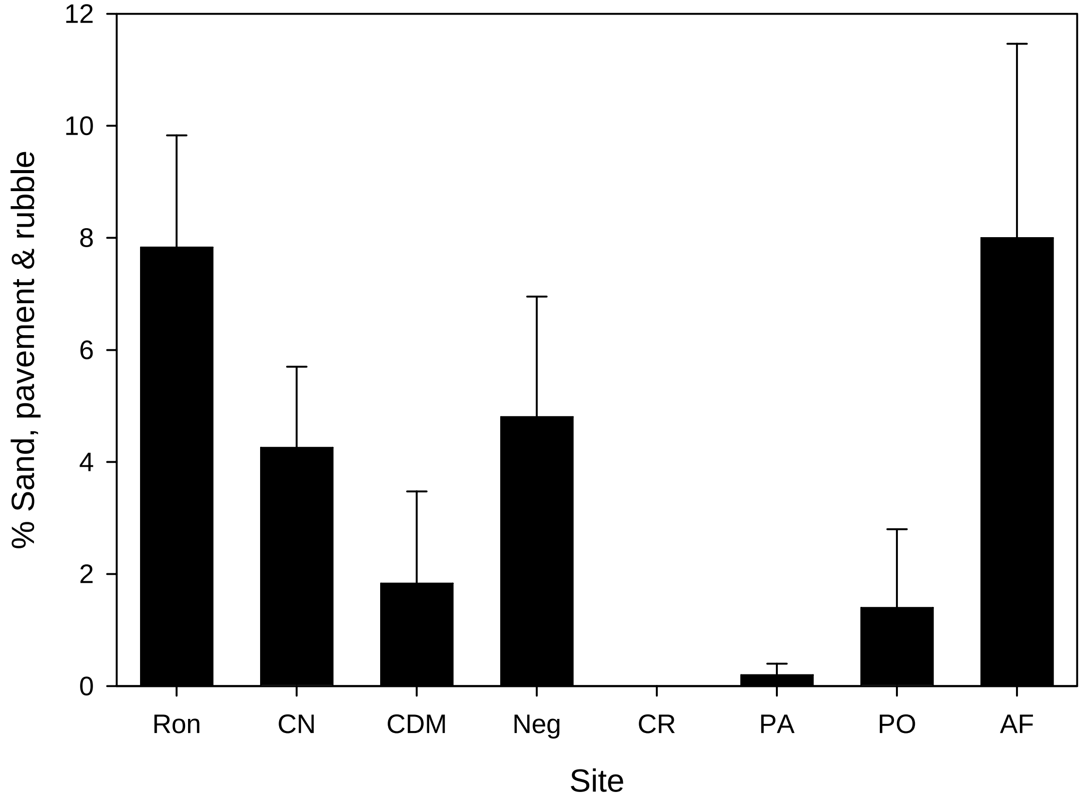

FIGURE 4.22. Percent sand, pavement and rubble (SPR) cover.

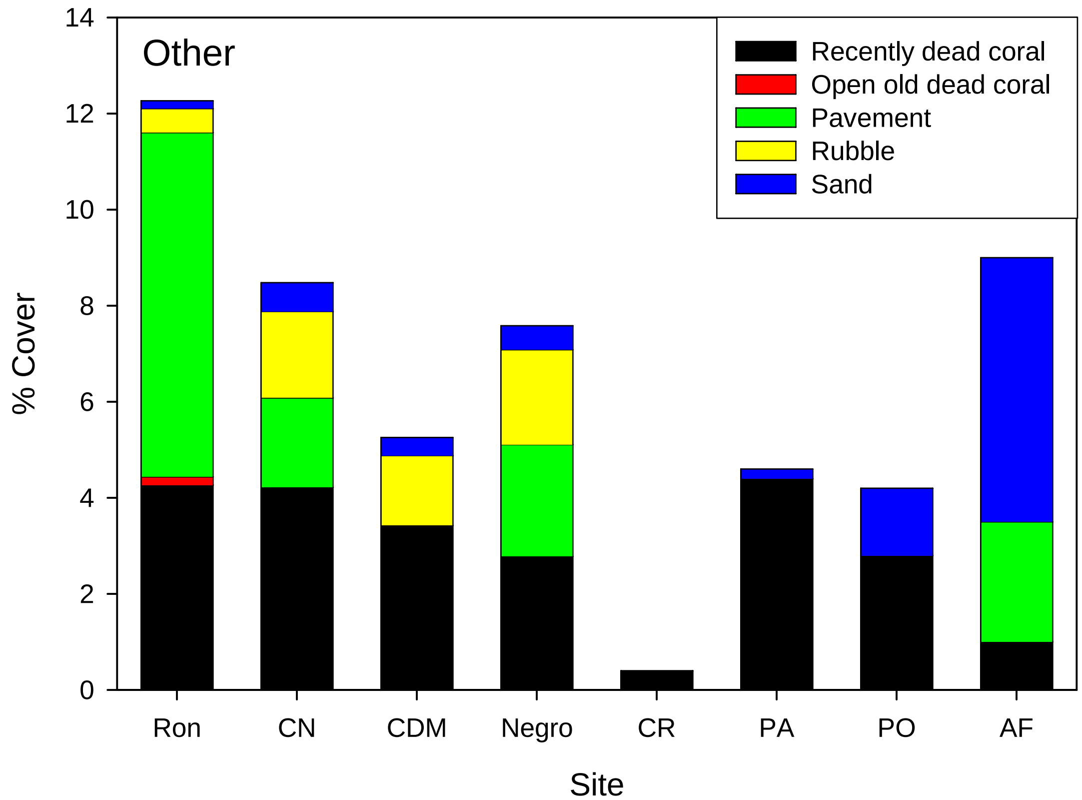

FIGURE 4.23. Percent relative cover of other categories.

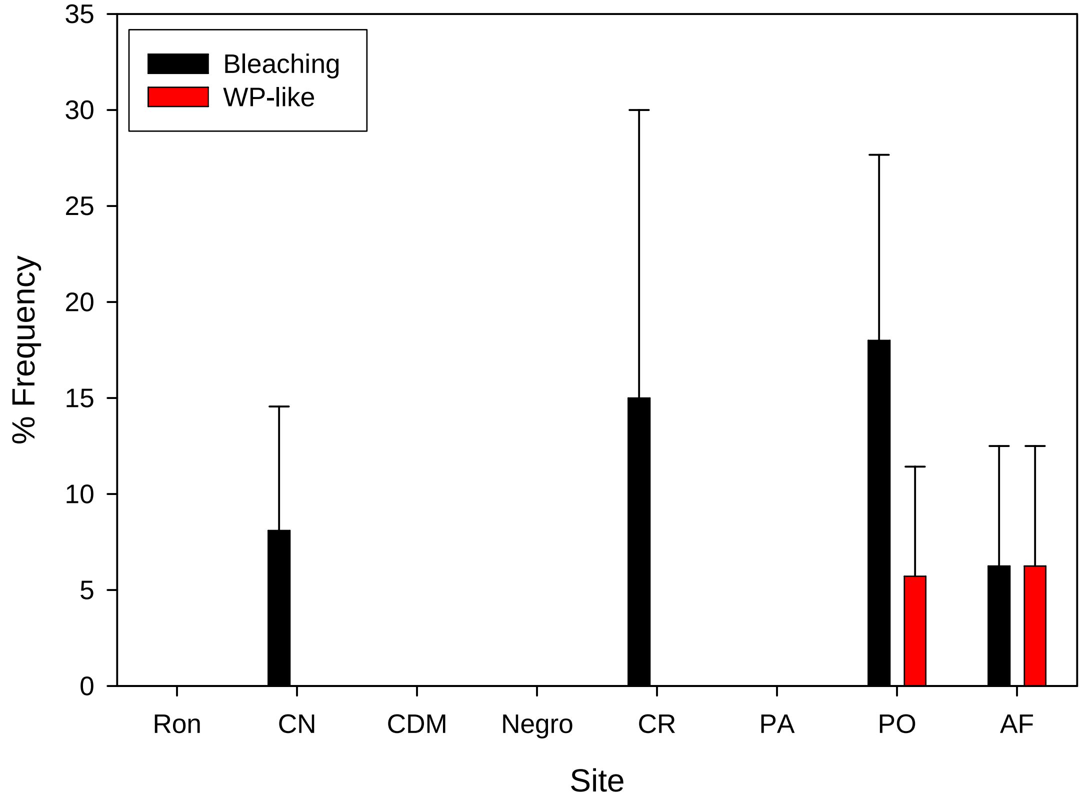

FIGURE 4.24. Percent frequency of bleached and white plague-like (WP)-infected colonies.

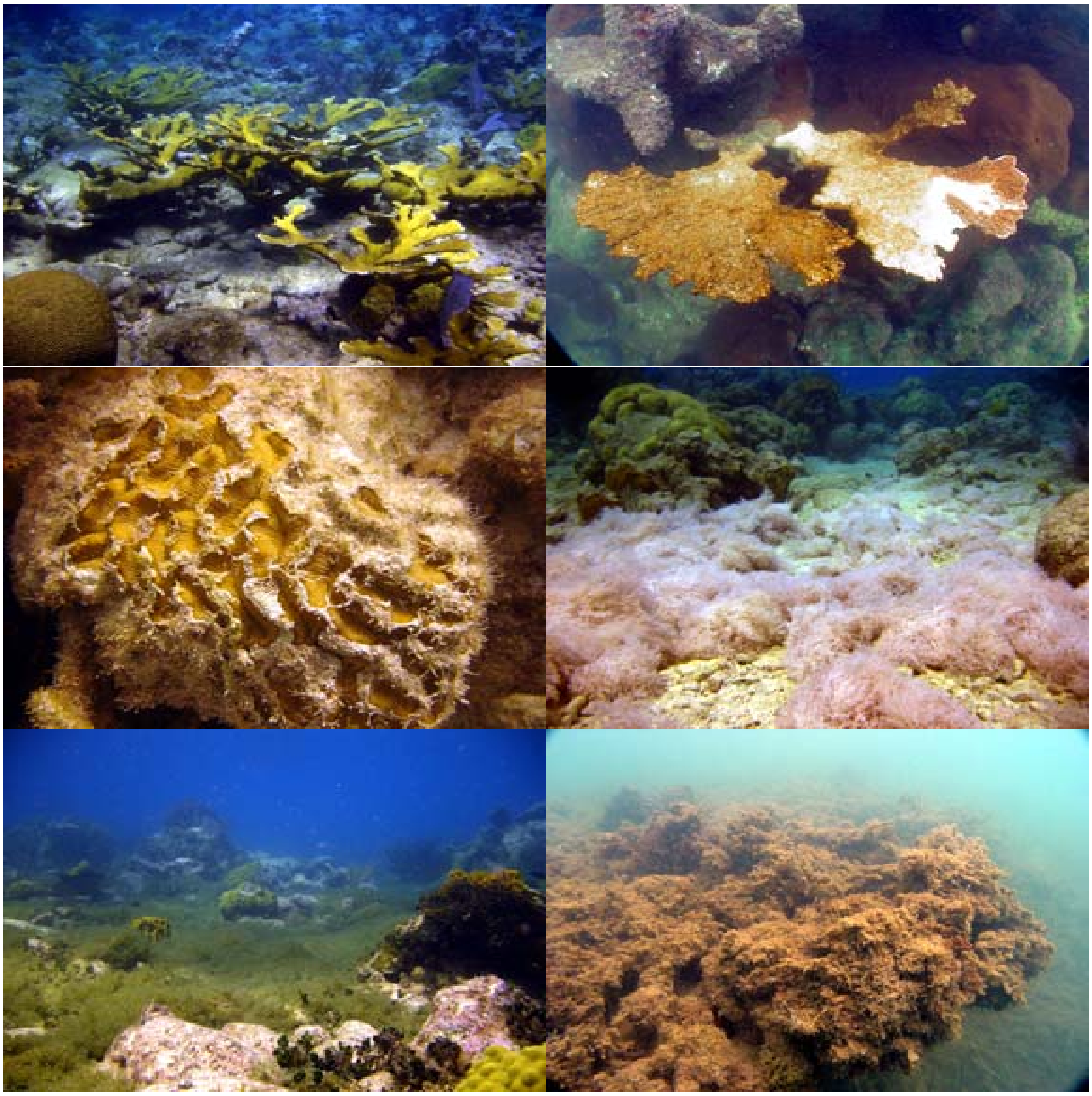

FIGURE 4.25. Examples of variable conditions along a distance gradient from non-point source pollution. From top left: (a) typical remnat patch of Elkhorn coral, *Acropora palmata* at Arrecife El Ron; (b) dying colony of *A. palmate* at Punta Ostiones, this was the only surviving acroporid at this site; (c) rapidly dying colony of *Colpophyllia amaranthus* at Corona del Norte; (d) Red macroalgae (*Liagora* sp.) bloom at Arrecife El Negro; (e) Brown macroalgae (*Dyctiota* sp.) bloom at Cayo del Medio; and (f) Macroalgal dominance of former *A. palmate* biotope at Cayo Ratones. Severe water quality degradation has resulted in a dramatic community phase shift at most sites.

disease/syndrome conditions. Figure 4.25 shows some examples of variable conditions at different coral reefs located a distance gradient from non-point pollution sources.

Multivariate effects at the scale of sites.

Multivariate statistics were applied to individual matrices of the entire coral reef benthic communities of all sites to test for spatial patterns of community structure along a non-point source pollution gradient. Figure 4.26 shows a hierarchical cluster ordination analysis plot of the community structure of shallow-water coral reef benthic communities among sites. Data shows two basic clustering patterns at the 50% community similarity cutoff level between inshore (upper four sites) and offshore locations (lower four sites). Such patterns result from macroalgal, octocoral and zoanthid dominance at the upper inshore cluster, and from coral and CCA dominance at the lower offshore cluster. This cluster analysis clearly reflects impacts associated to environmental stress gradients resulting from non-point source pollution affecting the spatial patterns of coral reef benthic community structure across the southwestern Puerto Rico shelf.

Figure 4.27 shows a multi-dimensional scaling (MDS) analysis plot of the community structure of shallow-water coral reef benthic communities among sites. Data shows two similar basic clustering patterns at the 50% community similarity cutoff level between inshore and offshore locations. But four clustering patterns emerged at the 60% community similarity cutoff level. Again, all four offshore sites clustered within a single group as a result of coral and CCA dominance. But inshore groups clustered in three different groups. Punta Ostiones and Cayo Ratones grouped together as a result of macroalgal dominance. Punta Arenas and Arrecife Fanduco formed two individual clusters as a result of zoanthid and octocoral dominance, respectively. As above, MDS analysis confirms impacts associated to environmental stress gradients resulting from non-point source pollution.

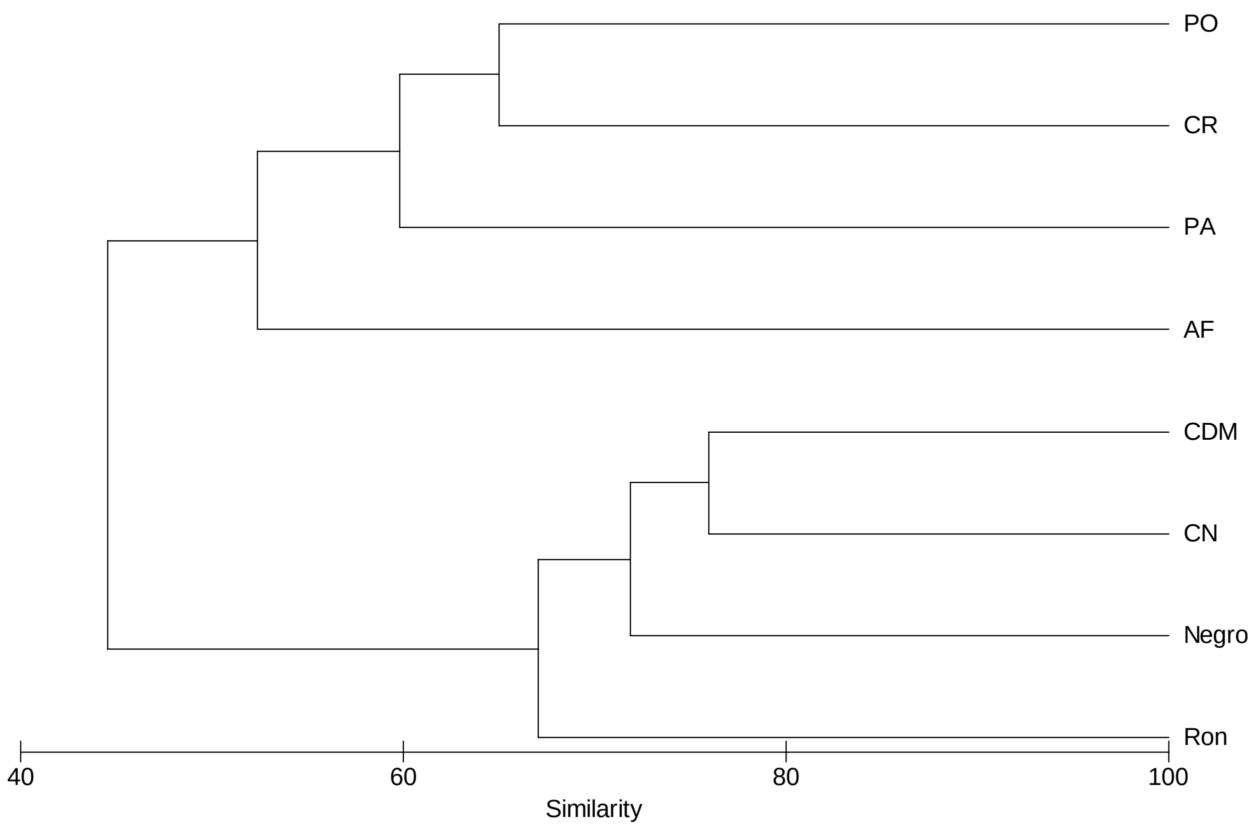

FIGURE 4.26. Hierarchical cluster ordination analysis of the community structure of shallow-water coral reef benthic communities among sites. Data shows two basic clustering patterns at the 50% community similarity cutoff level between inshore (upper four sites) and offshore locations (lower four sites).

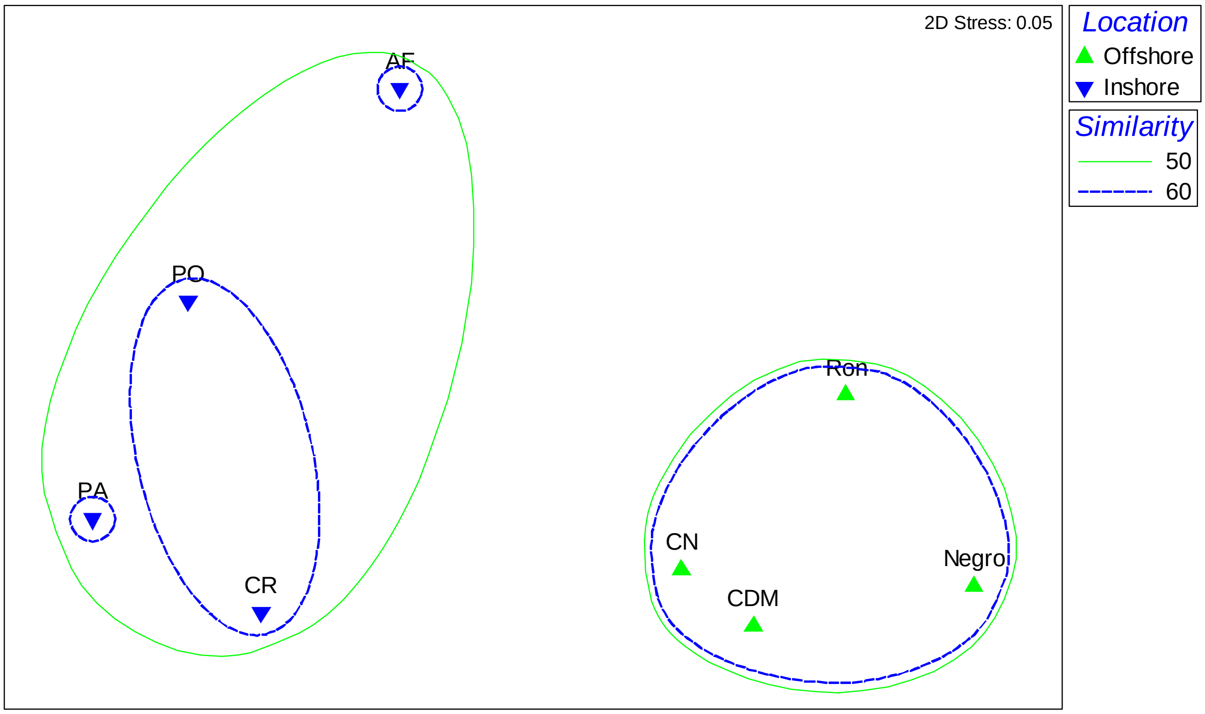

FIGURE 4.27. Multi-dimensional scaling analysis of the community structure of shallow-water coral reef benthic communities among sites. Data shows two basic clustering patterns at the 50% community similarity cutoff level between inshore and offshore locations. Four clustering patterns emerge at the 60% community similarity cutoff level. Again, all four offshore sites clustered within a single group as a result of coral and CCA dominance. But inshore groups clustered in three different groups, PO and CR grouped together as a result of macroalgal dominance. PA and AF formed two individual clusters as a result of zoanthid and octocoral dominance, respectively.

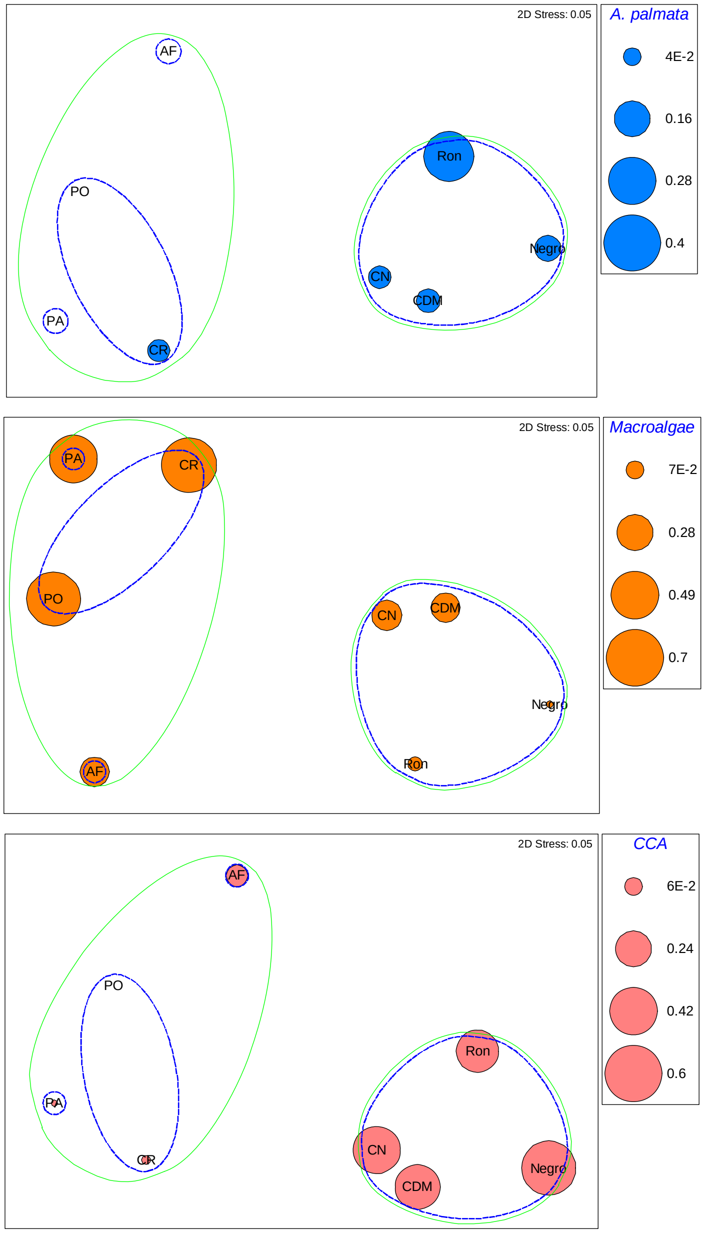

FIGURE 4.28. Bubble plots for the distribution patterns of: (a) Elkhorn coral, *Acropora palmata*; (b) macroalgae; and (c) CCA among sites. Data shows two clustering patterns at the 50% community similarity cutoff level between inshore and offshore sites based on the percent distribution of each group. Four clustering patterns emerged at the 60% community similarity cutoff level. Inshore reefs with very limited Elkhorn coral growth or where this species is absent clustered independently of those where this coral is still abundant to some extent (offshore sites). This plot clearly suggests potential environmental gradients associated to non-point sources affecting the spatial distribution of *A. palmata*.

Bubble plots were used to demonstrate three additional examples of the impacts of non-point source pollution gradients in the spatial patterns of shallow coral reef benthic community structure along the southwestern Puerto Rico shelf (Figure 4.28). These involve the spatial distribution patterns of: (a) Elkhorn coral, *Acropora palmata*; (b) macroalgae; and (c) CCA among sites. Data shows two clustering patterns at the 50% community similarity cutoff level between inshore and offshore sites based on the percent distribution of each group. Four clustering patterns emerged at the 60% community similarity cutoff level. Inshore reefs with very limited Elkhorn coral growth, or where this species is absent, clustered independently of those where this coral is still abundant at least to some extent (offshore sites). Inshore reefs with very dominant macroalgal growth also clustered independently of those where macroalgae were abundant only to some extent (offshore sites). Inshore reefs with very rare CCA growth clustered independently of those where CCA was abundant (offshore sites). This clearly suggests nutrification impacts by non-point source pollution affecting the spatial distribution of macroalgae. Also, it suggests that Corona del Norte and CDM were showing a moderate abundance of macroalgae as a possible combined result of occasional impacts by Guanajibo River plume pulses and overfishing of large herbivore fishes (Hernández-Delgado, pers. obs.). Further, *A. palmata* and CCA wer largely excluded or eliminated from chronically degraded reefs. Dead coral substrates became rapidly dominated by macroalgae and/or other non reef-building taxa.

TABLE 4.2. Results of ANOSIM test for significant differences of coral reef epibenthic community structure. Data was √x-transformed. Based on 5,000 permutations.

Compared factors*	Global R value	*p*
One way ANOSIM		
Site	0.725	**0.0002**
Geographic location	0.875	**0.0269**
Management regime	0.052	0.3710 NS**
Two-way nested ANOSIM		
Site x Location	0.512	**0.0012**
Site x Management	0.48	0.3333 NS
Location vs. Management	0.097	0.1300 NS

*Geographic location (Offshore vs. Inshore); Management regime (MPA vs. non-MPA control).
**NS= not significant.

TABLE 4.3. Matrix of key discriminating taxa among sites.

Sites	Ron	CN	CDM	Negro	CR	PA	PO	AF
Ron	-	-	-	-	-	-	-	-
CN	*Apal*	-	-	-	-	-	-	-
CDM	*Apal*	*Halim*	-	-	-	-	-	-
Negro	Cyan	Macro	Macro	-	-	-	-	-
CR	Macro	CCA	CCA	Macro	-	-	-	-
PA	*Apal*	CCA	CCA	CCA	*Zsoc*	-	-	-
PO	Macro	CCA	CCA	CCA	Turf	*Zsoc*	-	-
AF	*Apal*	*Psdpt*	*Psdpt*	*Psdpt*	Macro	*Zsoc*	Macro	-

The above discussed spatial patterns of benthic community structure were significantly different among sites (*p*=0.0002). Key discriminating taxa that explained differences in spatial patterns among sites were summarized in Table 4.3. It turned out that 8 different benthic categories explained spatial variation in community structure among sites. CCA explained most of the differences among sites (28.6%), closely followed by macroalgae (25%). *Acropora palmata* explained 14.3% of the variation, while *Pseudopterogorgia* spp. and *Zoanthus sociatus* explained 10.7% of the variation, each one. Cyanoabcteria, *Halimeda* spp., and filamentous algal turfs explained 3.6% of the variation, each one.

TABLE 4.4. Summary of mean values of coral reef community parameters between geographic locations.

Parameter	**Offshore**	**Inshore**
Species richness	4.3±0.3	3.5±0.3
Colony abundance	30.5±2.4	10.1±1.5
% Coral	20.8±2.7%	7.7±1.3%
% *Acropora palmata*	12.8±2.9%	1.6±1.2%
H'n	0.8894±0.0598	0.9906±0.0884
J'n	0.6825±0.0425	0.7953±0.0499
% Octocoral	1.7±0.4%	18.1±5.4%
% Sponges	4.0±0.9%	1.0±0.4
% Zoanthids	1.3±0.4%	6.7±2.9%
% Macroalgae	21.7±3.7%	59.8±5.5%
% CCA	45.9±3.8%	4.6±1.2%
% Cyanobacteria	2.2±0.7%	0.6±0.3%
% SPR	4.6±1.0%	2.1±1.0%

TABLE 4.5. Summary results of one-way ANOVA analysis of coral reef community parameters between geographic locations.

Parameter	**D.F. (within, between)**	**F statistic**	***P* value**
Species richness	1,39	3.13	0.0849
Colony abundance	1,39	59.1	**<0.0001**
% Coral	1,39	18.0	**0.0001**
% *Acropora palmata*	1,39	19.0	**0.0001**
H'n	1,39	0.94	0.3376
J'n	1,39	3.00	0.0909
% Octocoral	1,39	12.1	**0.0012**
% Sponges	1,39	12.2	**0.0012**
% Zoanthids	1,39	1.46	0.2347
% Macroalgae	1,39	34.6	**<0.0001**
% CCA	1,39	118.0	**<0.0001**
% Cyanobacteria	1,39	1.55	0.2212
% SPR	1,39	5.94	**0.0194**

TABLE 4.6. Identification of key discriminating taxa between geographic locations and management options.

Category	% contribution*	Mean abundance	Mean abundance
Offshore vs. Inshore	MD=55.49%	*Offshore*	*Inshore*
CCA	14.31	6.44	1.25
Macroalgae	10.85	2.99	6.82
Acropora palmata	7.53	3.42	0.62
Pseudopterogorgia spp.	6.03	0.41	2.69
Plexaura spp.	3.75	0.46	1.87
Reserve vs. Control	MD=47.80%	*MPA*	*Non-MPA*
CCA	11.22	4.88	2.81
Macroalgae	9.03	4.73	5.08
Acropora palmata	8.59	3.31	0.74
Pseudopterogorgia spp.	6.18	0.61	2.49
Zoanthus sociatus	3.95	0.00	1.21

*MD= Mean % dissimilarity.

Variation of community parameters between geographic locations.

Table 4.4 summarized community parameters between geographic locations. Offshore coral reefs showed significantly higher coral colony abundance (p<0.0001), % coral cover (p=0.0001),, and % cover of *Acropora palmata* (p=0.0001) (Table 4.5). Also, sponges (p=0.0012), CCA (p<0.0001) and SPR (p=0.0194) were significantly higher in offshore reefs. Octocorals (p=0.0012) and macroalgae (p<0.0001) were significantly higher at inshore locations. Further, coral reef benthic community structure was significantly different (p=0.0269) between sites (Table 4.2). CCA explained 14.3% of the variation between geographic locations, followed by macroalgae (10.9%), *A. palmata* (7.5%), *Pseudopterogorgia* spp. (6%), and *Plexaura* spp. (4%). There was a significant site x location interaction (p=0.0012), which suggests that differences in geographic locations were large the result of large variation among sites (Table 4.2). These results also revealed unequivocal impacts of non-point source pollution along an inshore-offshore geographical gradient that have affected the benthic community structure of coral reefs in the southwestern shelf of Puerto Rico.

Variation of community parameters between management regimes.

Table 4.7 summarized community parameters between management regimes. But interestingly, most parameters showed no significant differences between MPAs and non-MPA sites (Table 4.8). One exception was % cover in *Acropora palmata* (p=0.0462) that showed significantly higher % cover at MPA sites, particularly, within Tourmaline Natural Reserve, in comparison to Cayo Ratones and Adjacent Waters Natural Reserve. No living *A. palmata* colonies were identified within Finca Belvedere Natural Reserve marine extension. The other two exceptions were % zoanthid cover (p=0.0475) and % cyanobacteria (p<0.0001) that resulted higher at non-MPA sites. Coral reef benthic community structure showed no significant difference between management regimes (Table 4.2). Also, no significant site x management, or location x management interactions were observed. CCA also explained 11.2% of the variation in benthic community structure between management regimes, followed by macroalgae (9%), *A. palmata* (8.6%), *Pseudopterogorgia* spp. (6%), and *Zoanthus sociatus* (4%). These results revealed that impacts of non-point source pollution have not discriminated between management regimes, further implying that pollution pulses can potentially affect widespread geographic areas.

TABLE 4.7. Summary of mean values of coral reef community parameters between management regimes.

Parameter	MPA	Non-MPA
Species richness	3.8±0.3	4.1±0.2
Colony abundance	20.8±2.7	21.4±3.6
% Coral	16.8±2.7%	11.4±2.1%
% *Acropora palmata*	10.4±2.7%	3.3±1.7%
H'n	0.8882±0.0716	1.0115±0.0708
J'n	0.7412±0.0465	0.7247±0.0464
% Octocoral	10.4±4.2%	7.6±2.8%
% Sponges	3.0±0.9%	2.0±0.5%
% Zoanthids	1.1±0.4%	8.1±3.3%
% Macroalgae	36.1±6.0%	44.6±6.2%
% CCA	28.5±4.7%	24.2±6.9%
% Cyanobacteria	0.2±0.1%	3.4±1.0%
% SPR	4.1±1.0%	2.3±1.0%

TABLE 4.8. Summary results of one-way ANOVA analysis of coral reef community parameters between management regimes.

Parameter	D.F. (within, between)	F statistic	*P* value
Species richness	1,39	1.24	0.2724
Colony abundance	1,39	0.04	0.8363
% Coral	1,39	1.36	0.2501
% *Acropora palmata*	1,39	4.24	**0.0462**
H'n	1,39	1.36	0.2523
J'n	1,39	0.06	0.8127
% Octocoral	1,39	0.12	0.7262
% Sponges	1,39	0.09	0.7711
% Zoanthids	1,39	4.19	**0.0475**
% Macroalgae	1,39	0.92	0.3442
% CCA	1,39	0.49	0.4893
% Cyanobacteria	1,39	24.5	**<0.0001**
% SPR	1,39	2.06	0.1594

TABLE 4.9. Summary results of Pearson correlation analysis between selected coral reef benthic community parameters and fecal pollution indicators.

Parameter	r	*p*
Zoanthids vs. enterococci	0.9951	**0.0004**
Cyanobacteria vs. enterococci	0.9537	**0.0119**
Macroalgae vs. GB32	0.9384	**0.0182**
Macroalgae vs. HF183	0.8739	0.0527
SPR vs. HF183	-0.8519	0.0669
SPR vs. Macroalgae	-0.9224	0.0257

Relationship of coral reef community parameters and non-point source fecal pollution.

One of the most significant questions that emerge is whether human-associated non-point source fecal pollution is directly impacting coral reef communities. A Pearson correlation analysis revealed several significant correlation patterns (Table 4.9). Zoanthids and cyanobacteria were strongly correlated with enterococci counts (data from Appendix A-2), suggesting that zoanthids and cyanobacteria are dominant taxa under hypertrophic, polluted reef conditions. Macroalgal cover was significantly correlated to the frequency of *Bacteroides* GB32 molecular marker (data from Appendix A-3). *Bacteroides* is an anaerobic microorganism that grows in human and other warm-blooded animals gut. Therefore, its presence in environmental samples reveals a recent fecal pollution event. Further, macroalgae showed a strong but marginally significant relationship with *Bacteroides* molecular marker HF183. This probe is specific for human-derived fecal pollution. This suggests that inshore coral reefs that have undergone significant long-term phase shifts from coral towards macroalgal dominance are subjected to non-point source sewage pollution, mostly from human sources. Finally, SPR showed a strong but marginally significant negative correlation with HF183 molecular marker, as well as a significant negative correlation with macroalgae, suggesting that open reef substrates dominate at remote reefs located far from non-point source human sewage pollution.

Discussion.

This study has shown unequivocal evidence that coral reefs along a significant portion of the southwestern Puerto Rico shelf are being severely impacted by non-point source sewage pollution, mostly from human origin. The combination of historic natural factors (i.e., hurricanes), with long-term non-point source pollution pulses, and other potential anthropogenic reef degrading factors, such as sedimentation pulses and overfishing, have contributed to a dramatic phase shift in coral reef community structure. Phase shifts have favored dominance by macroalgae and non reef-building taxa. Such changes are generally irreversible at least in a human generation time scale (Knowlton, 1992; Hughes, 1994; Bellwood et al., 2004). Most often this is due to a phenomenon known as hysteresis, which is a phenomenon where a recovering community follows a different trajectory from that observed during the decline (Hughes et al., 2005). For instance, a declining reef previously dominated by massive corals might have shifted towards other community dominated by macroalgae or other non-reef building taxa. In the long-term, it may show some signs of recovery towards another alternate state such as dominance by octocorals. This is the case of Arrecife Fanduco, and most probably Punta Ostiones and Punta Arenas.

Declining coral reefs by sewage pollution along the western Puerto Rico shelf have resulted in significant declines of entire coral assemblages. This has resulted in a dramatic loss of functional redundancy (sensu Bellwood et al., 2003). The most dramatic example is the extirpation of Elkhorn coral from most inshore coral reefs. *Acropora palmata* constitutes a functional mono-specific group responsible of constructing an entire reef zone. Losing the *palmata* zone might have most probably resulted in a major biodiversity decline at the entire reef ecosystem level due to the net loss of nursery, shelter and feeding grounds of a myriad of fish and invertebrate species. Ludwig et al. (1993) referred to such irreversible trends as ratchets. Birkeland (2004) defined reef ratchets as "*self-reinforcing or positive feedback mechanisms that do not allow the process of resource degradation to cease or reverse, even when the original activities that set the trajectory toward deterioration are removed*". Based on Birkeland (2004) view, any anthropogenic factor, such as non-point source sewage pollution, in combination with overfishing or other conditions, may result in a severe coral decline below a critical threshold at which corals become susceptible to: (a) dilution of gametes at spawning (Allee effect); (b) disproportionate survival of coral predators; (c) algal abundance at levels that can perpetually swamp the abilities of grazers; (d) facilitation of bioerosion; (e) decreased topographic complexity; (f) decreased prevalence of CCA; and (g) alteration of habitats and food webs in ways that facilitate the establishment of invasive species. Such disparate ecological ratchets can act synergistically (Birkeland, 2004), significantly reducing coral reef ecosystem resilience, thus causing a stronger hysteresis in the system that may prevent recovery to a previous state (Hughes et al., 2005).

Another significant finding is that local MPAs had no significant impact on the status of coral reef benthic communities. Current management activities by the PR Department of Natural and Environmental Resources (DNER) at Tourmaline Natural Reserve, Cayo Ratones and Adjacent

Waters Natural Reserve, and at Finca Belvedere Natural Reserve marine extension are largely limited. There are no specific management actions implemented either by DNER or the PR Environmental Quality Board (EQB) to address the imminent negative impacts of non-point source sewage pollution in coral reef ecosystems. This study showed that coralline communities along the entire southwestern Puerto Rico coast are being constantly impacted by sewage pollution. Even offshore remote reefs are being often impacted by pollution pulses from the Guanajibo River plume, and during heavy sediment resuspension associated to variable oceanographic dynamics (addressed at Appendix A-1). According to ISRS, this could have paramount long-term negative impacts in reef communities by: (a) reducing coral larval production; (b) reducing coral recruitment; (c) increasing incidence of coral disease; (d) reducing coral skeletal density; (e) increasing coral mortality; (f) reducing coral species diversity; (g) producing a community phase shift; (h) enhancing bioerosion; (i) enhancing macroalgal growth and biomass; and (j) and by probably enhancing coral predators abundance.

It is probable that most inshore coral reefs along the southwestern Puerto Rico shelf have degraded beyond recovery within a human time scale. Further, water quality degradation is of such magnitude that recovery may never occur. Stronger efforts are needed from the government of Puerto Rico to prevent further degradation of remote reefs through the region. There is an immediate need to implement a sound management strategy to reduce and/or prevent non-point source sewage pollution impacts in coral reef habitats before we witness a reef ecological and socio-economic collapse within the next few years.

Conclusions.

Coral reefs have shown a relatively high stability through the recent geological time scale (220,000 years), but human activities have caused sudden dramatic habitat degradation and possibly irreversible changes in community structure (Pandolfi and Jackson, 2006). Factors such as non-point source sewage pollution have contributed to a major coral reef benthic community phase shift along the western Puerto Rico shelf that has favored dominance by macroalgae and non reef-building taxa, and altering ecosystem functions.

Controlling and managing pollution will require a definite strong political will and commitment from the government of Puerto Rico aimed at establishing stringent controls over land use patterns and over land-based pollution. Stringent pollution controls should result in increased water quality and long-term benefits of reduced algal competition with coral, and possibly reduced incidence of coral diseases/syndromes (Pandolfi et al., 2005). It will also require a moratorium on coastal development in proximity to coral reefs and associated ecosystems (i.e., seagrasses, mangroves). It is also very important to raise awareness how land-based activities and non-point source sewage pollution can negatively impact adjacent marine environments.

There is also a need to design and implement a national policy of integrated and sustainable coastal and watershed management. Any management activity should involve local stakeholders by means of participatory or co-management approaches. Further, there is a need to rehabilitate, restore and protect riparian and coastal vegetation, wetlands, and other areas of the watersheds that actively filter out suspended sediments, nutrients, and other types of pollution (ISRS, 2006).

There is a need to quantify the economic value of environmental goods and services, as well as to document the economic impacts of coral reef degradation due to non-point source sewage pollution. Finally, specific integrated monitoring programs should be designed to address hypothesis-driven questions regarding the long-term impact of sewage pollution, characterizing water and sediment quality, implementing microbial source tracking methods to address sources of fecal pollution, and quantifying environmental impacts on coral reefs. These approaches can be adapted to experimentally test concrete management actions to reduce and/or eliminate sources of sewage pollution. This would be the only way to determine and quantify how effective are management measures. Failing to recognize and manage non-point source sewage pollution impacts on coral reefs may result in a further loss of extensive remote reef systems along the western Puerto Rico shelf that are already showing the early signs of degradation.

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Appendix B

An evaluation of Guanajibo River suspended sediment distribution in Mayaguez and Cabo Rojo nearshore waters using Remote Sensing techniques (1999, 2001, 2003 and 2004)

Maritza Barreto-Orta, Ph.D

Introduction

Terrigenous sediments are important sources to feed sedimentological components as beaches, cliffs, alluvial plains in coastal systems. These materials are coming from erosion and weathering products that are transported from upland sites to coastlines areas by river discharge and runoff. Terrigenous sediments were also transported to the insular shelf by longshore currents and waves. Longshore sediment transport direction will be defined by the shelf morphology and local oceanographic conditions. Suspended sediments can be transported to deeper waters by submarine canyons producing loss of sediments in the insular platform.

A river plume is defined sometimes in nearshore waters. The river plume is produced when less dense fresh water underlying denser seawater. Suspended sediment and river plume distributions are affected by magnitude of river discharge, precipitation, channel profile, occurrence and magnitude of tropical storm systems, cold fronts, and topographic profile, among others. Man-made activities as deforestation, slope modification, constructions, presence of quarries, constructions of dams are also important variables that can cause changes in sediment transport in a geographic zone.

This study evaluates qualitatively the behavior of the Guanajibo River plume in the nearshore area for a specific date in 1999, 2001, 2003 and 2004. The main goal is to identify the distribution and direction of river sediment plumes in nearshore areas at Mayaguez-Cabo Rojo region.

Study Area

The study area includes the coastal and nearshore area of Mayagüez and Cabo Rojo, located in the west coast of Puerto Rico. The relative geographic location is from Guanajibo River mouth at Punta Guanajibo, Mayagüez, to Puerto Real at Cabo Rojo. The study area includes coastal and nearshore sites of Guanajibo River at Mayagüez, Joyudas, Belvedere and Puerto Real Bay. Evaluation site is included the insular platform up to 6 miles from the shoreline.

Figure 1. Joyudas Coastline (Photo by Jack Morelock)

Figure 2. Puerto Real Coastline (Photo by Jack Morelock)

Guanajibo River, Mayagüez

Guanajibo River has drainage basin area of $311 km^2$ and it does not have dams. (Earne, To, G., Webb, R. M. and Larsen, M. C., 2005). The river and sediments discharge is demarcated by seasonal patterns (Miller, R. L. and ET al., 1994).

Figure 3. River sediment plume at Guanajibo River (Photo by Morelock)

Major river discharge was identified during the passage of tropical storms in the region. As an example, major river discharge was observed in September 22 of 1998 during the passage of Hurricane Georges. Average river discharge of the Guanajibo River during the passage of storm systems was 991 m^3/s. This discharge is greater than Culebrinas (481 m^3/s) River, Río Grande de Arecibo (419 m^3/s), Portugues River (158 m^3/s), Jacaguas River (242 m^3/s), Fajardo River (249 m^3/s), Cibuco River (414 m^3/s), among others, for the same period.

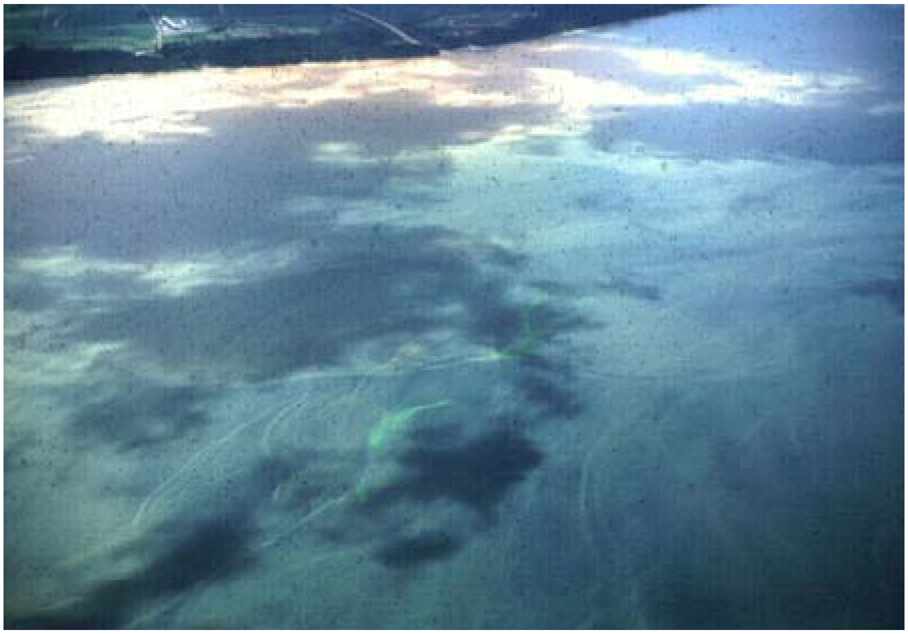


Figure 4. Suspended sediment and re-suspension at Guanajibo River (Reference: Status of Coral reef in P.R-Jack Morelock)

Major land uses found in the Guanajibo River drainage basin are agricultural (cane of sugar, coffee), grassland and forests (Thousand, R. L., and ET al., 1994). Major agriculture areas as coffee areas are confined to the areas located in mountains (Cruise, J. F. and ET al., 1994).

The U.S. Geological Survey has 3 sampling stations in the drainage basin of the Guanajibo River. These are San Germán (131990) station, Rosario River (50136400) station and in Guanajibo River main channel (50138000). The Rosario and Guanajibo River station collects information of river discharge and suspended sediments.

The main channel Guanajibo river station is located in highway 100, in the low part of the Rosario River. Lower river discharge was occurred during February and March period. Major river discharge was identified during September to November. River discharge showed a seasonal distribution apparently related to the distribution of the precipitation of the region. Based on USGS data major

Guanajibo river discharge during the studies period was occurred in 1999, following 2001 and 2003 periods.

Figure 5. Location of Guanajibo River channel river station (USGS, water Resources data of Puerto Rico and US Virgin Island)

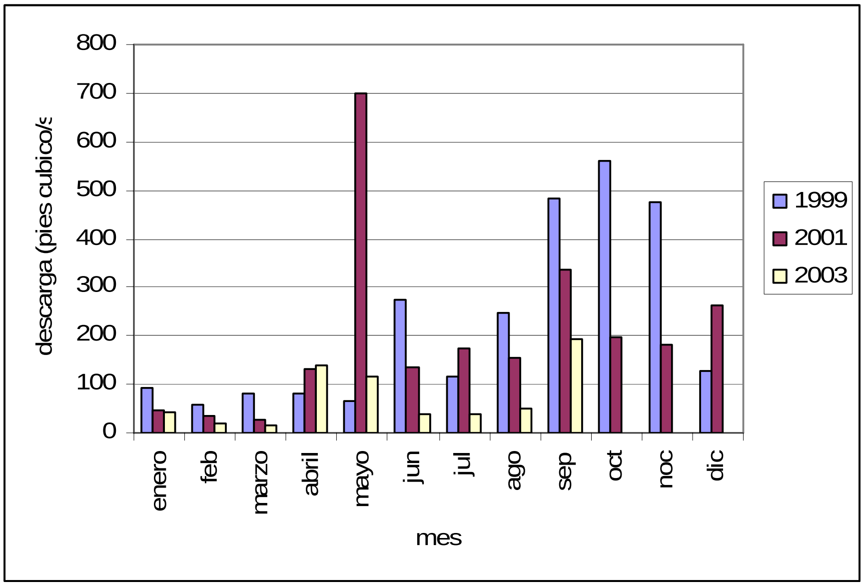


Figure 6. . Guanajibo River channel river station data (USGS, water Resources data of Puerto Rico and US Virgin Island, 1999, 2001 and 2003)

Rosario River is the major tributary of Guanajibo River drainage basin. The Rosario station is located 12.9 km upward from the connection with Guanajibo River. This tributary is located mainly in the mountainous zone that contains slopes ranging from 36% and 56%.

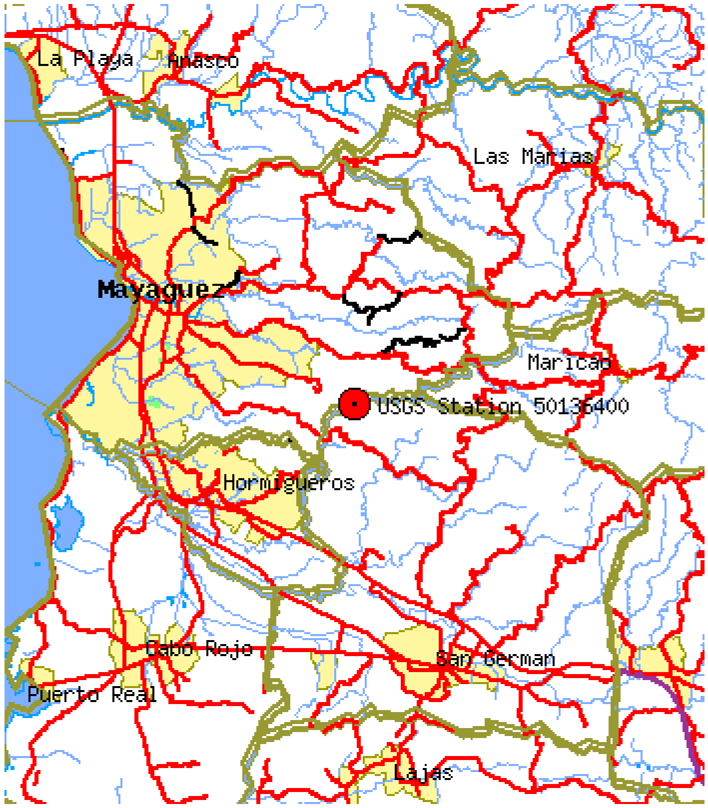


Figure 7. . Location of Rosario river station (USGS, water Resources data of Puerto Rico and US Virgin Island)

Lower river discharge at Rosario occurred for the period between February and March. Major river discharge was occurred during September and November. USGS data includes for the study period indicated that major river discharge was occurred in 1999. The major sediment discharge in Rosario River station is observed in May, June, August and September.

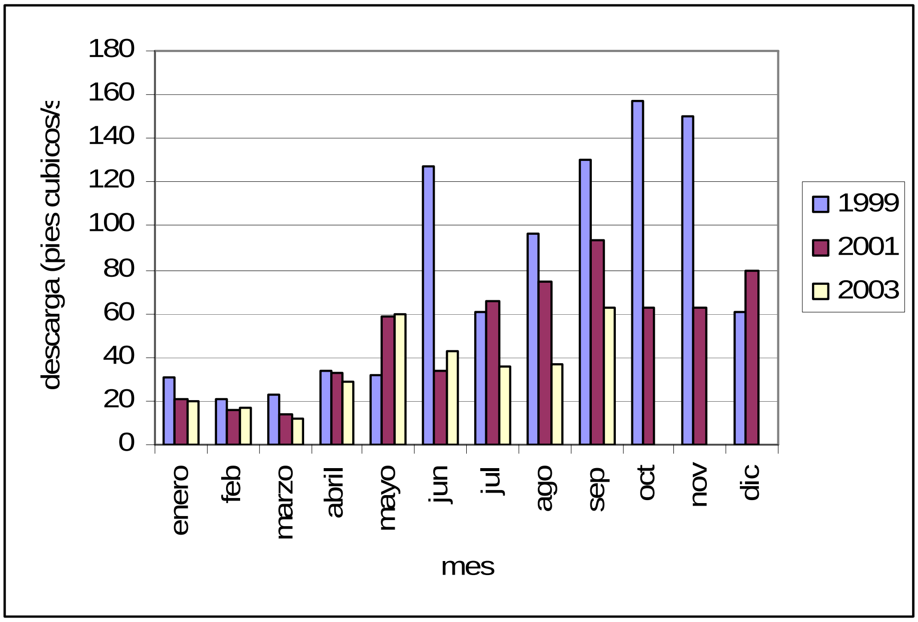


Figure 8. Rosario River station Data (USGS, water Resources data of Puerto Rico and US Virgin Island)

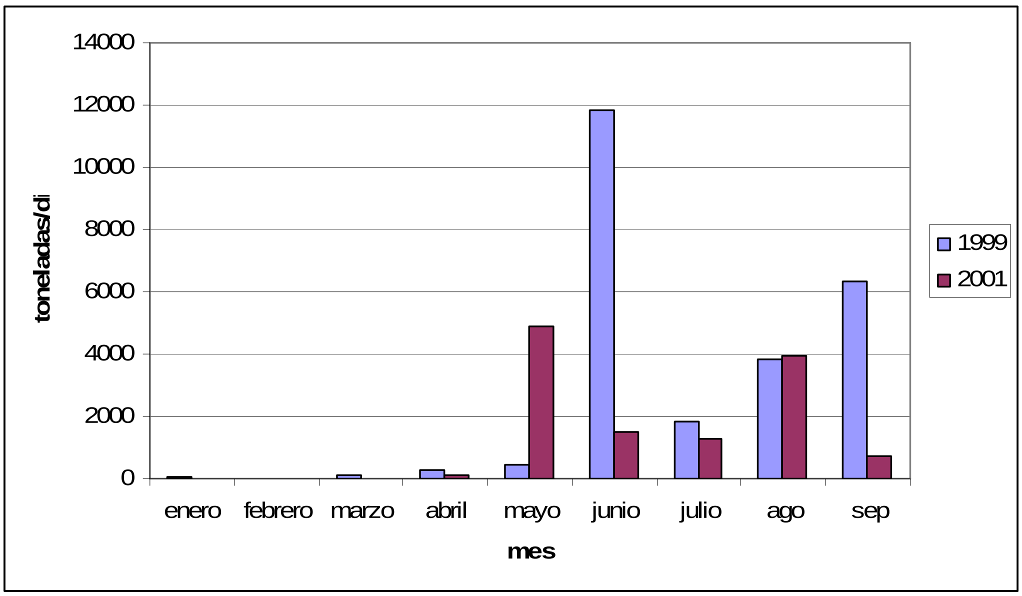


Figure 9. .Rosario river station, sediment discharge data (USGS, water Resources data of Puerto Rico and US Virgin Island)

Joyudas is located from south Punta Guanajibo to north Belvedere at Cabo Rojo. This area showed narrow beach sites and beachrock shoreline types. Joyuda do not showed river system in the area. Hotels and restaurant were found along Joyudas shoreline.

Belvedere is located south Joyuda town. Puerto Real is located southward Belvedere. The area includes a lunate bay between carbonate outcrops. No river system is found in the area.

PREVIOUS STUDIES

Several studies were done evaluated suspended sediment, river sediment plume morphology and nearshore characteristics using remote sensing techniques in the last decades. Major studies were done using multispectral images as Landsat Thematic Mapper (TM), Landsat Enhancement Thematic Mapper (ETM), Seawiff, SPOT, CAMS, IKONOS, among others. Color Ortophotos were also used to identified river sediment plumes and nearshore components as coral reef, mangroves and seagrass beds.

There are several studies made focused in the identification and characterization of suspended terrigenous sediments in coastal and nearshore waters using remote sensing techniques. These studies include the characterization of the length and morphology of river sediment plumes. Suspended sediment studies were mainly done using multiespectral images as Landsat TM and ETM, Seawiff, using spectral bands that ranges between 550 to 690 nanometers (visible bands green and red) (Doxaran and et al., 2003).

Hochberg and et al. (2003) uses high spatial resolution IKONOS images to improve bottom mapping in near shore environments at Lee Stocking Island, Bahamas. In this study he reveal an improvement of user's accuracies for critical benthic habitat classes such as coral-dominated habitat , dense seagrass beds after specular reflectance elimination. Contrast of subsurface features was enhanced, sharpened between sediments and coral-algal framework.

Houholis (2001) uses Landsat Thematic Mapper (TM) and Landsat 7 Enhanced Thematic Mapper (ETM) to identified coastal habitat at Charlotte Harbor along the Coast of Florida south of Tampa. In this study Houholis indicated that TM and ETM have not be used as extensively for coastal zone applications due to its spatial resolution (30 meters), but also due to the difficulty of imaging aquatic environments (low reflectance ranges, atmospheric effect, etc). In this study she applied masking procedure to improve mangrove and other coastal features discriminations.

Previous Work at Puerto Rico

Few works were published related with identification of suspended sediment, assessment of river plume length and morphology through time, and nearshore characterization using remote sensing in Puerto Rico.

Various studies were evaluating water quality and suspended sediment distribution in the west nearshore areas of Puerto Rico. Cruise, J. F. and Miller, R. L. (1994) made a study on the quality of water integrating Remote sensing tools, hydrologic models and field work (water sampling, etc.) in the Mayagüez Bay. In this study, they identified suspended sediment along nearshore areas and land use changes. Land use changes and suspended sediments discrimination were done using CAMS images and Landsat Thematic Mapper (MT) with 30 meters of space resolution. General results showed that suspended sediments of the Guanajibo River are transported to reefs located in the external platform of the Mayagüez Bay zone.

Miller, R. L. and et al., (1994) monitoring water quality in Mayagüez Bay integrating remote sensing tools and field measures. They conducted field measures as radiances reflectance, chlorophyll pigments, temperature and salinity. These data were related with images taken at the same period. Data showed that Mayagüez Bay waters are oligotrophics with maximum chlorophyll a concentrations . Maximum values are associated with river discharge particularly with Yaguez River. The chlorophyll concentration declines with values of water clarity <0.25 μ/l. The data collected in the analysis of spectral images demonstrate that the river sediment plum is narrow, approximately of 100 to 300 meters of the coast.

Cruise and Miller (1994) also indicate that the greater sediment discharge of the Guanajibo River in Mayagüez occurred in during the presence of storm systems in the area. They indicate that for 1989 sediment plumes was extended 12 kilometers seaward from the coast .

Warne, A. G., R. M. and Larsen M. C. (2005) evaluated water characteristics, sediments and discharge of nutrients in the rivers of Puerto Rico and their potential influence in coral reef areas. In this study, they evaluated the relation between precipitation, water discharge and sediments of main rivers of the Island of Puerto Rico. General results showed that major suspended sediment transport by river occur during storm systems periods in the area. Major suspended sediments were found during high major precipitation events.

METHODOLOGY

Remote sensing technique is used as main tool to identify suspended sediment and river sediment plume in nearshore areas at Mayaguez-Cabo Rojo Region

River sediment plume definition

River sediment plume can be easily discriminate using remote sensing technique Suspended sediment can be easily defined in spectral ranges between 560 a 670 nanometers. Sediment plumes were easily separated from clear water characteristics.

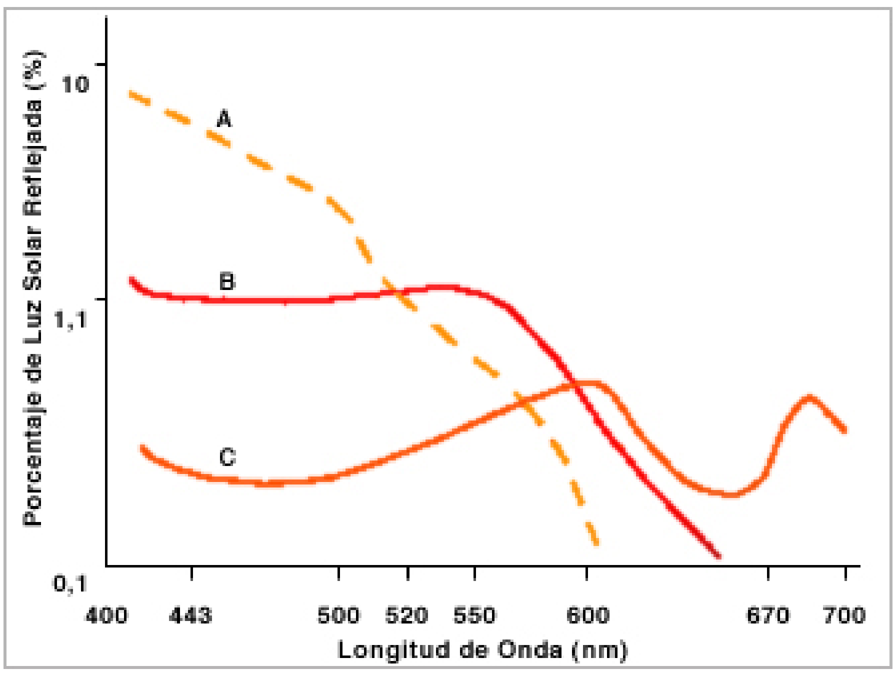


Figura 10. Suspended sediment and clear water spectral definition (Reference.http://www.uc.cl/sw_educ/geo_mar?html/h541.html.

River sediment plumes morphology and distribution can easily identified using multispectral images in visible range through time. River plume morphology and distribution changes can be cause by wave, longshore currents, wind regime, and availability of sediments. Studies in changes in river plume morphology can helps to identified plume direction and extension.

Images

In this study we used color ortophotos and multispetral images. Ortophotos were collected by the National and Oceanic and Atmospheric Administration (NOAA, 1999) and USDA Forest Service (2004). Landsat Enhancement Thematic Mapper (ETM 2001 and 2003) were the multispectral images used in this study.

These images have different spatial resolution that ranges between 2 to 30 meters.

Atmospheric differences among images were found due to variability in solar azimuth and period of time when were taken the image. For instance, an qualitative evaluation of suspended sediment distribution was done for each individual image for period.

Color Ortophotos (NOAA, 1999)

Color Ortophotos were taken during February and March 1999 with spatial resolution from 1 to 9 meters. These images have a spectral resolution of three visible spectral bands. These are band blue, green and red.

Figure 11.Color ortophoto, NOAA, 1999, band 1,2,3

Landsat Enhancement Thematic Mapper

LANDSAT Enhancement Thematic Mapper image (ETM) has flown on the Landsat 7 satellite from 1999 to present (responsible Center NASA Goddard Space Flight Center). This provides repetitive multispectral, high resolution digital imagery from the earth.

This sensor includes 8 spectral bands that cover the visible, near infrared, shortwave and thermal infrared spectral bands of the electromagnetic spectrum in 30 meters spatial resolution. Panchromatic band is defined for 15 meters spatial resolution. Thermal band has a 60 meter spatial resolution. Overall radiometric accuracy is to 5 percent (Earth Observing System, 2005). The ETM will provide data are sufficiently consistent in terms of geometry, spatial resolution, spectral characteristics and calibration from previous LANDSAT data to meet requirements for global change research (The earth observing system).

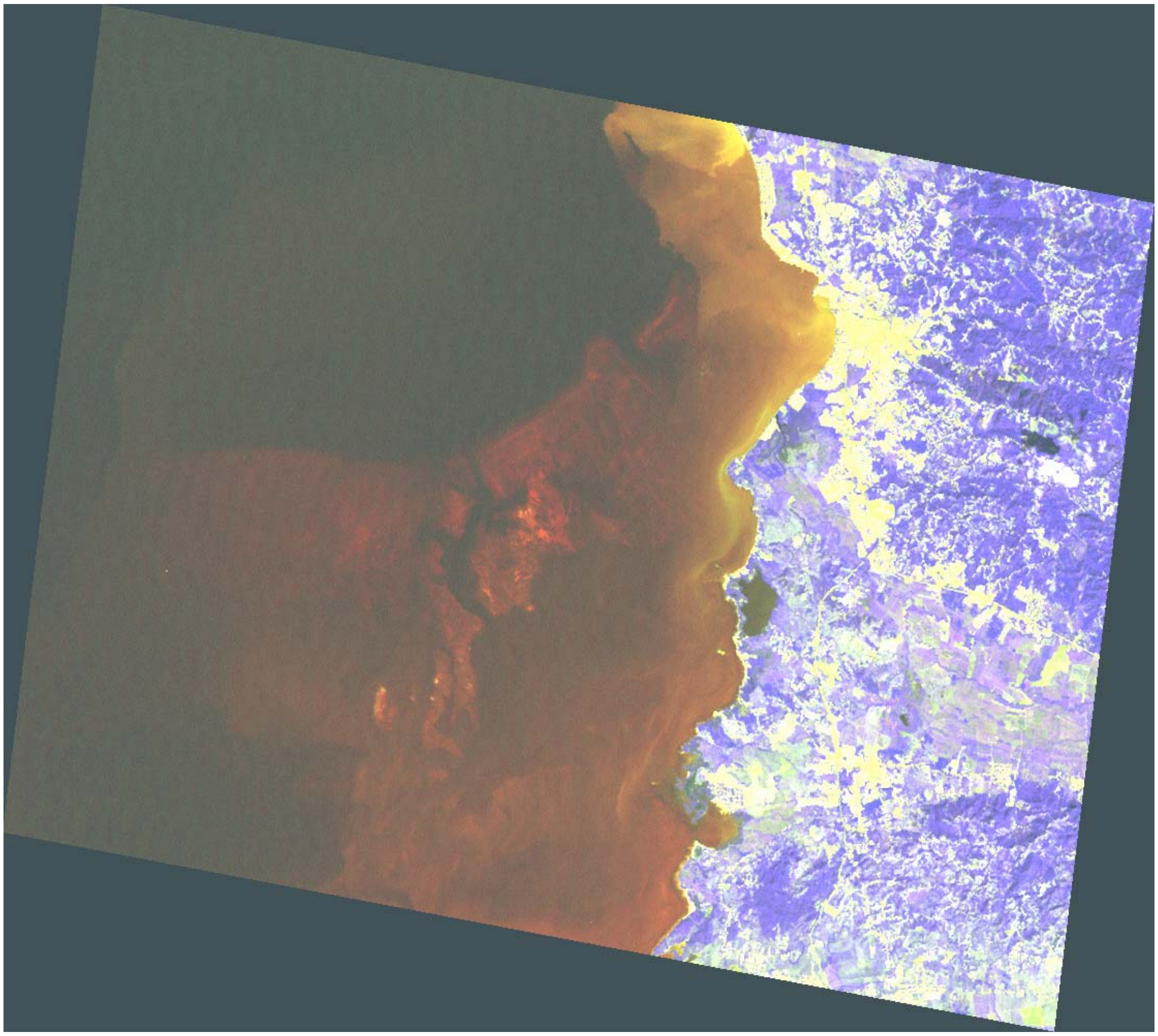


Figure 12 Landsat ETM Image, 2001, band 3,3,2

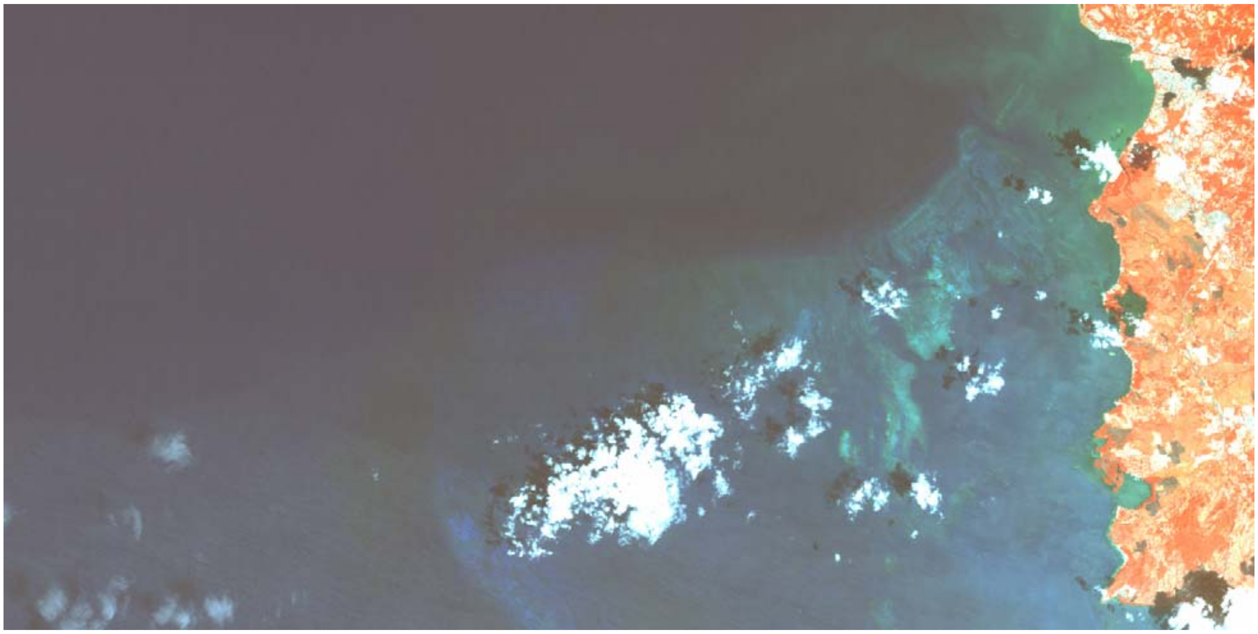


Figure 13 Landsat ETM Image, 2003, band 2,3,4

Table 1. **Spectral Resolution of LANDSAT Enhancement Thematic Mapper (ETM)**

Band	Spectral Range		Spatial Resolution
1	0.45 to 0.52	Blue	30
2	0.53 to 0.61	Green	30
3	0.63 to 0.69	Red	30
4	0.78 to 0.90	Near infrared	30
5	1.55 to 1.75	Infrared	30
6	10.4 to 12.5	Infrared	60
7	2.09 to 2.35	Infrared	30
8	0.52 to 0.90	PAN	15

Band combinations used in this study were: Band 1 (blue), band 2 (green), bands 3 (red) and band 4 (near infrared). Band 4 was used mainly to separate sea and land environments. Band 1 and 2 were used mainly to discriminate shallow water environment. Band 3 was used to discriminate suspended sediments.

Color ortophotos USDA (2004)

Color ortophotos were obtained from USDA. These images were collected by Leica ADS40 sensor. The images were defined in a 3.75 minutes of latitude and longitude grid with 8 bit of resolution for October 2004. Spatial resolution is 1 meter. Images projection are UTM, 19 zone NAD 83. The image has three spectral band in the visible spectrum (blue, green and red).

Figure 14. Color Ortophoto, USDA, 2004.

Remote Sensing Software

Imaging Software from ERDAS Company was used as remote sensing software to conduct multispectral image analysis. This software include 6 main modules that can manipulate and analyze different values of digital values include in the image. These digital values correspond to the reflected radiance obtained from the objects. View, Importing/exporting, data processing and classification modules were used in this study.

Image Preprocessing

Rectification, mosaic preparation and images edition were done as a image preprocessing. Color ortophoto obtained from USDA (2004) was used as image base for rectified all images used in this study. This image is projected in UTM, 19 zone NAD 83. Rectification processing was done using polynomial algorithm included in the viewer module of ERDAS imaging software version 7.4.

Various images mosaics were done to define the study areas. Mosaic procedure was applied to color ortophotos obtained by NOAA (1999) and USDA (2004) using ERDAS Imaging software.

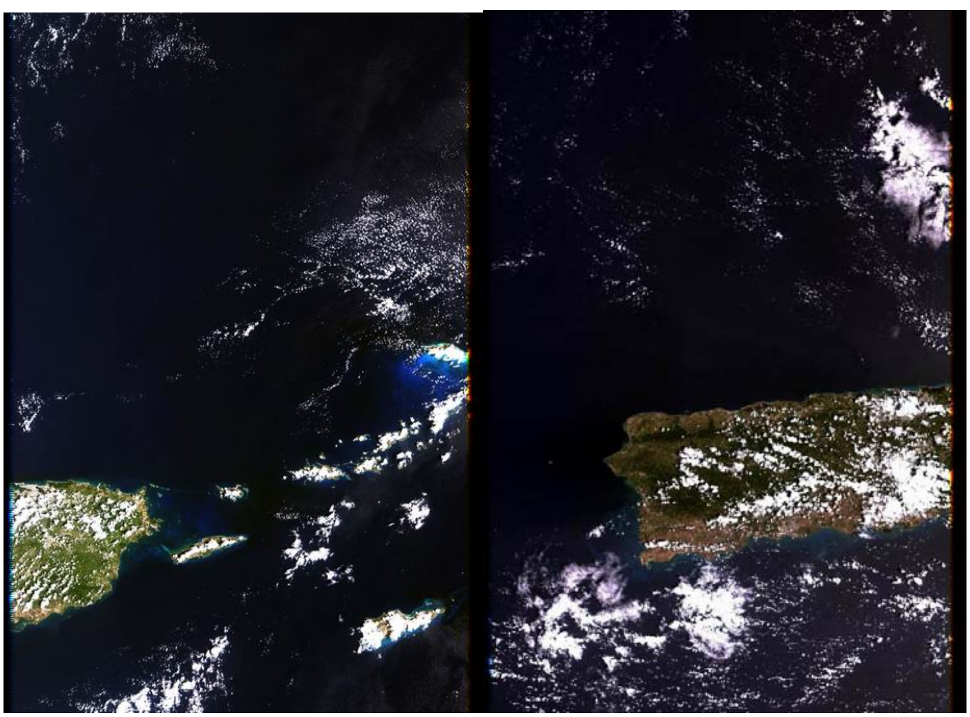


Figure 15. Landsat ETM (obtained from Electrical Engineering Department, RUM-UPR, 2003)

Image Processing

Image data are used to define thematic files through multispectral classification. (ERDAS, Field Guide, 1999). A unsupervised classification procedure was used to identify suspended sediments and river plumes in this study.

A unsupervised classification algorithm defines classes or categories based on digital spectral values of the features. Minimum distance algorithm was used as a base classification model. A classification map was done using these classifications.

RESULTS

Qualitative evaluation of ETM multispectral images and ortophotos identified the presence of the river plume of Guanajibo River in nearshore areas of Mayaguez for 1999, 2001, 2003 and 2004 period. Suspended sediments also were observed at Puerto Real Bay and nearshore areas for all periods.

Three major spectral categories were identified in this study. These are: 1) high digital values (high reflectance) category; 2) moderate digital values (moderate reflectance) category; and 3) lower digital values (reflectance values) category.

High digital value category (red polygon) includes digital values that range from 100 to 150 (based on 8 bit image format). This category includes areas that showed higher concentration of suspended sediment. It is possible that sediment includes in this category showed major grain diameter than other categories identified in this study. This category also includes barren land located inland.

Moderate digital value category (dark brown polygon) could be related with areas with less suspended sediment concentration with finer sediments.

Lower digital values category (lighter brown and yellow polygons) could be related with less suspended sediment concentration with/ or finer sediment grain size. It is possible that this category also includes other type of suspended solid or particles.

These categories includes objects characterization using spectral bands between 530 to 690 nanometers (visible spectrum-green and red) that identified suspended material in water column. Suspended sediments were mainly identified in the 630 to 690 nanometers spectrum range.

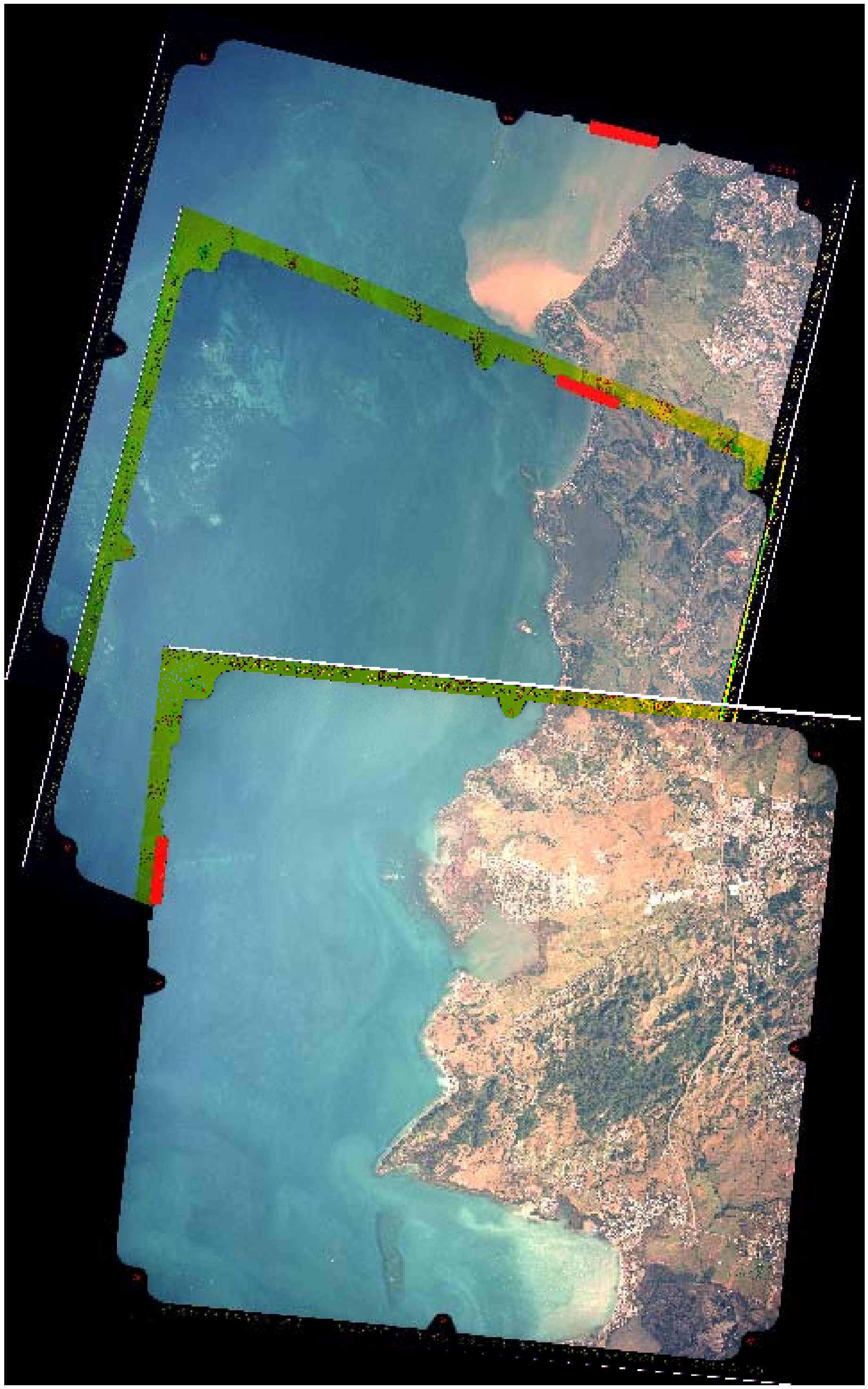
Identification of Guanajibo River plume

Guanajibo River plume showed differences shapes and lengths in each image where it was identified in this study. Changes in shape and length could be related with date of image acquisition, seasonal variations in precipitation and river discharge, availability of sediments, land use changes, among others.

River plume, NOAA, 1999

Guanajibo River showed a very well defined sediment plume in the ortophoto collected by NOAA in 1999. River sediment plume showed a west drift with south east component. Longshore river plume component is observed from Punta Guanajibo to Joyudas. But major river plume drift is toward west. It is also observed a river plume toward Belvedere. River plume was not observed near to Puerto Real for this period. It is possible that part of Guanajibo River suspended sediment can be transported toward Guanajibo River and Yaguez River submarine canyon located in the insular shelf.

Figure 16. Ortophoto collected by NOAA, 1999



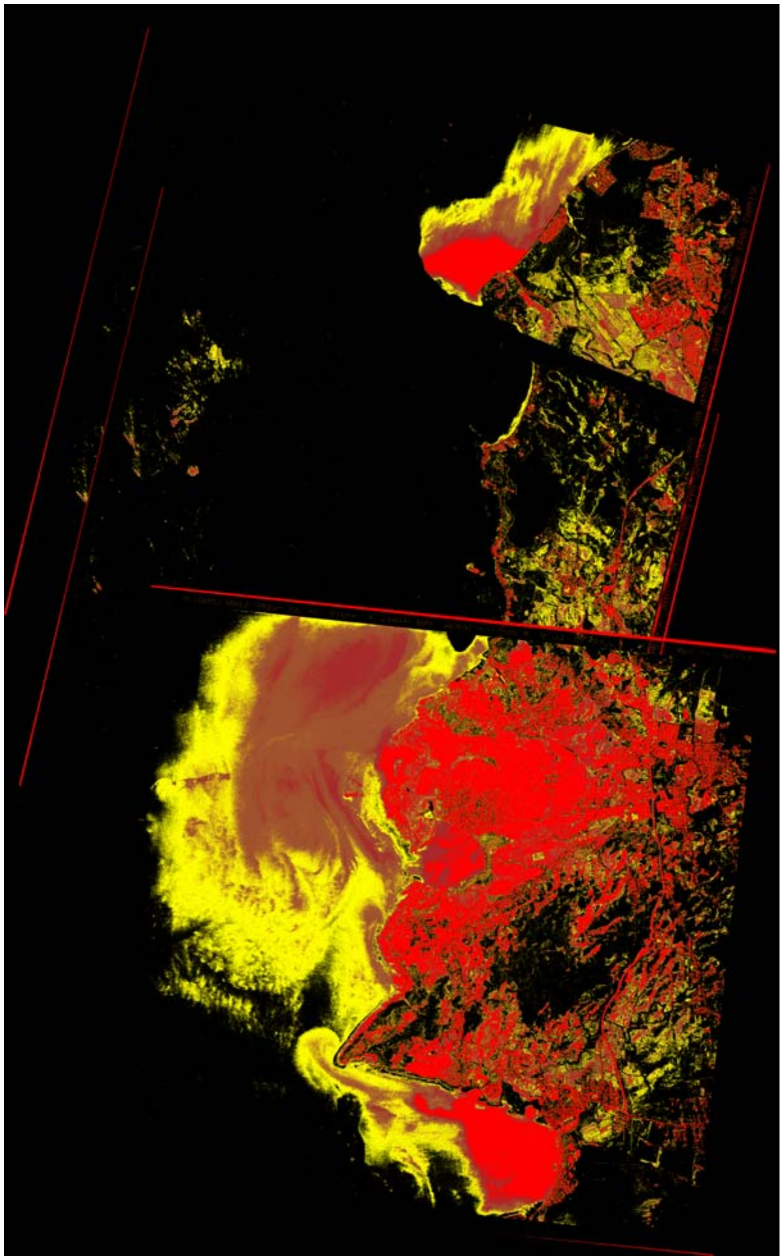


Figure 17. Unsupervised classification, ortophoto NOAA, 1999. Guanajibo River plume, ortophoto collected by NOAA, bands 3,3,and 2.

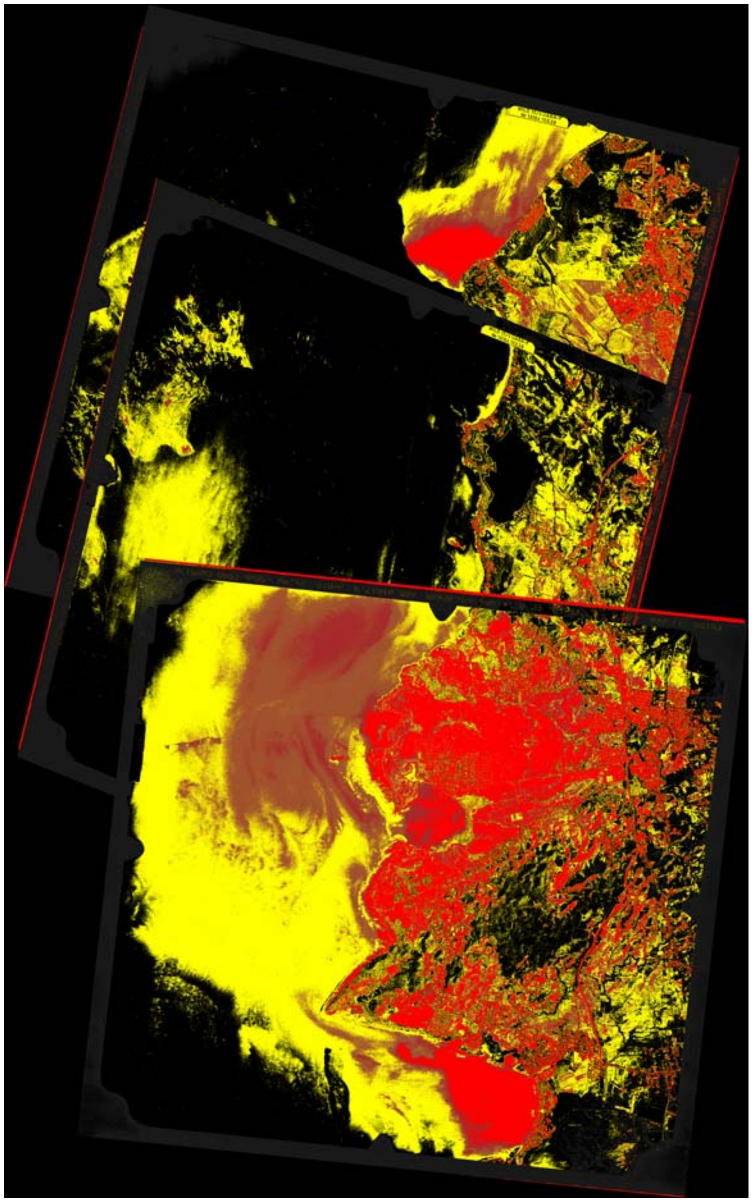


Figure 18. Unsupervised Classification, ortophoto NOAA 3,2,1

Evaluation of spectral band combination 3 (red),2 (green),1 (blue) showed a similar distribution of river plume as band combination 3 (red),3 (red),2 (green).

River plume, Landsat ETM 2001

Añasco, Yaguez and Guanajibo Rivers plumes were identified using Landsat ETM (2001) with band combination between 560 to 690 nanometers. Guanajibo River plume showed a higher concentration of suspended sediment than Añasco and Yaguez River for this period. Guanajibo River plume is transported toward south. A well defined river plume was identified from Guanajibo River mouth to Joyudas nearshore area. A minor and discontinuos longshore river plume transport was observed from Joyudas to Belvedere in this period.

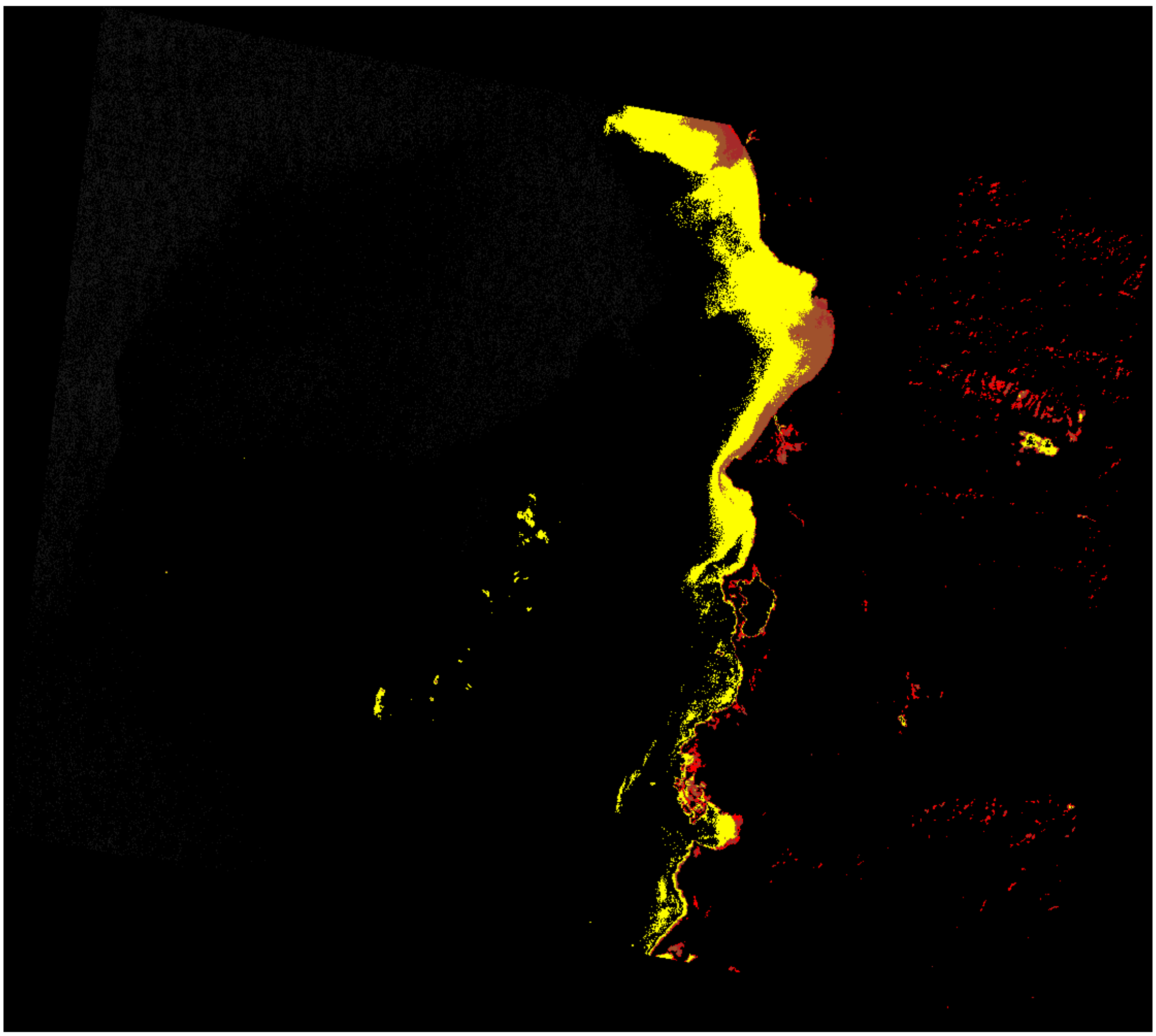


Figure 19. Unsupervised Classification, Guanajibo River plume, Landsat ETM, 2001

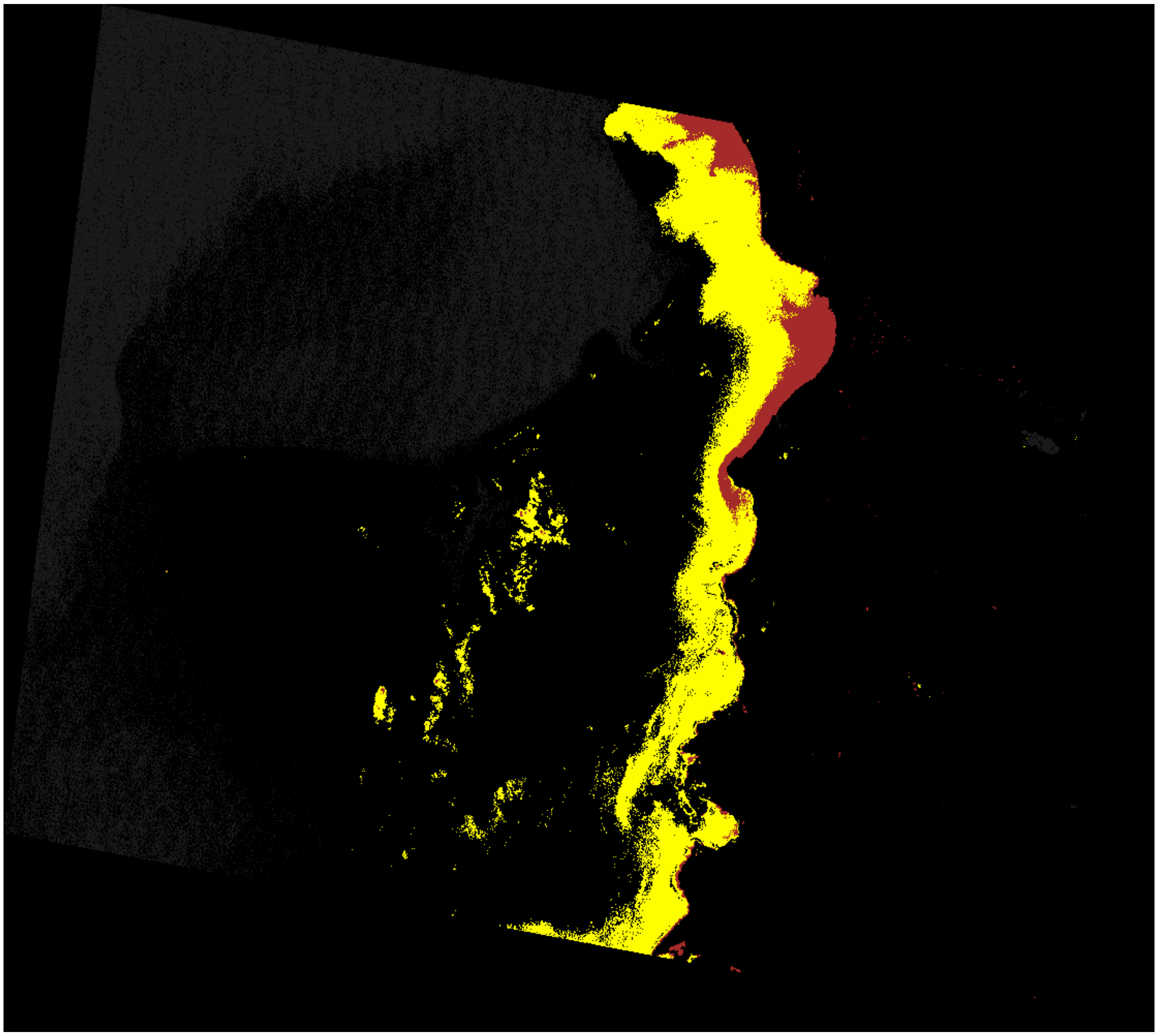
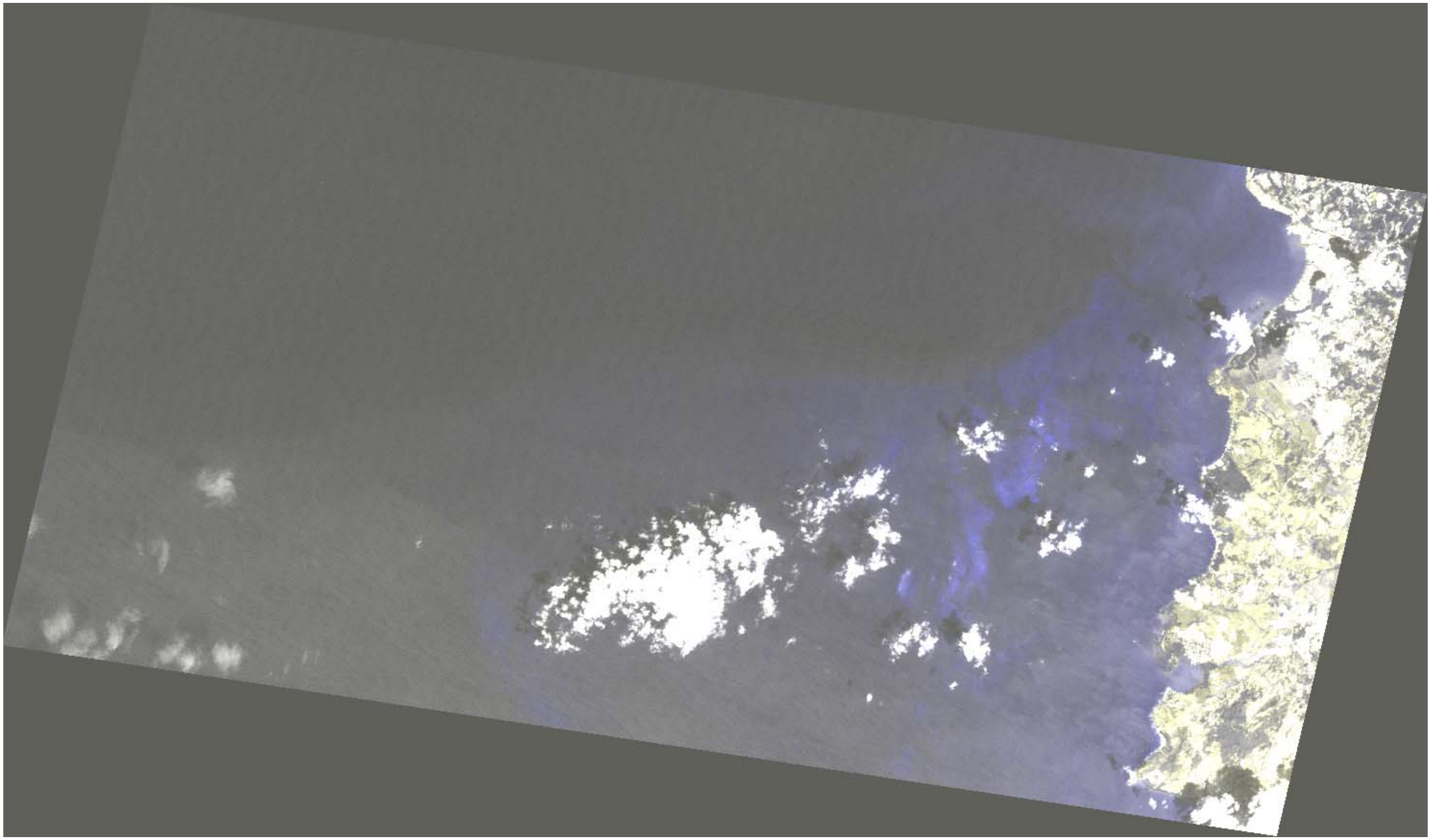


Figure 20. Unsupervised classification, Guanajibo River plume, ETM 2001, bands 1,2,3

River plume, Landsat ETM 2003

Yaguez, Caño Corazones and Guanajibo Rivers plumes were observed in Landsat ETM images for 2003 period. Caño Corazones River plume was transported toward south (toward Guanajibo River mouth). Guanajibo River mouth can not identified in this image due to cloud cover. But, Guanajibo River sediment plume was identified from southward Punta Guanajibo. A narrow suspended sediment longshore transport was identified from Punta Guanajibo to Joyudas. Guanajibo River plume was not observed alongshore at Belvedere for this period.

Figure 21. Landsat ETM image, 2001.



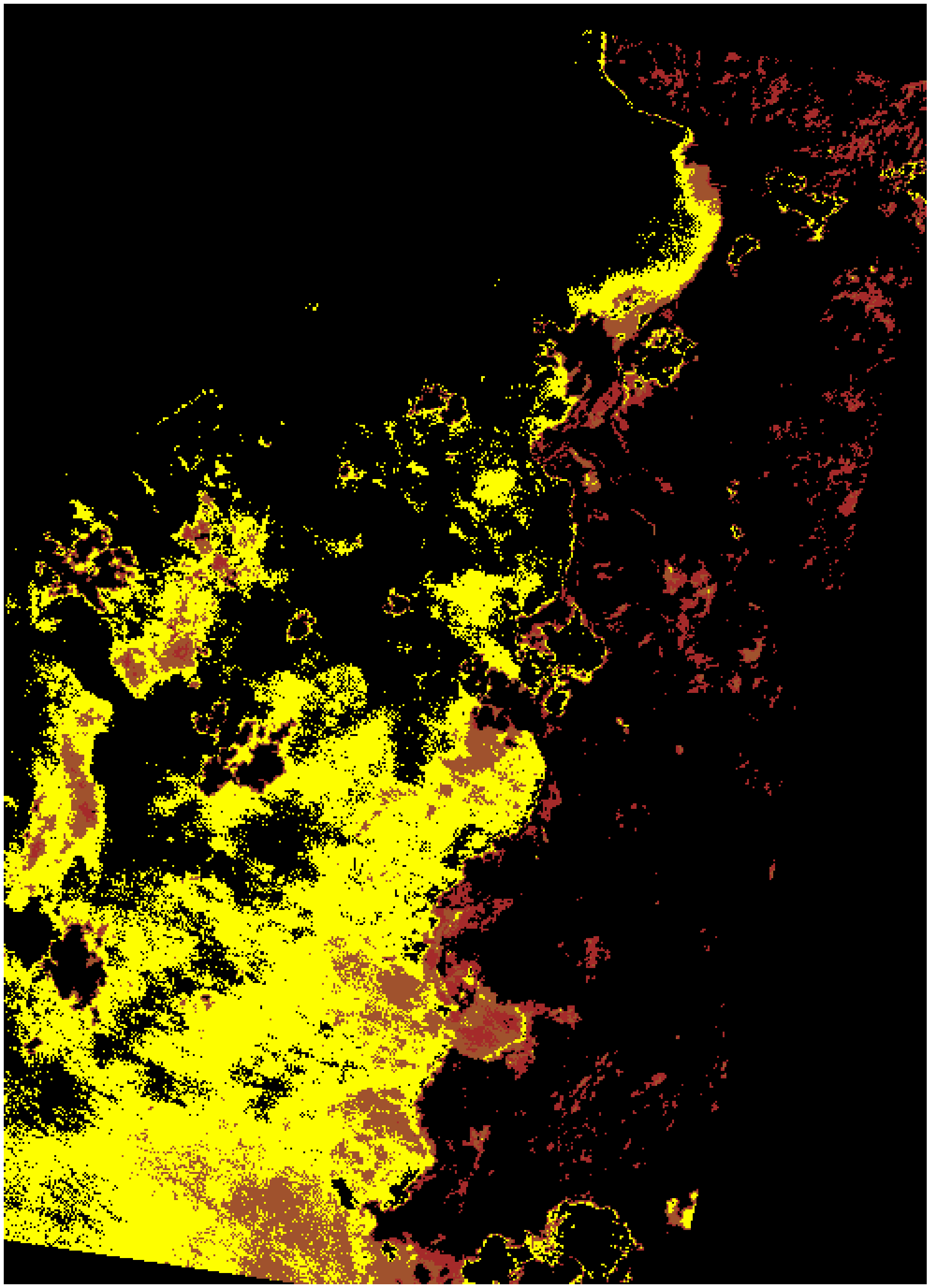


Figure 22. Unsupervised classification, Landsat ETM 2003, bands 3,3,2

River plume, Ortophotos collected by USDA, 2004

Guanajibo River plume was identified in the ortophoto collected by NOAA in 2004. River plume showed a transport toward west with a southward component. A longshore suspended sediment transport was identified from Guanajibo river mouth to Belvedere, Cabo Rojo. In this period, Guanajibo River plume showed the larger transport for the study period. This transport covers from Guanajibo River mouth to Belvedere, Cabo Rojo.

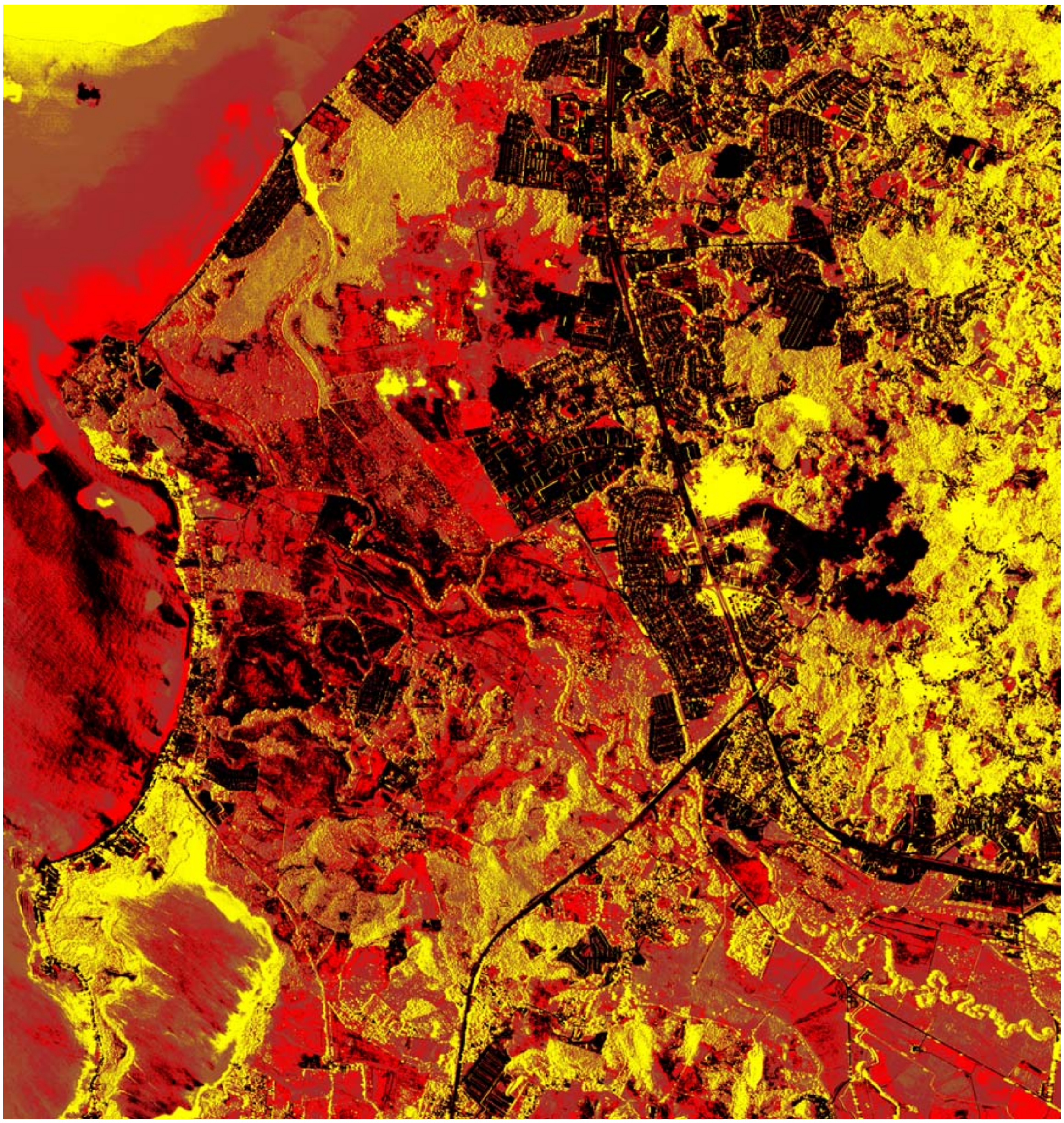
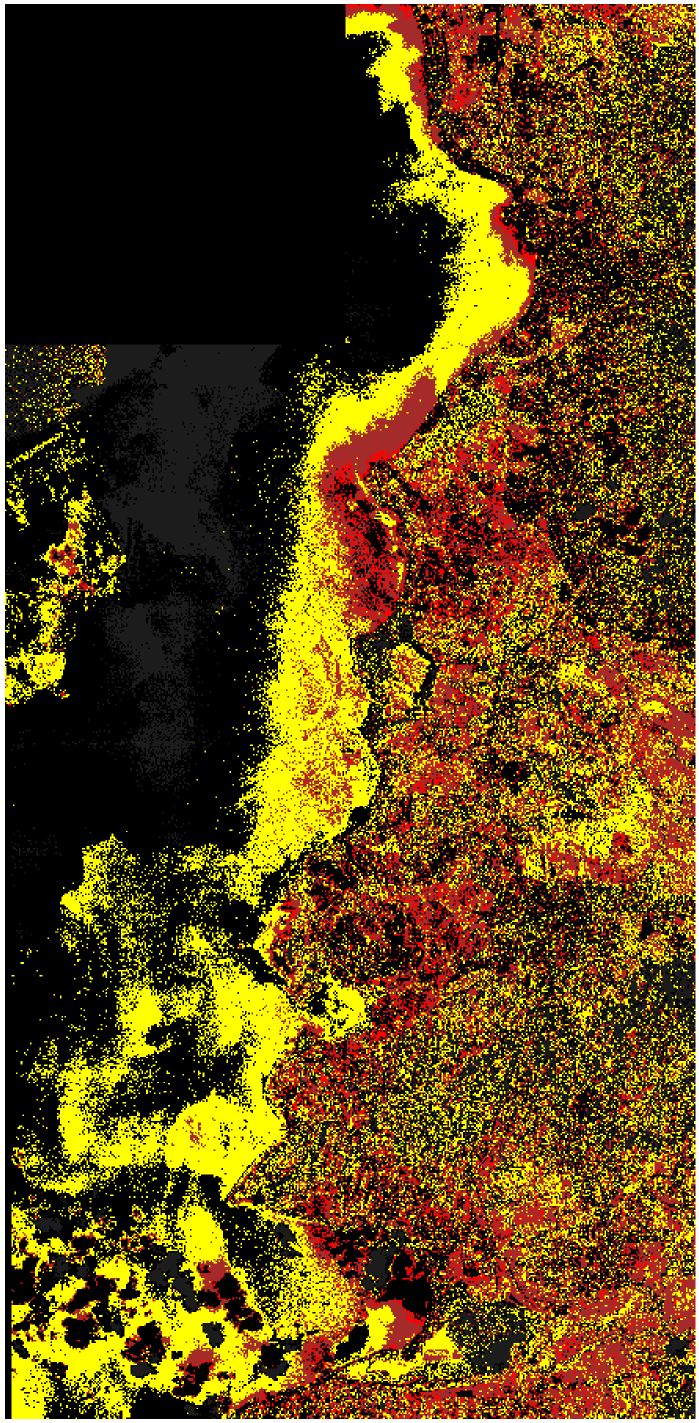


Figure 23. Unsupervised classification, Guanajibo, Mayaguez, USDA, 2004

Figure 24. Ortophoto, USDA, 2004

Figure 25. Unsupervised classification, Mosaic, Images Mayaguez and Cabo Rojo, USDA, 2004.



Identification of suspended sediment at Puerto Real Bay, Cabo Rojo

A suspended sediment concentration was observed in Puerto Real Bay in all images includes in this study. Suspended sediment identified was not related with Guanajibo River suspended sediment source. These sediments were apparently related with erosion and weathering material transported from Puerto Real inland areas. It is possible that this material was removed from areas that were modified by man-made activities as changes in land use and constructions.

Major sediment suspension concentration was identified for 2003 and 2004 period in Puerto Real Bay and nearshore waters. Increase in suspended sediment can be related with increase in inland alteration as changes in land use and constructions.

CONCLUSIONS

1. Guanajibo River plume was identified in all images includes in this study (1999, 2001, 2003 and 2004).
2. Guanajibo River plume showed variations in shape and length in all periods.
3. Major Guanajibo River plume transport toward south was identified for 2004 period. River plume transport arrived close to Belvedere, Cabo Rojo shoreline.
4. Caño Corazones River plume was identified in all images include in this study.
5. Spectral definition of suspended sediment is difference between rivers. These differences can be related with grain size, sediment type and suspended sediment concentration.
6. A second suspended sediment concentration was identified in Puerto Real Bay and nearshore waters. Suspended sediment distribution was not apparently related with Guanajibo River plume distribution.
7. Major suspended sediment was identified at Puerto Real for 2004 period.
8. Major suspended sediment can be related with inland modification at Puerto Real as changes in land use and constructions.

Period 1999

9. Guanajibo River plume transport is toward west with south component for 1999 period.
10. It is possible that part of suspended sediment transported to the west during this period could be lost through Yaguez- Añasco-Guanajibo submarine canyon system.
11. Suspended sediments transported alongshore to the south were observed closed to Belvedere for 1999.

Period 2001

12. Guanajibo River plume was transported toward south for 2001.
13. Major river plume was identified from Guanajibo River plume to Joyudas shoreline.

Period 2003

14. River plume was not well identified in the river mouth due by cloud cover.
15. Guanajibo River plume transport was toward south.
16. River plume showed a narrow shape from Guanajibo River to Joyudas shoreline.

Period 2004

17. Guanajibo River plume showed a transport to the south.

RECOMMENDATIONS

1. Develop a man-made activities databank for Joyudas, Belvedere and Puerto Real Area. This databank will be help to evaluate the possible relation between man-made activities and suspended sediment presence in Puerto Real Bay.
2. Conduct a superficial and longshore currents study for the Mayaguez and Cabo Rojo nearshore areas. Currents study will be help to evaluate in detail suspended sediment paths.
3. Conduct a study in changes in land use at Belvedere and Puerto Real area. This study will be help to evaluate possible relation between changes in land use and suspended sediments locations.

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Web Pages

Remote Sensing Data

www.uc.cl/sw_educ/geo_mar?html/h541.html.

Appendix C

Inventory of Septic Tanks

Nilda Luhring

Introduction

In the research for determining a non point source of pollution it is imperative to study multiples variables that can bring a more comprehensive and precise conclusion. In this report it is presented some of the various parameters needed for it, the study of infrastructure using septic tanks, the geology analysis, the soil type analysis and the hydrology analysis. These parameters are only some of the total that were analyzed. It was also analyzed the water quality in function of the marine ecosystem, the health of the coral reefs, the extent of the Guanajibo River, the population, demography.

The importance of study the infrastructure using septic tanks is imperative because the location of those infrastructures needs to be evaluated in order to establish that they can be a source of pollution. The geological and soil types analysis are useful to determine the exposures routes of contaminant and if the land in joint with the previous study of infrastructure with septic tanks can be one for determined a source of pollution.

Methodology

A. Study the infrastructure using septic tanks

In an area focused in part of the Puerto Real town, in Cabo Rojo, Puerto Rico, we analyzed a total of 2,060 houses for determine the number of houses with septic tanks. First, we delimitated the area of study using an aerial photo with the Geographic Information System Program, Arc Map, version 9.1.

B. Geology of the Study Area

For the geological analysis we use geodata from the Planning Board of Puerto Rico, an aerial photo from the Office of Management and Budget of Puerto Rico as the main tools using the Geographic Information System program, Arc Map, version 9.1. To the purpose of creating an area of one kilometer from the coastline, was created a shapefile, a polyline, drawing the coastline from the aerial photo. The next step was created a buffer of an area of one kilometer in function to the polyline created. With the buffer zone as the study area, an area representative to be a sample, the third step was to clip the geodata of geology and presented as categories. For the attributes, it was use the table of the geodata.

C. Soil Types

For the soil types analysis were use geodata from the Planning Board of Puerto Rico, an aerial photo from the Office of Management and Budget of Puerto Rico as the main tools using the Geographic Information System program, Arc Map, version 9.1. To the purpose of creating an area of one kilometer from the coastline, was created a shapefile, a polyline, drawing the coastline from the aerial photo. The next step was created a buffer of an area of one kilometer in function to the polyline created. With the buffer zone as the study area, an area representative to be a sample, the third step was to clip the geodata of geology and presented as categories. For the attributes, it was use the table of the geodata and the Soil Survey of Mayaguez Area of Western Puerto Rico, from the United States Department of Agriculture, Soil Conservation Service.

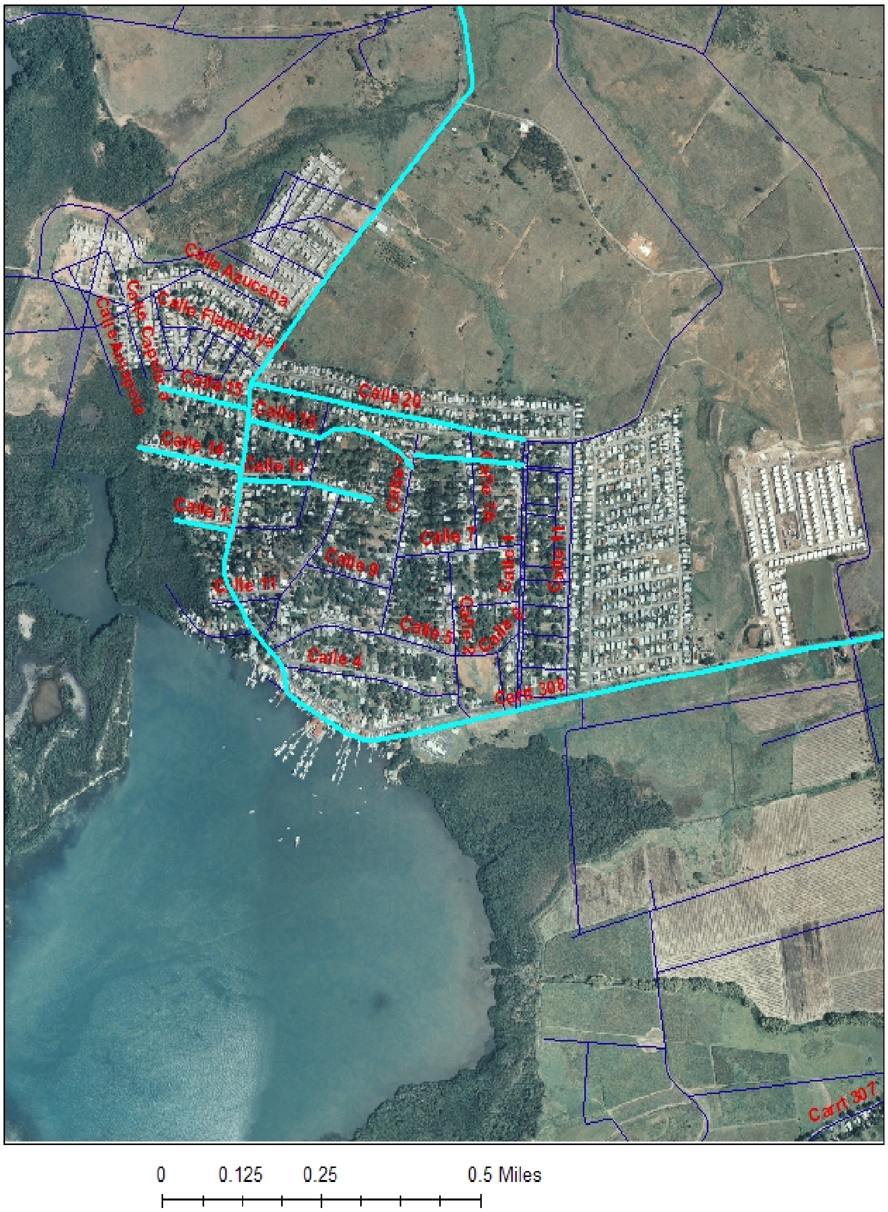
Analysis

A. Study the infrastructure using septic tanks

In the area of study we identified 435 septic tanks. Is pertinent to notice that the streets with the more number of houses/commerce with septic tanks in decreasing order are: Road 308, with 81 infrastructures; Puerto Real, Street 20 with 33 infrastructures; Puerto Real, Street 13, with 30 infrastructure; Puerto Real, Street 15 with 27 infrastructures; Puerto Real, Street 14 with 26 infrastructures.

From the aerial photo we notice that the road 308 is adjacent to the coast, and is the one that have the more houses with septic tanks in the area evaluated. It is pertinent to mention that the major parts of the streets named before are near of a water body with a possible effect in respect to the water quality. So, it may be one of the variables that can be implicated with the water quality pollution.

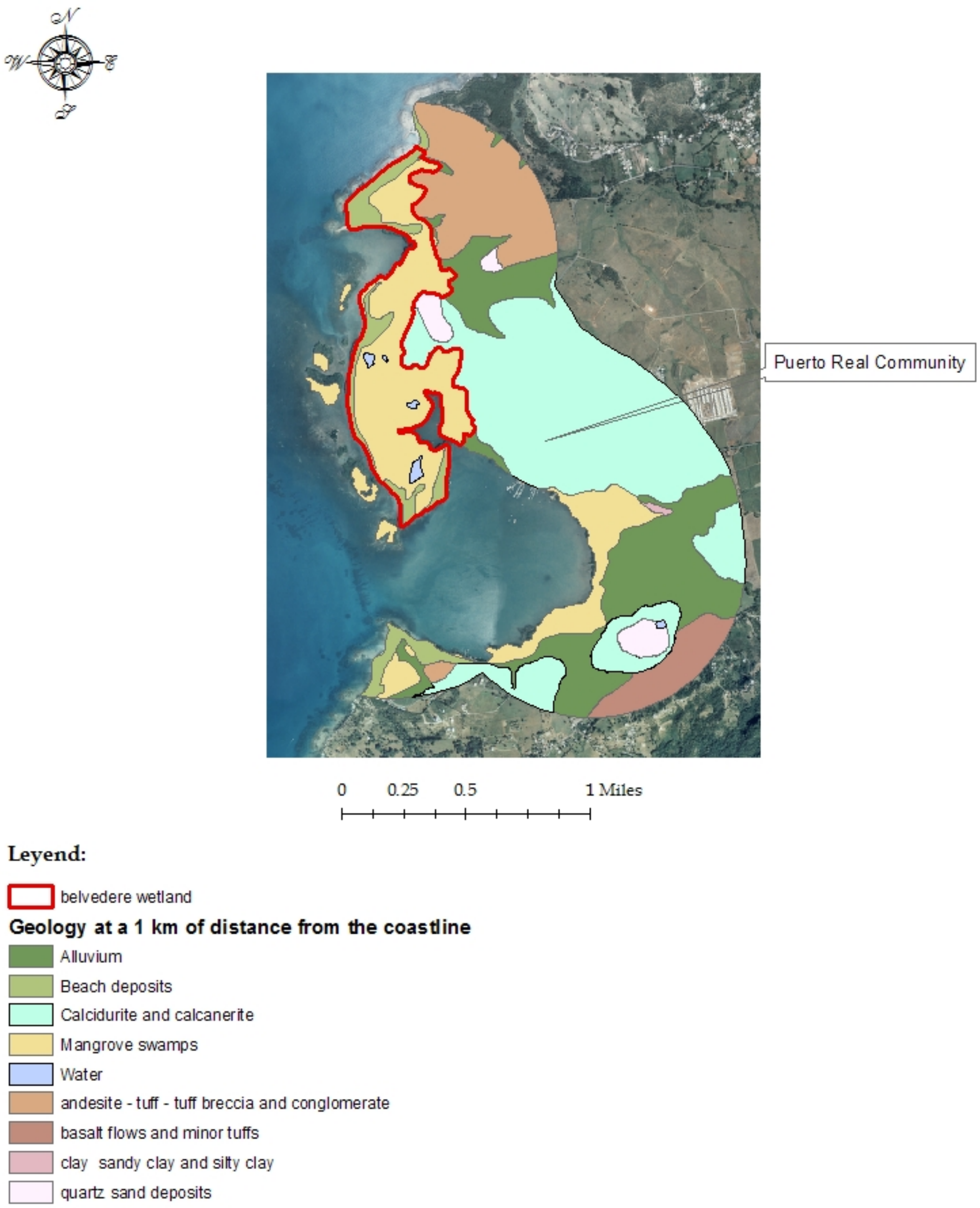
Aerial Photo of Puerto Real with Some of the Streets
Described in the Preliminary Report



B. Geology of the Study Area

We analyzed an area of 1 kilometer of distance in function of the coastline to take a representative sample of the study area. We encounter the following eight geological description: alluvium, beach deposits, mangrove swamps, andesite-tuff-tuff breccia and conglomerate, basalt flows and minor tuffs, calcidurite and calcanerite, clay sandy clay and silty clay, quartz and deposits. The alluvium was encounter meeting most of the area southeast- east and north of Puerto Real, it is from the Middle Quaternary Period and doesn't have a formation survey. The beach deposits were found in the coast, and are from the Middle Quaternary Period and from the Beach Deposits Formation. The mangrove swamp were found near the coast from west and south west of Puerto Real, there are from the Middle Quaternary Period and doesn't have formation survey. The andesite-tuff-tuff breccia and conglomerate were found north of the area of study, there are from the Middle Createaceous Period and from the Sabana Grande Formation. The basalt flows and minor tuffs were found south in land from the area of study, there are from the Middle Cretaceous Period and from the Lajas Formation. The calcidurite and calcanerite were found in the area of study and are from the Middle Terciary Period and from the Guanajibo Formation. The clay sandy clay and silty clay were found in less magnitude at the south of the area of study, it is from the Middle Quaternary Period and from the Swamp Deposits Formation. The quartz and deposits were found in less magnitude to the north, south and in part of Puerto Real, it is from the Middle Terciary Period and doesn't have formation survey. For a more comprehensive visualization of the geology of the area we provide the following figure.

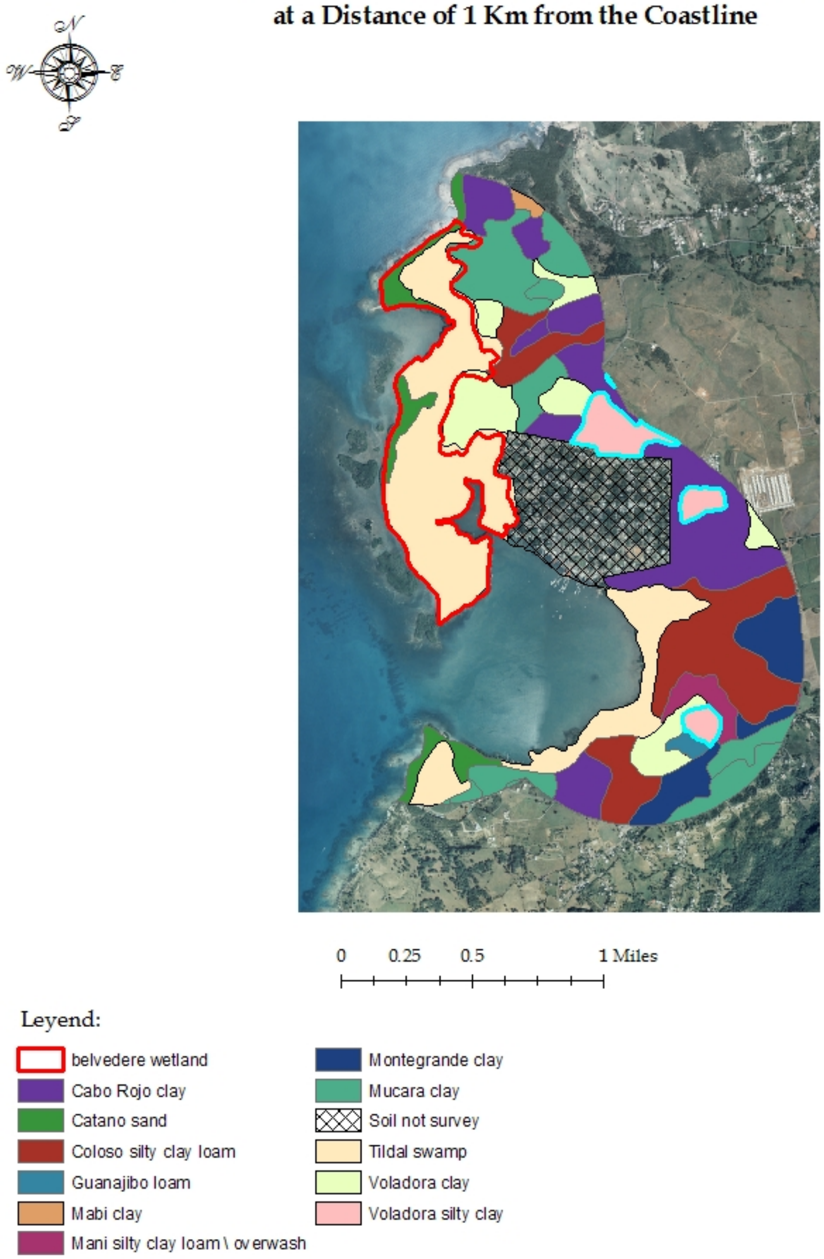
Aerial Photo of Puerto Real with the Description of the Geology at a Distance of 1 Km from the Coastline



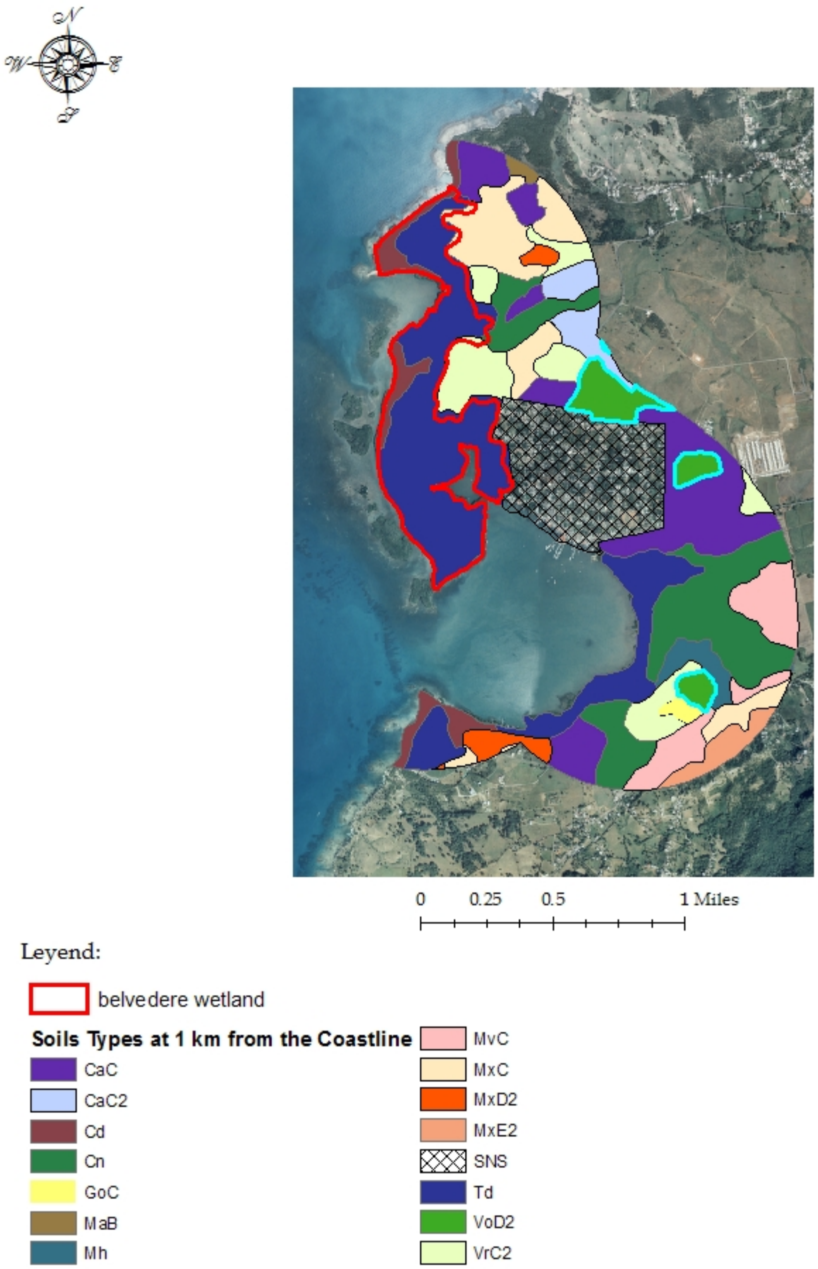
C. Soil Types

As with the geology description, we analyzed an area of 1 kilometer of distance in function of the coastline to take a representative sample of the study area for soil types description. The area of study encounter the following twelve different soil types: Cabo Rojo clay, Cataño sand, Coloso silty clay loam, Guanajibo loam, mabi clay, mani silty clay loam (overwash), Montegrande clay, Mucara clay, Tidal swamp, Voladora clay, Voladora silty clay. The Cabo Rojo clay, CaC, is a type of soil with a 2-12 percent of slopes, runoff is medium, moderate permeability and the erosion is a hazard. The Cataño sand, Cd, is nearly level and a rapid permeability, so present low runoff. The Coloso silty clay loam, Cn, is nearly level, are located in flood plains, so its exposure to floods and have moderate permeability. The Guanajibo loam, GoC, has a 2-12 percent slopes, the erosion is a hazard, and have a moderated permeability. The Mabi clay, MaB, are organics soils, with 2-5 percent slope, slowly permeable, so present high runoff. The Mani silty clay loam (overwash), Mh, are soils nearly level, moderately slowly permeable, located in alluvial fans and slightly above flood plains. The Montegrande clay, MvC, has a 2-12 percent slope, are moderately slow permeable, and the erosion is a hazard. The Mucara clay has some different sub-types: MxC, MxD2, MxE2. The Mucara clay, MxC, has a 2-12 percent slope, runoff is medium and a moderate permeability. The Mucara clay, MxD2, has 12-20 percent slope, moderate permeability, runoff is medium, and the erosion is a hazard. The Mucara clay, MxE2, has a 20-40 percent slope, moderate permeability, rapid runoff, and the erosion is a hazard (the more susceptible for erosion between the other Mucara clays). The Tidal swamp, Td, are areas covered mostly with mangroves, there are most of the time under salt water. The Voladora clay, VrC2, has 5-12 percent slope, are moderately permeable with a medium runoff and an erosion hazard. The Voladora silty clay, VoD2, has a 12-20 percent slope, moderate permeable, medium runoff and present hazard for erosion. Following are two layouts for describing in general and in a more detailing frame the soil types of the area.

Aerial Photo of Puerto Real the Description of the Soil Types at a Distance of 1 Km from the Coastline



Aerial Photo of Puerto Real the Description of the Soil Types in Simbology at a Distance of 1 Km from the Coastline

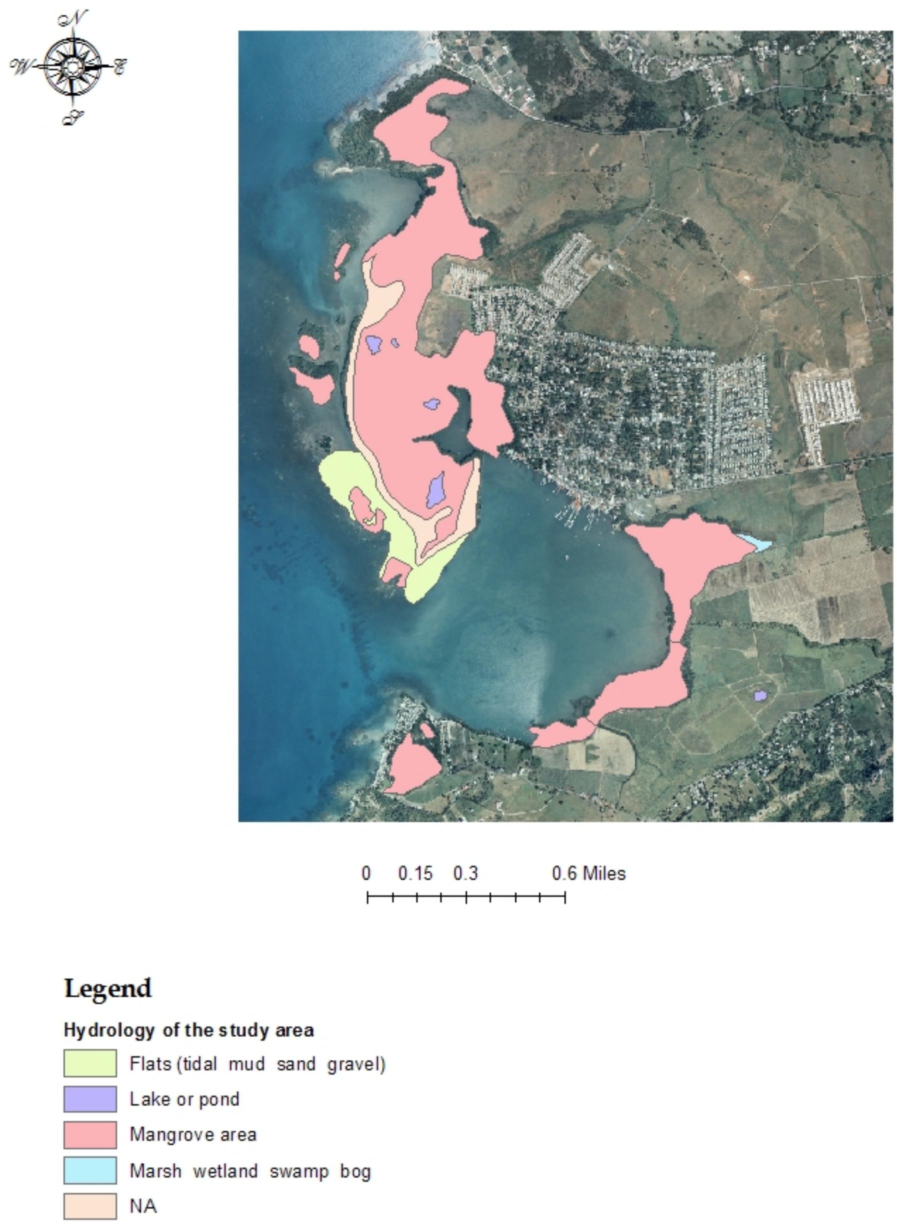


SNS: Soil Not Survey

D. Hydrology

In general, based in a sample area of study it was found four qualitative attributes: Flats (tidal, mud, sand, gravel), lake or pond, Mangrove area, Marsh wetland swamp bog. Following is a visualization of it:

Aerial Photo of Puerto Real with the Description of the Hidrology
at a Distance of 1 Km from the Coastline



Conclusion

The area of study, with respect to the infrastructure using septic tanks, contains soil types Cabo Rojo clay (CaC), Tidal swamp (Td) and the Voladora silty clay loam (VoD2). The CaC has a percent slope of 2-12, a moderate permeability, a medium runoff and a hazard for erosion, so this kind of soil can bring pollution as sediments to the marine ecosystem. Also, this kind of soil in structure is a mix of clay, with sticky texture of high plasticity. The movement of water or other substances in it, will be slow. The Tidal swamp, is covered mostly with mangroves. It is most of the time under salt water, it has a high water table and any substance can be transported to the marine ecosystem. By this extrapolation it can be said but not established that the soil in the study area represents a route of exposure of contaminant related to water quality in slow movement or a direct one, as with the tidal swamp. It can be established from the soil type analysis that the area can be a source of pollution in terms of sedimentation. For a precise explanation of the behavior of substance in the soils within the area of study survey of it is needed. Also it will help the contour lines of underground water. There are no previous studies in the area of focus in terms of underground water movement.