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A Septic Waste Index Model to measure the impact of septic tanks on coastal water quality and coral reef communities in Rincon, Puerto Rico

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Abstract

The impact of fecal contamination of coastal waters and coral reefs is a major cause of concern in marine reserves in Puerto Rico. The measurement of the association between septic tank frequency in watersheds of creeks draining into these reserves and coastal water quality and coral reef condition is of importance in configuring pollution control policy. Fecal coliforms and enterococci assays were used to measure the density of fecal contaminants across the Tres Palmas Marine Reserve (TPMR) in Rincon, Puerto Rico. Inshore waters are intermittent creeks, receiving fecal pollution only from faulty septic tanks. Fecal indicators measurements showed higher densities near the southernmost creek mouth emptying into TPMR, a finding consistent with a larger amount of dwellings with septic tanks within this watershed (Vista Azul creek). A Septic Weight Index was constructed to analyze sewage impact from all watersheds draining into the marine reserve. Linear Regression analyses showed a significant association between these non-point source fecal pollution sources and both coastal water quality and impact on some parameters measuring the condition of *Acropora palmata* coral reef colonies.

Key words: Coral reefs; Non-point source coastal fecal pollution; septic tank pollution; watershed analysis

1. Introduction

Water pollution, and particularly sewage from land-based nonpoint sources, is one of the principal threats to coral reefs and its associated ecosystems, not only because of its long-term ecological impact on coral reefs (Prog et al., 1985), but also on water-based recreation (Cordero et al., 2012). Land-based source pollution (LBSP) is one of the most critical concerns for the conservation of coral reefs (Cloern, 2001; Díaz-Ortega and Hernández-Delgado, 2014), and for the protection of public health of bathers. Population growth and rapid urban development, particularly in small islands, often results in increased deforestation and construction of coastal residential and tourist projects. This may lead to a significant increase in the rates of soil erosion and sediment delivery, in the increased discharge of wastewaters, watershed degradation, and in the impact of urban runoff on coastal waters (Ramos-Scharrón, 2012; Ramos-Scharrón et al., 2012, 2015). Although impacts of coastal water pollution and eutrophication have been well documented in the scientific literature, there are still important gaps in addressing nonpoint LBSP impacts in small tropical islands coastal ecosystems.

The main island of Puerto Rico (PR) is the smallest of the Greater Antilles across the northeastern Caribbean and showed a significant increase in human population over the second half 20th century, leading to a concomitant increase in non-sustainable coastal development (Hernández-Delgado et al., 2012). Previous studies have shown that coral reefs along a significant portion of the southwestern and northern region of PR have been severely impacted by wastewater pollution from nonpoint sources. (Bonkosky et al., 2009; Hernandez-Delgado et al., 2010, 2011; Díaz-Ortega and Hernández-Delgado, 2014). Main rivers of both regions represented some of the main sources of sediment and sewage pollution that caused increased levels of turbidity and declining microbiological water quality along the coast. An important element associated with coastal fecal contamination in areas of coral reefs and recreational beaches located adjacent to human settlements is the malfunctioning and poor maintenance of on-site septic treatment systems. Leaking septic tanks have been previously identified as significant non-point sources of sewage pollution adversely impacting adjacent coral reef systems (Lewis, 1987; Bonkosky et al., 2009; Méndez-Lázaro et al., 2012; Norat-Ramírez et al., 2012; Díaz-Ortega and Hernández-Delgado, 2014). There is evidence that coastal groundwater and surface waters throughout developed urban areas are characterized by increasing elevated concentrations of N and P through time (Lewis et al., 1987; Lapointe et al., 1990). However, impacts of leaking septic tanks on coastal coral reefs and recreational beaches have still been poorly addressed.

Protected coastal areas, such as marine reserves, offer public opportunities for relaxation, recreation, and nature appreciation. Under idealized conditions these areas also present researchers with a rich variety of terrestrial and aquatic habitats for study under (relatively) un-impacted conditions (Mallin et al., 2012). The Tres Palmas Marine Reserve (TPMR), in Rincon, PR (Figure 1), offers the opportunity of studying the isolated impact on coastal water quality and nearshore coral reefs of low density mostly residential (and some guest houses) septic tanks in small, steep, highly forested watersheds. The creeks flow into a tropical small sized marine reserve. Inadequate septic systems pollute surface waters with pathogenic bacteria, viruses, parasites and excess nutrients affecting the water quality of watersheds that empty in coastal waters, potentially impacting the use of receiving coastal areas, as well as the condition of adjacent coral reefs.

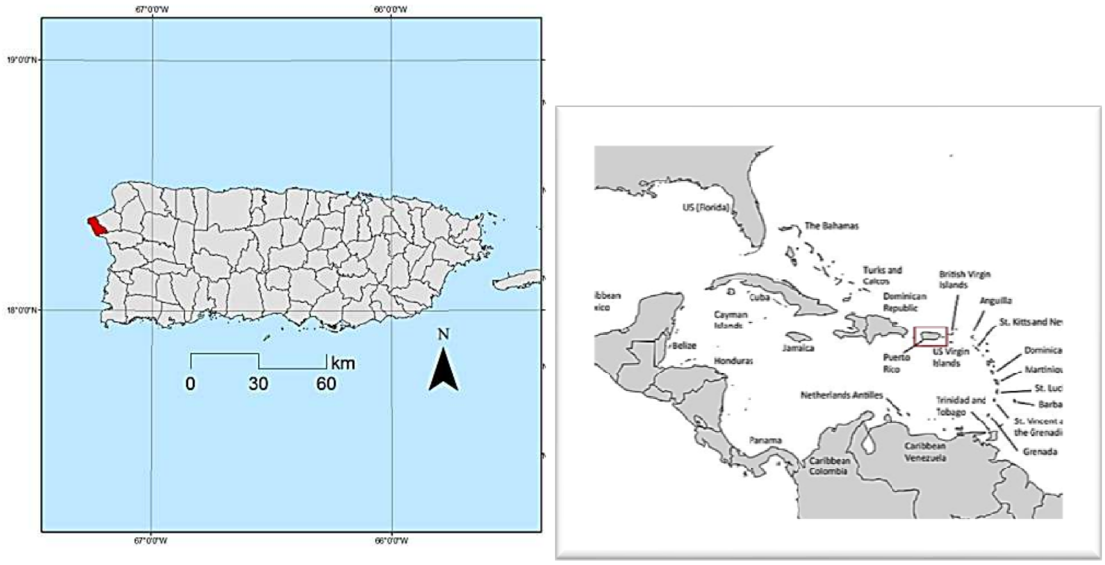

Figure 1. Left Panel: The Main Island of Puerto Rico with its 78 municipalities. In red, the municipality of Rincon showing the study area near the Northwestern tip of the main island of Puerto Rico. (Puerto Rico Planning Board) Right panel: Regional map of the Caribbean presenting Puerto Rico regional location (red rectangle) as the smallest island of the Greater Antilles across the northeastern Caribbean. ("Map of the Wider Caribbean Region with 25 countries/island nations that were included in the final analyses investigating the effect of mangrove forest area on reef fish abundance.", Figure posted on 04.11.2015, 00:28 by Joseph E. Serafy Geoffrey S. Shideler Rafael J. Araújo Ivan Nagelkerken,)

Previous studies in Puerto Rico indicate significant widespread fecal pollution in coastal waters that represent a potentially serious threat to the marine environment and human health (Bachoon et al., 2010). A recent study in the North coast of Puerto Rico (Cordero et al., 2012) measured the water quality at a recreational beach affected intermittently by non-point sources of fecal pollution. Mean daily average density (CFU/100 mL) of Enterococcus by culture method was significantly higher ($p < 0.001$ ANOVA analysis) in the autumn, or rainy season (log10 = 0.33) than in the summer season (log10 = 0.19). Mean daily average density of Fecal coliforms was significantly higher ($p < 0.001$ ANOVA analysis) in the autumn season (log10 = 0.82) than in the summer season (log10 = 0.64).

The Puerto Rico Environmental Quality Board (PREQB) reported 437 impaired km of regularly monitored coastlines in Puerto Rico in 2012. A water body is considered "impaired" if any of its designated uses is not met due to water quality problems. This represented 64.2% of the total extension of 680 km of the assessed coastline, which represented 77% of the total coastline in PR. A total of 382 km of coastline (56%) was impacted by onsite wastewater systems (PREQB, 2013). Also, 158 km (23%) of the coastline failed to meet Enterococci water quality standards, while 137 km (20%) of coastline failed to meet Fecal coliforms standards. The Puerto Rico Water Quality Standards Regulation (PREQB, 2010) classifies the marine coastal waters of TPMR as Class SB waters. The "Usages and Description" of this class of waters is as follows: "Coastal waters and estuarine waters intended for use in primary and secondary contact recreation, and for propagation and preservation of desirable species, including threatened or endangered species." The Puerto Rico water quality standard for Class SB waters is that the geometric mean of a series of representative samples (at least five samples) of the waters taken sequentially shall not exceed 200 colony forming units (CFU/100 mL) of Fecal coliform bacteria. Not more than 20 percent of the samples shall exceed 400 colonies/100 mL. (PREQB, 2010, Rule 1303.2.B.2.b.)

The problem is not confined to Puerto Rico, since it has been reported that approximately thirty five percent of the impaired rivers and streams of the U.S. are polluted by Fecal coliforms, which is listed as the leading factor of water quality impairment in the rivers and streams and second leading factor in estuaries (USEPA, 2000; as cited by Liu, et al., 2010). Twenty percent of all water quality samples at U.S. Geological Survey's main sampling stations across the U.S. exceeded the Fecal coliform standard of 200 MPN per 100 mL in the 1980's (Smith, et al., 1992; as cited by Liu, et al., 2010).

State and tribal agencies in the United States report that onsite septic systems currently constitute the third most common source of ground water contamination and that these systems have failed because of inappropriate siting or design or inadequate long-term maintenance (USEPA, 1996a). In the 1996 Clean Water Needs Survey (USEPA, 1996b), states and tribes also identified more than 500 communities as having failed septic systems that have caused public health problems (USEPA, 2002).

Over 50% out of the 3.5 million residents in PR depend on septic tank systems. However, there is a lack of data about onsite septic systems design and operations. Examples abound of septic tanks constructed with holes for percolation, in practice carrying out untreated wastewater injection into the soil. Tanks typically lack sufficient volume for adequate retention times and drainage fields are typically never used. Septic tanks require between 2 and 5 feet (0.6–1.5 m) of aerated soil (the vadose zone) beneath the septic drain field to completely treat the pollutants in sewage (US EPA, 2002, as cited by Mallin & McIver, 2012). Raw sewage can overflow or enter the ground and reach the saturated zone during the frequent intense rain storms that occur throughout the year in PR. The wastewater plume can then migrate downstream through the watershed in response to the prevailing large hydraulic gradient typical of the PR surface. Within small tropical island scenarios with multiple high-density human settlements, raw sewage can reach coastal waters and coral reef ecosystems very rapidly.

There are some 562,000 septic tanks across Puerto Rico, disposing of about 170 million gallons of wastewater daily, versus the 230 million gallons a day flowing into the Puerto Rico Aqueducts and Sewers Authority sanitary sewer system. Most septic tanks were constructed under unregulated conditions, with little knowledge and crude technology, and placed in inadequate areas. Once they start malfunctioning, it is very difficult to make repairs. Bringing a septic tank into compliance, when feasible, costs $5,000 to $10,000, so the cost of the undertaking is another vexing issue, given the battered finances of government agencies and household finances following six years of recession (Marino, 2012).

Pollution problems associated with onsite septic systems in Rincon follow similar trends previously documented in PR and elsewhere across the tropics (Norat et al., 2006). This study investigated how the patterns of human settlements with septic tanks near TPMR affect water quality, coral reefs and beaches for recreational use. The hypotheses of this study were that: (1) Due to the steep slopes and local hydrology, during storm events, untreated sewage drains from septic tanks located in the adjacent residential areas affecting water quality in TPMR through overland runoff of septic systems overflows or leachate; (2) chronic nonpoint source pollution have adverse ecological impacts in coral reefs along a distance gradient from creek outlets; and (3) such pollutants also could present a potential human health problem to bathers in nearby Rincon Public Beach. A model predicting the water quality and ecological impact of septic tanks on a watershed basis using the Septic Weight Index parameter was constructed to prove these hypotheses.

2. Methods

2.1. Description of study area

This study was carried out across TPMR, off the town of Rincon, in northwestern PR (Fig. 1). The TPMR was designated through PR Law 17 of 2004 to protect some of PR's healthiest remnant populations of Elkhorn coral (*Acropora palmata*), which was designated in 2006 as threatened under the U.S. Endangered Species Act. About thirty species of corals have been documented in this Reserve. Adjoining TPMR to its south is the town of Rincon, with a residential area extending north along the seashore for about 1.18 km and east towards a mountain range parallel to the coast next to TPMR. This part of Rincon is a specially protected land area, mostly zoned CR at the coast, or Resource Conservation (less than 2% of total area in occupation, no subdivision allowed, 1 or 2 families per farm); RE, or Scenic Route(20,000 m^2 lots), and RT-B, or low density tourist – residential (2,000 m^2 lots) (Junta de Planificación, 2010). Dwelling density within the seven small watersheds draining into the coast within TPMR ranges between 0.12 units/ha and 2.9 units/ha according to our data. Seasonally, Rincon has high summer visitor usage, and sewage treatment in the area abutting the park consists of septic systems.

Geologically, the Rincon region belongs to the Río Culebrinas Formation, of a mixed carbonate/clastic rock lithology. It is described as having moderate permeability (Mitchell, 1954; McIntyre, 1970). The region is dominated by limestone and epiclastic outcrops, and the predominant soils are the Colinas and Mabi clays (Ithier-Guzmán, 2010). The soils in the Tres Palmas area of Rincon are primarily:

(1) a coastal band reaching around 30 meters inland from shoreline of flat beach deposits, of a beach sand lithology (Cd, Cataño sand);

(2) an adjacent nearly flat band reaching around 100 meters inland of Mabi clay (MaC2), 5 to 12 percent slopes, eroded, somewhat poorly drained and;

(3) steep slopes and hilly terrain up until a mountain ridge divide running north - south parallel to the coast, with peaks close to 2 km. from shore, classified as Malaya clay (McF2), 20 to 60 percent slopes, eroded and well drained.

The mean annual precipitation is 175-200 cm, and depth to the water table in this area is more than 2 m (USDA, 2014). The TPMR limits include 1,180 linear meters of coastline from its north end to its South end. The maritime limit (towards the West) is the depth contour of 20 m. The TPMR area has a perimeter of approximately 3.8 km and a total area of 80.4 hectares (Valdés-Pizzini, 2008, p.23).

A municipal recreational beach lies just 600 m south of TPMR. Microbiological water quality at this beach is monitored by the PR Environmental Quality Board (PREQB) at a routine coastal water quality sampling station 1400 m south of TPMR. However, the most recent water quality data published by PREQB in STORET (EPA, 2014) for this station were collected in 2008, and showed Enterococci densities for that year ranging from below quantification limit to 80 colony forming units (cfu)/100 ml, which exceeded the PREQB water quality standard for recreational waters (Class SB) of 35 cfu/100 ml (PREQB, 2010), implying the existence of sewage pollution pulses impacting the area.

The rocky shoreline of the Tres Palmas Marine Reserve leads to a narrow backreef lagoon with coarse sandy sediments. The lagoon is a semi-protected environment associated with an extensive *Acropora palmata* (Elkhorn Coral) reef formation that has developed along a hard ground platform fringing the shoreline. The top of the platform is found at depths between 2 - 5 m. (García-Sais el al. 2009). Rainfall runoff with heavy loads of terrestrial sediments has been previously reported to reach this fringing reef (García-Sais et al., 2004).

Sampling sites were selected within and outside TPMR, covering near shore (east) and offshore (west) of the Reserve, and stretching along the shore from north to south, covering an array of creek mouths with different probable pulse sewage pollution influences. A hydrologic characterization allowed us to identify and delineate the sub-watersheds of creeks draining into the TPMR. They were named according with salient geographic features for identification (Fig. 2). Figure 2 presents a map showing the location of water quality sampling points within and outside TMPR, as well as the location of coral reef sampling points (red dots). The watersheds of the creeks flowing into TPMR are color shaded. Water quality sampling station names indicate their location in terms of south, north, east and west cardinal points, as well as whether they are located within the Reserve or are control points.

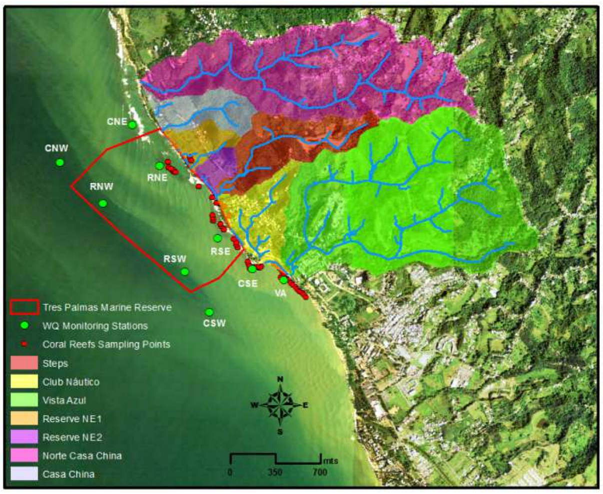

Figure 2. Map showing Rincon municipality (complete cutout) and sub-watersheds discharging into *Tres Palmas Marine Reserve* (marked with a red perimeter line, near the Northwestern tip of the main island of Puerto Rico). It also shows the location of Reserve and both water quality (green dots) and coral reef sampling points in red. The map shows the location of the Reserve and the marine water quality sampling points used in the study.

A total of nine microbiological water quality sampling stations were used, four within the TPMR and five outside it as control stations in adjacent areas. The control sites included three south of the Reserve and two north of the Reserve. The inshore sampling sites (East) locations were less than 100 m from shore. The offshore sampling sites (West) locations were 300-500 m from shore. A coastal water sampling station was located near the public beach and named Vista Azul (VA), indicating its proximity to the outlet of a creek draining into the ocean near a residential development with that name.

2.3. Methods for water quality analyses

Samples were collected monthly or bi-monthly between May and December 2011, for a total of ten sampling dates including the rainy and dry season. The following environmental parameters were measured in triplicate at each station on each sampling date: surface seawater temperature, pH, salinity and conductivity, and dissolved oxygen concentration. Multiparameter water quality probes linked to a Thermo Scientific Orion 5 Star Meter display unit were used. Triplicate marine water samples were collected at each station during each visit to measure Fecal coliforms and *Enterococcus* bacteria density, Turbidity, and Suspended Solids at the laboratory. Turbidity readings were made in the laboratory using a Hach 2100P turbidimeter.

Suspended solids were measured with a glass fiber filter disk using a Denver Instrument Co. Analytical balance A200D and a Lindberg/blue M Oven (Standard Method 2540D, APHA, 1995).

Water samples were placed in coolers and maintained in ice at 1–4 ºC during the time before analyses. Water samples were analyzed within 6 hours of collection using membrane filtration and culture methods. Fecal indicator bacteria by culture methods, Enterococcus and Fecal coliforms, were quantified in cfu/100 mL. Viable Fecal coliforms were enumerated by the American Public Health Association (APHA) Method 9222D on Membrane-Fecal coliform Agar (mFC) plates (APHA 1995). The M-FC medium was used; incubating cultures prepared with membrane filtration techniques for 24 ± 2 hours at 44.5 ±0.2 ºC.

Viable Enterococci were enumerated by the USEPA Method 9230C (Id.). Membrane filtration techniques were used to prepare cultures using the m Enterococcus agar. Prepared cultures were incubated for 48 hours at 35 ±0.5 ºC. Confirmation tests were carried out using Brain-heart infusion broth for 48 hours at 45 ±0.5 ºC.

2.4. Methods for hydrologic and land use analyses

A hydrological characterization of the study area was carried out based on river basins and sub-basins with the aim of identifying the drainage areas which may have impact on the TPMR. These basins have a very close relationship with human activities taking place in their environment (Méndez-Lázaro, 2011). Geographic information systems (GIS) (ArcGIS 9.3), the HEC-GeoHMS v.6.0 *Geospatial Hydrologic Modeling Extension* tool of the US Corps of Engineers, the digital elevation model (DEM), and the database of the *National Hydrography Dataset* and the *National Land Cover Dataset* were used to characterize the hydrographic network for the analysis. The already calibrated DEM product of remote sensing was used for GIS analysis of runoff drainage patterns. To permit the construction of an index of exposure of coastal water sampling stations to septic tank pollution, distances were measured between each of the coastal water sampling stations and the mouths of the seven creeks flowing into TPMR. The Spider Diagram Tool of the Spatial Analyst Extension of Arc GIS 10.0 was used for this purpose.

GIS tools allowed us to count the number of septic tanks in each watershed, therefore permitting us to run a model measuring the effect on water quality of septic tank pollution load. Aerial photographs of the West of Puerto Rico and the municipality of Rincon were used to identify the amount of residenses and businesses with septic tanks in the sub-watersheds. Similarly, information from the Puerto Rico Aqueducts and Sewers Authority was used to learn which communities were connected to sanitary sewer service. We assumed that every residential and business unit had one septic tank. Therefore, knowing the number of houses and commercial buildings within the sub-watershed, the number of septic tanks that lie within the hydrological units that discharge directly into the TPMR could be estimated.

2.5. Methods for coral reef benthic habitat characterization

The part of the study was also aimed at determining the current distribution and condition of threatened *A. palmata* stands within TPMR to provide a basis to assess its population status and trends, and to determine if there were impacts associated to runoff and sewage pollution on coral colonies. Coral reef ecosystems within TPMR support one of the most important remnant populations of this species across the entire northeastern Caribbean region. GIS-based tools were used to randomly select 52 sampling stations within and outside the TPMR, subdivided on several replicate stations per site as follows: *Within Reserve* - Northeast Reserve-*NR* (n=7), Mid Reserve-*MR* (n=10), South Reserve-*SR* (n=8), and *Control Outside Reserve* - South control-*SC* (n=8), and Public Beach control-*BC* (n=19). Sampling stations were limited to 5 m depth contours or less which constitute the dominant habitat by *A. palmata* across the zone. Bathymetric and benthic habitat layers (NOAA 2001) were used to select areas between 0.5 and 5 m of linear reef and hard-bottom habitats. All map calculations were conducted at 10 m cell size.

Randomly selected points were uploaded to a handheld GPS. In the field, a leaded line with a surface buoy was used to mark the randomly chosen point to center of each 100 m^2 survey area. At each sampling station we quantified the presence of *A. palmata* and dead standing skeletons within the 100 m^2 by delimiting the area with a floating line used to delineate a 5.6 m radius of each circular plot following the methods described by Mayor et al. (2006) and Schärer et al. (2010). When living *A. palmata* were present the number, size (maximum length, width and height), percent live coral cover of each colony, and colony condition (i.e., % old/recent mortality, % bleaching, % disease incidence) was measured for those colonies with their center within the survey area. A Live Area Index (LAI) was calculated (length*width*% live/100) for each colony as an estimate of total coral tissue (m^2). Data on colony area (m^2) and volume (m^3) were also documented, as well as total abundance of damselfishes (Pomacentridae), corallivore snails (*Coralliophilla abbreviata*), and fireworms (*Hermodice carunculata*). A total of 620 colonies of *A. palmata* colonies were sampled through the project including 179 at NR, 259 at MR, 83 at SR, and 99 SC. No colonies were documented at BC. Also, colonies of *A. cervicornis* and hybrid *A. prolifera*, which were present only at SC, were assessed as above.

2.6. Construction of Septic Weight Index Model and methods for statistical analyses

Statistical analyses of data were carried out using the Intercooled Stata for Windows package, version 12.1. Statistical distribution of physical-chemical parameters was calculated for values of marine water turbidity, suspended solids, temperature, salinity, conductivity, pH, and dissolved oxygen. The purpose was to detect differences in physical-chemical water quality parameter values across marine water quality sampling stations and whether trends coincided with differences in microbiological parameter values. Averages of Log_{10} of physical-chemical parameters were calculated by sampling station. Box plots were constructed for these parameters also. The association between selected marine physical-chemical parameters and microbiological densities was studied using linear regression techniques.

For microbiological results, range and statistical distribution parameters of daily microbiological density values (CFU/100mL) were calculated (mean, percentiles 25, 50 and 75, minimum, maximum, and standard deviation). Microbiological density values were log_{10}-transformed to normalize parameters (McDonald, 2014). Box plots were constructed for these parameters.

The association between exposure to septic tank pollution from watersheds and microbiological coastal water densities was studied. Statistical analysis was carried out using a model based on the Septic Weight Index (water quality stressor) as a water quality predictor. This is an original index created for this project in order to assess the impact of each of the creeks draining into TPMR on each of the marine water quality monitoring stations within the TPMR. To construct the variable Septic Weight, the number of septic tanks in watersheds of creeks draining into TPMR was estimated using GIS and aerial photography and the distance from the creek mouth and the coastal water sampling stations was calculated using GIS techniques. The index sums the influence of all creeks on water quality at each marine sampling station measured by number of septic tanks in each watershed divided by the distance of the sampling station to the creek mouth. A dummy variable was used for the sampling date in the regression model to adjust for the different conditions between sampling dates. The Septic Weight Index has the following form:

$$Septic\ Weight\ j\ (water\ quality) = \sum_{1}^{7} \frac{(\#\ septic\ tanks\ i)}{(distance\ ij)}$$

Where Septic Weight = total effect of septic flow from creeks 1 to 7 on water quality sampling station j;

\# Septic tanks= total number of septic tanks in watershed for creek i;

Distance ij=distance in meters from mouth of creek i to water quality sampling station j.

Finally, analysis of impact of septic tanks on coral reef in Rincon was carried out using a regression analysis of Septic Weight Index and variables describing the current distribution and condition of Elkhorn coral within the TPMR. Statistical analyses results are shown in Table 7.

3. Results

3.1. Results of land cover and hydrologic analyses of study watersheds

In this study GIS allowed us to delineate catchment basins draining into the marine reserve where there are septic tanks and where local communities have no wastewater sewer service (Méndez Lázaro et al., 2012). It was found that population density is low within these watersheds, and land use is almost exclusively residential in nature, while several guest houses exist near the coast. The results obtained under these conditions serve to ascertain the effect of malfunctioning septic tanks on coastal water and consequently coral reef formations. These small, steep watersheds rapidly drain storm water contaminated by septic tank overflow and leachate.

With the intention of identifying the amount of residences and commercial establishments in the sub-watersheds with septic tanks, aerial photographs of the West of Puerto Rico and the municipality of Rincon were used. Similarly, information from the Puerto Rico Aqueducts and Sewers Authority (PRASA) was used to identify which communities were connected to the sanitary sewer system. Once concluded that none of the residences in the watersheds draining into the TPMR were connected to a PRASA sewer, we hypothesized that every residential and business unit had at least one septic tank.

As a whole, the hydrological units include 5.83 km^2, spatially distributed in 7 different sub-watersheds. A total of 426 residential and business units with their respective septic tanks were found among all the sub-basins (Table 1). This represents an average of 73 septic tanks per square kilometer. However, when analyzing each basin separately, it is worth mentioning that Norte Casa China sub-basin, although not the largest basin, has the highest density of residences/business and septic systems among all study basins, with an estimated 100 units/km². In Table 1 one can observe that 274 of the 426 septic tanks identified in the study area are located in the watersheds of the three southernmost creeks.

Table 1

Sub-watershed characteristics, number of residences and septic tanks by hydrological unit. Sub-watersheds are ordered from top to bottom according to location of creek mouth from North to South.

Sub-Watersheds	AREA (Ha)	ELEV. MAX (m)	N° of Septic Tanks	N° of Residential and Commercial Buildings
Sub-watersheds in coastal area from Piletas to Los Ramos creeks	1114	370	N/A	N/A
Norte Casa China	132	111	132	132
Casa China	52	94	9	9
Reserva NE1	35	93	9	9
Reserva NE2	17	86	2	2
Steps	38	104	20	20
Rampa Pescadores	26	82	76	76
Vista Azul	283	110	178	178
∑ Sub-watersheds	583	n/a	426	426

Sanitary sewer service near Rincon's coast stretches only as far North as Vista Azul residential project, south of the southernmost study watershed. Intermittent creeks serve as conduits of septic tank overflows or bypass discharges from several "barriadas" within the selected sub - watersheds (Fig. 2). Most of these creeks with mouths immediately draining into the Reserve were relatively dry during the study period, and surface water flowed only immediately after it rained.

The runoff capacity of the region is worth mentioning. Even though the micro-basins are small, all of them move a great volume of water when it rains. As an estimate, creeks draining these micro-basins can flow an average of 3.8 m^3 over the surface of the seven hydrological units that discharge in the marine reserve with 1 inch of rain that falls in 1 hour (Table 2).

Table 2

Hydrological characteristics of the sub-watersheds at Tres Palmas Marine Reserve. Sub-watersheds are ordered from top to bottom according to location of creek mouth from North to South.

Sub-watersheds	Creek length (m)	Creek Slope (m/m)	Runoff Capacity (25.4mm/1h) peak flow (m^3/sec)
Norte Casa China	2815	0.06	9.85
Casa China	627	0.15	4.93
Reserva NE1	91	1.02	3.65
Reserva NE2	168	0.51	1.75
Steps	1442	0.07	3.14
Rampa Pescadores	744	0.11	2.39
Vista Azul	1750	0.08	1.15

3.2. Results of Physical-chemical analyses of water quality

The average temperature of coastal marine waters in TPMR during the study period was 28.5 ºC, while the median was 28.7 ºC and standard deviation 0.8. While there was no significant difference found in water temperature among water quality sampling stations, there was an increase of almost 3°C between the lowest temperature sampling day (May 9, 2011) and the highest (October 17, 2011). This is a common pattern at these latitudes and in these months. Values were observed in excess of 29 ºC on October 17, 2011 at all sampling points. The maximum water temperature recorded was 29.7 ºC at Vista Azul Station on sampling day August 29, 2011. Salinity of coastal water had a very small variance among observations, from a minimum of 34.9 to a maximum of 36.5, with a median of 35.5 ppt and a standard deviation of 0.46 for all sites. There was a slightly smaller average salinity in the south easternmost water quality sampling stations, those nearest the mouth of the creek draining the largest watershed of the study area. Conductivity of coastal water also had a very small variance among observations, with a minimum of 52.6 and a maximum of 54.9 µS/cm. Median was 53.6 and standard deviation 0.65. pH did not vary much among observations either, with a median of 8.14 and a range from 8.05 to 8.87, standard deviation 0.1. Dissolved oxygen concentrations were high, and ranged from 5.52 to 6.83 mg/L, median 6.27 and standard deviation 0.23 among all observations. High turbulence associated with large wave action in this surfing activity area could help explain the high levels of dissolved oxygen of coastal water. There was a slightly smaller average dissolved oxygen concentration (6.1) in the south easternmost water quality sampling stations, those nearest the mouth of the creek draining the largest watershed of the study area. This creek drains the watershed with the largest amount of septic tanks in the study area. Coastal water had very low turbidity, which ranged from 0.11 to 5.77 NTU among all observations, with a median of 0.4 and a standard deviation of 0.7 for all sites. Being a marine reserve, this is expected at TPMR. Average turbidity values higher than at other sampling stations were observed in the south easternmost water quality sampling stations (up to 1.3 NTU), indicating a possible larger effect of storm water runoff events on sampling stations near the mouth of Vista Azul Creek. Figure 3 shows the distribution of turbidity values (NTU) by coastal water sampling points. In these box plots, boxes are bound by the 25th and 75th percentiles ($x_{[25]}$ and $x_{[75]}$) of measured values. The white horizontal lines represent the median. Whiskers represent the upper and lower adjacent values as defined by Tukey (1977): the upper adjacent value is defined as $x_{[75]} + 1.5\ (x_{[75]} - x_{[25]})$. The lower adjacent value is defined as $x_{[25]} - 1.5\ (x_{[75]} - x_{[25]})$. Dots represent outside values.

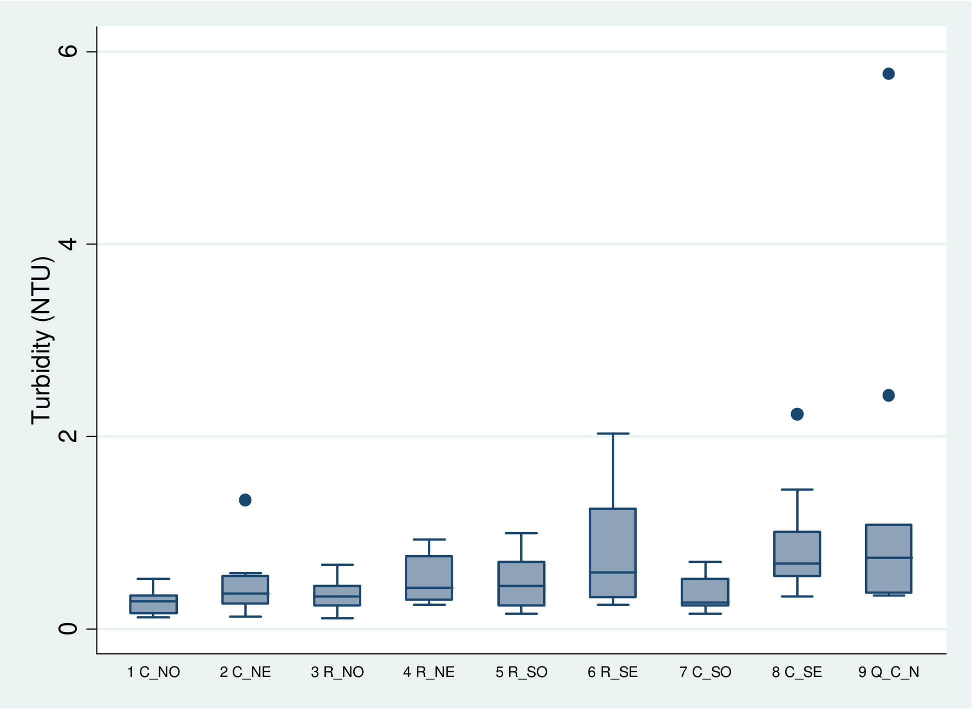

Figure 3. Box plot of turbidity (NTU) by coastal marine sampling station from May to December, 2011 in TPMR, Rincon. Sampling stations are ordered in a North-South direction from left to right in box plot.

In the figure, sampling stations were ordered in a general North-South direction from left to right. The Vista Azul Creek drains the largest watershed of the study area, containing the largest number of residences with septic tanks. The mouth of this creek is closest to the south easternmost sampling stations. Dispersion of observed turbidity values for coastal marine stations to the south of TPMR was larger than for stations to the north of the Reserve.

Marine suspended solids showed a pattern of higher variability than other water quality parameters between the TPMR coastal water sampling stations during the study period. They ranged from 128.0 to 290.0 mg/L. The median was 237.5 mg/L and the standard deviation 36.8. The variability of this parameter, along with turbidity, best describe the differences in water quality as a function of sampling date and station. One would expect higher levels of these contaminants in sampling stations closer to the mouths of creeks draining watersheds with the largest number of residences served by septic tanks. We would also expect to find higher contaminant levels during the rainy season due to higher levels of storm water runoff. The average physical chemical parameter values by sampling station are shown in Table 3.

Table 3

Summary data for physical chemical measurements, marine water samples, Tres Palmas Marine Reserve, Rincon, Puerto Rico, May – December 2011, n = 10 measurements for most parameters, 9 for stations R_NO and R_SE for turbidity, and 4 for suspended solids, data as mean ± standard deviation/median, range, units as shown.

Sampling Station	Dissolved Oxygen	Suspended Solids	Conductivity	pH	Salinity	Temperature	Turbidity
	(mg/L)	(mg/L)	(µS/cm)		(ppt)	(°C)	(NTU)
C_NO	6.4 ± 0.1/ 6.4, 6.3-6.5	221.0 ±47.8/ 239, 168-279	53.6 ±0.7/ 53.6,52.8-54.8	8.2 ± 0.0/ 8.2,8.1-8.2	35.6 ± 0.5/ 35.6, 35.0-36.4	28.6 ± 0.8/ 28.8, 26.9-29.6	0.3 ± 0.1/ 0.3, 0.1-0.5
C_NE	6.0 ± 0.3/ 6.0, 5.5-6.3	186.0 ± 21.7/ 188, 161-209	53.6 ±0.7/ 53.6,52.8-54.8	8.1 ±0.1/ 8.1,8.1-8.2	35.6 ± 0.5/ 35.6, 34.9-36.4	28.5 ± 0.8/ 28.7, 26.7-29.4	0.5 ± 0.4/ 0.4, 0.1-1.3
R_NO	6.3 ± 0.1/ 6.4,6.2-6.5	257.8 ± 34.1/ 250, 219-310	53.6 ±0.7/ 53.7,52.8-54.8	8.1 ±0.0/ 8.2,8.1-8.2	35.6 ± 0.5/ 35.6, 35.0-36.4	28.6 ±0.8/ 28.8, 27.0-29.5	0.4 ± 0.2/ 0.3, 0.1-0.7
R_NE	6.1 ± 0.2/ 6.2, 5.7-6.3	226.0 ± 58.5/ 235, 128-276	53.6 ±0.7/ 53.6,52.8-54.8	8.1±0.0/ 8.1,8.1-8.2	35.6 ± 0.5/ 35.6, 35.0-36.5	28.5 ± 0.8/ 28.7, 26.8-29.5	0.5 ± 0.3/ 0.4, 0.3-0.9
R_SO	6.3 ± 0.1/ 6.3, 6.0-6.5	246.0 ± 27.1/ 254, 211-278	53.6 ±0.7/ 53.5,52.8-54.8	8.2 ±0.0/ 8.2,8.1-8.2	35.6 ± 0.5/ 35.2, 35.1-36.4	28.6 ± 0.8/ 28.8, 27.0-29.6	0.5 ± 0.3/ 0.5, 0.2-1.0
R_SE	6.1 ± 0.2/ 6.1, 5.7-6.5	263.8 ± 34.0/ 259, 225-306	53.6 ±0.7/ 53.6,52.7-54.8	8.1 ±0.1/ 8.1,8.1-8.3	35.6 ± 0.5/ 35.6, 34.9-36.4	28.5 ± 0.9/ 28.7, 26.8-29.4	0.8 ± 0.6/ 0.6, 0.3-2.0
C_SO	6.4 ± 0.1/ 6.4, 6.0-6.5	247.8 ± 43.3/ 239, 190-308	53.6 ±0.7/ 53.6,52.8-54.8	8.2 ±0.1/ 8.2,8.1-8.4	35.6 ± 0.5/ 35.5, 35.0-36.4	28.6 ± 0.8/ 28.9, 27.0-29.6	0.4 ± 0.2/ 0.3, 0.2-0.7
C_SE	6.2 ± 0.3/ 6.1, 6.0-6.8	227.0 ± 47.4/ 228, 166-275	53.6 ±0.7/ 53.6,52.7-54.9	8.2 ±0.2/ 8.2,8.1-8.7	35.6 ± 0.5/ 35.6, 34.9-36.4	28.5 ± 0.8/ 28.8, 26.9-29.3	0.9 ± 0.6/ 0.7, 0.3-2.2
Q_C_N	6.1 ± 0.1/ 6.1, 5.8-6.3	262.0 ± 22.6/ 260, 240-297	53.6 ±0.7/ 53.5,52.7-54.7	8.2 ±0.2/ 8.1,8.1-8.9	35.6 ± 0.5/ 35.5, 35.0-36.4	28.5 ± 0.9/ 28.8, 26.9-29.7	1.3 ± 1.7/ 0.7, 0.4-5.8
Total	6.2 ± 0.2/ 6.3, 5.5-6.8	237.5 ± 42.8/ 240, 128-310	53.6 ±0.7/ 53.6,52.6-54.9	8.2 ±0.1/ 8.1,8.1-8.9	35.6 ± 0.5/ 35.6, 34.9-36.5	28.5 ± 0.8/ 28.8, 26.7-29.7	0.6 ± 0.7/ 0.4, 0.1-5.8

The main difference in turbidity and suspended solids between sampling dates was probably due to the amount of rainfall-driven runoff.

3.3. Results of microbiological analyses of water quality

As shown in Table 4, Fecal coliform densities were higher near the mouths of creeks draining the watersheds with the largest amount of septic tanks along the south part of the TPMR. The difference in Fecal coliforms densities between the four northern stations and the five southern stations showed to be statistically significant (ANOVA, $p < 0.01$). Also, the difference in Fecal coliforms densities between the four stations within the Marine Reserve and the five control stations showed to be statistically significant (ANOVA, $p < 0.02$).

Table 4

Summary statistics for Fecal coliform densities by sampling station (n=10 for each sampling station). Sampling stations are ordered from north to south, with the western station (offshore) followed by the eastern station (near shore) for each pair of aligned sampling points.

Sampling Station	Average of Fecal coliforms (CFU/100 mL)	Median of Fecal coliforms (CFU/100 mL)	Minimum	Maximum
C_NO	0.2 ± 0.4	0.00	0.0	1.0
C_NE	0.8 ± 1.1	0.21	0.0	3.3
R_NO	2.2 ± 5.0	0.21	0.0	16.3
R_NE	1.7 ± 3.4	0.13	0.0	10.6
R_SO	0.6 ± 1.1	0.00	0.0	3.0
R_SE	2.1 ± 2.6	0.88	0.0	7.7
C_SO	0.6 ± 1.4	0.04	0.0	4.5
C_SE	9.8 ± 15.4	3.34	0.2	48.3
Q_C_N	20.2 ± 17.4	16.34	0.8	51.7
Total	4.2 ± 10.0	0.33	0.00	51.7

The box plot shown below (Figure 4) reflects pattern of Fecal coliform densities by sampling station in graphic form. The box-plot figures show the median values with a white horizontal stripe that dissects the gray vertical box, which shows the distribution of the central half of the observations. The thin vertical lines that extend up and down the central box (whiskers) represent the distribution of the adjacent observations.

Figure 4 shows a wider distribution of observed values of Fecal coliform densities in the southernmost sampling stations.

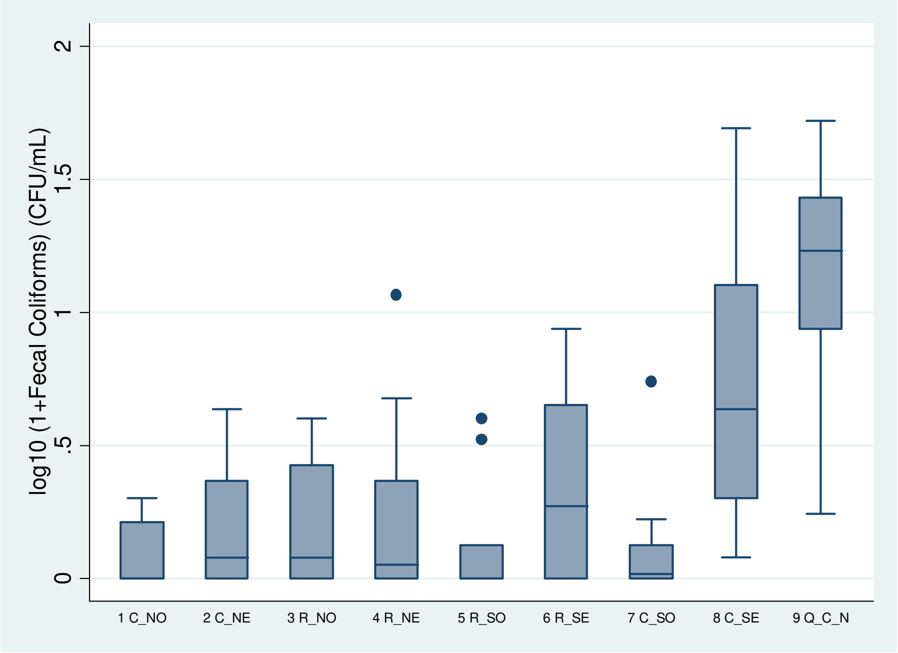

Figure 4. Distribution Log$_{10}$ modified Fecal coliform densities (1+ Fecal coliforms) (CFU/100 mL) by sampling station in TPMR in Rincon, during May – December, 2011.

Average daily densities of enterococci at the different coastal marine stations ranged from 0 to 110 colony forming units (CFU/100 mL) along the insular platform where the TPMR is located. The mean daily density was 11.1 and the median was 3.5 (CFU)/100 mL for all observations. Standard deviation was 11.5.

The EPA recreational water quality criterion was not exceeded on any sampling date during the study period (USEPA, 2012). For all marine sampling stations, the 90th percentile of Enterococci density measurements did not exceed the Statistical Threshold Value of 130 (cfu/100 mL). The highest densities occurred at stations near the south edge of the TPMR. This is near the mouth of the Vista Azul creek, draining the watershed with the largest number of septic tanks. This implies that measurements of higher densities of enterococcus reflect the lingering effect of pulses of storm water runoff carrying bacteria of fecal origin. In repeated land field visits most of the creeks were dry or with very small flow the same day water quality samples were taken. Septic tanks in the watersheds of creeks flowing into TPMR are the only significant source of these bacteria observed in the area.

As seen in Table 5, Southern stations within the Reserve and controls close to the south edge of the Reserve showed averages of up to 25.3 CFU/100 mL of enterococci, while the North sites furthest from the coast reached an average down to 3.1 CFU/100 mL of enterococci after analyzing samples from the ten sampling visits. The difference in Enterococcus densities between the four northern stations and the five southern stations showed to be statistically significant (ANOVA $p < 0.01$) Table 5 shows mean Enterococcus densities in CFU/100 mL ranged from 4.3 to 17.6 for sampling stations within the reserve. Maximum values in this table represent the 90th percentile for each station since this study used a total of ten sampling dates.

Table 5

Average Enterococcus densities by sampling station (n=10 for each sampling station). Sampling stations are ordered from north to south, with the western station (offshore) followed by the eastern station (near shore) for each pair of aligned sampling points.

Sampling Station	Average Enterococcus densities (CFU/100 mL)	Median Enterococcus densities (CFU/100 mL)	Minimum (CFU/100 mL)	Maximum (CFU/100 mL)
C_NO	3.1 ± 7.55	0.17	0.0	24.3
C_NE	5.4 ± 7.3	1.38	0.1	22.3
R_NO	7.9 ± 17.21	0.84	0.0	56.0
R_NE	4.3 ± 7.8	0.33	0.0	22.3
R_SO	5.5 ± 6.3	2.71	0.0	17.0

R_SE	17.6 ± 33.8	4.29	0.3	110.3
C_SO	15.1 ± 19.0	8.28	0.0	61.8
C_SE	15.8 ± 20.0	6.46	0.0	59.6
Q_C_N	25.3 ± 21.8	19.96	3.6	77.3
Total	11.1 ± 18.5	3.48	0.0	110.3

Figure 5 shows a box-plot of the distribution of measurements of Enterococcus densities by sampling station in TPMR during May – December 2011.

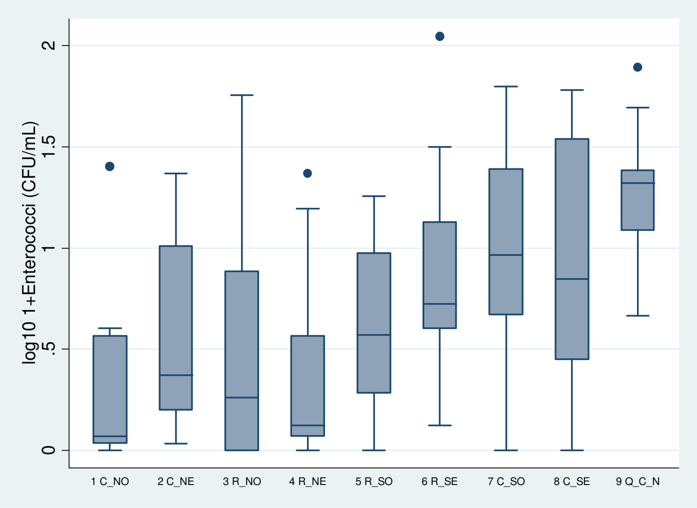

Figure 5. Distribution Log_{10} of modified Enterococcus densities (1+ Enterococcus) (CFU/100 mL) by sampling station in TPMR in Rincon during May – December 2011.

Station "Yacht Club Creek" (Q_C_N) represents the marine sampling station closest to the mouth of study creeks that drain just to the north of the recreational beach of Rincon and just to the south of the Southern limit of the Reserve. Together these two creeks drain about 309 hectares of land with residences served by septic systems. This catchment area is greater than that of any other creeks that drain into the Reserve. In the adjacent area to the south of the Reserve, near the Club Náutico (Yacht Club), the densities ranged from 0 to 110 CFU/100 mL of enterococci between days of sampling, demonstrating the effect of pulses of contaminated runoff during rainy days.

3.4. Results of the association between Septic Weight Index and marine water quality

Table 6 shows statistically significant results obtained associating Septic Weight Index with most of the water quality parameters.

Table 6

Summary of regression analysis of Septic Weight Index and marine water quality. Septic Weight Index was used as a predictor variable controlling for sampling date therefore controlling for rain events.

Parameter	**# of observations**	**Coefficient**	**Prob(F)**	**R^2**	**Adjusted R^2**	**95% Confidence Interval**
Fecal coliforms	90	5.98	0.00	0.48	0.41	4.46 - 7.50
Enterococci	90	5.09	0.00	0.35	0.26	1.89 - 8.28
Turbidity	88	0.29	0.00	0.50	0.43	0.19 - 0.40
Suspended Solids	45	3.90	0.43	0.35	0.26	-6.08- 13.89
Salinity	90	-0.03	0.00	0.99	0.98	-0.04 - 0.02
Conductivity	90	-0.03	0.00	0.98	0.98	-0.05 - 0.01
Dissolved Oxygen	90	-0.06	0.01	0.22	0.12	-0.11 - -0.02

All microbiological and physical chemical water quality parameters except suspended solids show a statistically significant linear association with the Septic Weight Index. This means that the number of septic tanks in each creek's watershed and the distance from the creek mouths determine to a large extent the density and concentration of water quality parameters at the marine monitoring stations. The statistical model using Septic Weight Index and using sampling date as a dummy variable accounted for forty-eight, thirty-five, and fifty percent of the variance (R^2), respectively, in the Fecal coliform, Enterococci, and Turbidity parameters of coastal marine water quality. The adjusted R^2, which takes into account the degrees of freedom in the linear model, was 0.41, 0.26 and 0.43, respectively. Suspended solids did not show a significant linear association with Septic Weight Index, probably due to other factors affecting this parameter such as oceanic coastal turbulence.

3.5. Results of association between Septic Weight Index and coral reef condition

Table 7 shows the results obtained associating Septic Weight Index with the parameters corresponding to condition of coral reef. The Septic Weight Index showed a significant (although partial) linear association with the parameter % Bleaching of colonies of Elkhorn coral (*Acropora palmata*). Two transects out of thirty showed above zero percent bleaching in the corresponding colonies. Both transects, number five (0.10%) and number 7 (0.03%) are located in the southern portion of the reserve. A marginal linear association was found with the parameters % Living tissue cover and % Old mortality.

Table 7

Summary of linear regression analysis of Septic Weight and coral reef quality.

Parameter	# of observations	Coefficient	Prob(F)	R^2	Adjusted R^2	95% confidence interval
% Living tissue cover	30	13.39	0.10	0.09	0.06	-2.84 - 29.63
% Old mortality	30	-13.62	0.10	0.09	0.06	-30.13 - 2.89
% Recent mortality	30	0.22	0.70	0.01	-0.03	-0.99 - 1.44
% Bleaching	30	0.16	0.03	0.17	0.14	-5.42-15.19
% Diseased	30	0.24	0.68	0.01	-0.03	-0.94 - 1.42
Snails/colony	30	0.62	0.60	0.01	-0.03	-1.80 - 3.05
Damselfish/ colony	30	-11.52	0.23	0.05	0.01	-30.86 - 7.82
Area (m^2)	30	-11.83	0.19	0.06	0.03	-29.84 - 6.18
Vol (m^3)	30	-10.25	0.22	0.05	0.02	-27.16 - 6.66
Live Area Index (m^2)	30	-7.53	0.15	0.07	0.04	-18.04 - 2.98
% Recruits	30	0.06	0.94	0.00	-0.03	-1.61 - 1.74
% Fragmented	30		0.19	0.06	0.03	

3.6. Results of analyses of rainfall and marine water quality in TPMR

Rainfall data were obtained from Coloso NWS rainfall station in Aguada, Puerto Rico. This is the nearest weather station for which continuous data was found for the study period. Linear regression analyses of 16-day rainfall and both Fecal coliforms and Enterococcus in marine sampling stations were carried out. A scatter plot of Log10 (1+Fecal coliforms) (CFU/100mL) in TPMR and 16-day rainfall (mm) by sampling station was constructed. A sum of rainfall during the 16 days prior to and including the sampling date from May to December 2011 was used. The same procedure was followed using Log10 (1+Enterococci). No significant association between rainfall and either of the microbiological indicators was found.

The rainfall data used were from a station located about 10 Km inland, outside of the sub-watersheds that drain into the Tres Palmas Marine Reserve (Figure 1). Since this region of the northwest coast of Puerto Rico is characterized by localized rain downpours, these data are not sufficiently representative of the rainfall within the sub-watersheds considered in this study to capture a statistical association between rainfall and water quality. The Coloso NWS rainfall station in Aguada is actually located in a different climatic subdivision of Puerto Rico than is the study area according to the USGS (Rico, n.d., p.11). Apparently, the fact that in this region (Mayagüez) prevailing winds come from the west, as opposed to the rest of Puerto Rico main island, might be related to the sparsity and small size of clouds producing precipitation in the study area. A rain gage was installed at the Surfrider Foundation office in downtown Rincon, about 1.5 kilometers form the study area, and even that data proved to be unrepresentative of rainfall events in these small study watersheds. Personnel from the Foundation attempted to gather water samples from the study area creeks while heavy downpours occurred in downtown Rincon and found no rainfall and the creeks dry within the study area at those times.

4. Discussion

4.1. Discussion of implications of fecal indicator bacteria densities in TPMR

The fact that the PREQB water quality standard was not exceeded at any station reflects both the high quality of the marine reserve waters and the ephemeral presence in TPMR of Fecal coliform indicators of marine water pollution of fecal origin. Mean densities of Fecal coliforms for sampling stations within the marine reserve were lower than those in control points outside and near the reserve.

Previous studies show a significant relationship between land use and stream water quality, especially for Fecal coliform (Tong and Chen, 2002**)**. In sites where non-point pollution sources predominate, high bacterial loads have been shown to increase in water bodies after rain events (Crowther et al. 2001; Lipp et al. 2001; Abdelzaher et al. 2011). Conventional Fecal Indicator Bacteria (FIB) concentrations in untreated sewage are approximately 10^7 CFU/100 ml for total coliforms and 10^6 CFU/100 ml for Fecal coliforms and enterococci (Harwood VJ, et al. 2005, as cited by McQuaig et al., 2012).

A study of urban and non-urban watersheds in southern California found that bacteria concentrations in storm water runoff varied based on the contributing land use in the following manner: Recreational with horse livestock > agricultural with animal fertilizer> urban > open space (Tiefenthaler et al., 2011). The study found the following Mean *E. coli* concentrations for storm flow from the corresponding land uses: 5.3×10^5 MPN/100 ml for recreational with horse livestock; 8.2×10^3 for commercial, 7.8×10^3 high density residential (HDR), 6.5×10^3 for low density residential.

In Hanoi, the capital of Vietnam, runoff in urban areas is the major source of fecal pollution of surface water bodies (Gruber et al., 2005, as cited by Quan, et al., 2010). In this case a total area of more than 3,300 km^2 housed 6.4 million inhabitants in 2009. The sewerage system is a combined type, where both wastewater and storm runoff water are served by one system. In a 2008 study of runoff during rainfall events there, *E. coli* densities of up to 5×10^4 CFU/100 mL and Total coliform densities of up to 1×10^5 CFU/100 mL were measured (Quan, et al., 2010).

In a Southern California study carried out at Doheny State Beach (Dana Point, CA), affected mostly by urban runoff, results showed the following average Log_{10} FIB levels (CFU/100 ml) (McQuaig et al., 2012): Total coliforms, 0.1 – 4.1; Fecal coliforms, 1.1 – 3.1; Enterococci 1.1 – 3.1. The same study showed the following results for Avalon Beach (Catalina Island, CA), affected by sewers, as average Log_{10} FIB levels (CFU/100 ml): Total coliforms, 2.5 – 3.0; Fecal coliforms, 0.7 – 2.7; Enterococci 0.6 – 2.0.

In our study, lower densities of Fecal coliforms were found in coastal marine waters than in the studies described, probably due to the low density, forested, mostly residential land cover in watersheds near TPMR.

There might a small but potential threat to adequate water quality at the Rincon public beach when affected by storm water pulses. The beach is located just south of the southern edge of the marine reserve, near where a 90th percentile value of 110 (cfu/100mL) was observed at random sampling times. Therefore Enterococci densities have the potential to exceed the 130 (cfu/100mL) STV during or shortly after rainstorms at that beach. Slightly lower Enterococci densities prevail within the reserve than in control points outside the reserve and where the public beach is located. This finding is consistent with results obtained with the Fecal coliforms indicator and suggests that the establishment of the Marine Reserve at Tres Palmas is potentially helping to maintain adequate marine water quality.

4.2. Discussion of the usefulness of the Septic Weight Index as a predictor of water quality of marine reserve

The Septic Weight Index proved to be a powerful predictor of water quality of marine reserve waters. A highly significant linear association was observed between this index and microbiologic and physical-chemical parameters, except suspended solids. This means that it is useful to measure the impact of malfunctioning septic tanks on marine water quality in this instance of a relatively clean near coastal marine reserve affected by several small creeks draining small forested watersheds with almost exclusive residential land uses. Of noteworthiness is the high significance of the linear association and high percentage of the variance of Fecal coliforms, Enterococci, and Turbidity explained by the model. These water pollution variables can all be associated with the effect of malfunctioning septic tanks.

These results are consistent with previous studies modeling the effect of land use on fecal pollution of marine waters. In a study in South Carolina where statistical association was also measured between land use and fecal pollution, R^2 values for the models tested indicated that 45–50% of the variability of Fecal coliform densities observed in a nearby estuary was explained by the variables representing seasonal precipitation and land use (Kelsey et al., 2004). The land use variables included in the regression model were the following: distance to urban area, distance to road, weighted septic tank/distance variable, weighted urbanized area in delineated watersheds/distance variable, weighted urbanized area in approximated watersheds/distance variable, and distance to marina area. The study found that specific land-use parameters can be identified as substantial contributors of fecal contamination and are important considerations for management of fecal pollution in an estuary.

The high energy coastline of the Mona Passage, where Tres Palmas is located, is subject to strong tidal currents (Segura-Torres, 2000, p.317). The question remains whether longshore currents bring suspended sediments and contaminants from areas outside the drainage areas considered in this study. The analyses of data from this study cannot fully address this issue, although the statistically significant differences between sampling sites and the low turbidity and fecal indicator densities at most sites point against a phenomenon regional in nature. Also, the barotropic tide (surface tide) propagates from northeast to southwest along Mona Passage. The semidiurnal current ellipses in the Mona Passage, with a clockwise rotation, are roughly aligned in a north–south direction (Rosario-Llantín, 2000, p. 82). Thus the main surface current in the area of Tres Palmas comes from the Atlantic flowing southward. This study measured fecal indicator densities near shore at Tres Palmas, which is protected from the region's principal source of contaminants (sediments and bacteria) to its north (up current). The Rincon peninsula stands in the way of contaminants coming from the largest contaminant source to the north in this area, the Culebrinas River mouth in Aguada (18 km north along shore). Meanwhile, the other large regional contaminant source, the Añasco River mouth, lies 10 km down current to the south. Tidal current effects on bacteriological water quality would be minimal under these conditions of large distances from pollution sources and direction of tidal currents.

4.3. Discussion of the usefulness of the Septic Weight Index as a predictor of conditions of coral reefs of Tres Palmas Marine Reserve

The fact that Septic Weight Index showed a significant linear association with percent bleaching (albeit explaining only part of the variance) in the Elkhorn coral (*Acropora palmata*) colonies suggests that the sewage-laden runoff pulses occurring at the Tres Palmas Marine Reserve might have some near shore ecological impact. In a different study, identification of *Serratia marcescens* strain PDR60 in sewage, in diseased *Acropora palmata*, and in corallivorious snails (Coralliophila abbreviata) within a discrete time frame in the Florida Keys suggested a causal link between white pox and sewage contamination on reefs and supported the conclusion that humans are a likely source of this disease (Sutherland, 2010). On the other hand, in our case of coral bleaching in Rincon, low salinity and sediments from fresh water pulses could be the physical factor responsible for part of the bleaching, in addition to the presence of fecal pollution.

Intense coral bleaching in Puerto Rico has been associated with sea surface temperature in other studies (Ballantine et al., 2008; Bonkosky et al., 2009: Hernández et al., 2008, 2011). The 2005 bleaching event and post bleaching mass mortality followed record- breaking sea surface temperatures up to 31.8°C at 30 m depths and 33.1°C at reef crests in La Parguera (Ballantine et al., 2008). While the data in our study show that the Septic Weight Index only explains 14-16% of the variability (R^2) in *Acropora palmata* bleaching, future studies may further explore the effect of sewage on Elkhorn coral bleaching. The marginal linear association found between Septic Weight Index and the parameters % Living tissue cover and % Old mortality reinforce the finding of a possible link between sewage-laden runoff and ecological impact on coral reefs at this site.

5. Conclusions

The Septic Weight Index proved to be a very accurate predictor of water quality in the near shore of Rincon, where the TPMR is located. The results of this study demonstrate the need to address the problem of ill-designed and malfunctioning septic systems in Puerto Rico in order to protect costal water quality and coral reefs, even in protected marine reserves where coastal water appears of high quality during average conditions. The need for government and private sector action in this matter is urgent to protect marine resources in Puerto Rico.

The coastal sites in this study are actually quite clean with low Fecal coliform counts. It can be concluded that studies can be carried out using this model to shed light on water quality impacts of residential and commercial malfunctioning septic tanks even at relatively clean sites. The Septic Weight Index was able to predict differences in water quality among marine sampling stations with high accuracy under these conditions. It also helped to explain part of the variance in some parameters of condition of coral reefs (Norat-Ramírez, 2012). The evidence suggests the need for further study on possible chronic impacts from storm water runoff from the southernmost study watershed on the population of Elk Horn coral, *Acropora palmata*. This species is listed as "threatened" under the Federal law of species in danger of extinction. These potential impacts warrant immediate corrective actions. At the specific areas where this problem is of concern, reconstruction of septic tanks following adequate engineering design would be of great benefit to coral reefs and the whole environment. The Puerto Rico government could also improve efforts to connect these residences to sewer lines where soil or other hydraulic conditions do not permit septic tank overhaul. Our study provides the location of these problem areas so that stakeholders can recommend specific actions. This will support petitions and recommendations from the Department of Natural and Environmental Resources (DNER) to other government agencies to act on malfunctioning septic tanks in Puerto Rico. The DNER has jurisdiction on the management of the Natural Reserves, but not on the regulation of septic tanks. This jurisdiction belongs to the Puerto Rico Department of Health within the construction permit process. The Environmental Quality Board (EQB) regulates pollutant discharges into water bodies, but its water quality program is inadequately funded at the present time. Extensive site inspections would be needed for EQB to intervene with all inadequate septic tank owners as violators of its "Regulation of Water Quality Standards". Additional resources would be needed for litigation costs, which the Puerto Rico government cannot afford within the present budgetary constraints in Puerto Rico. It is even politically risky for the Central Government to act against these mostly indigent septic tank owners if solid scientific evidence of imminent and extensive danger is not provided. Nearly 50% of the Puerto Rico population of 4 million people lives in residences not connected to sewer lines.

Global initiatives have been carried out regarding this matter such as “The Global Program of Action for the Protection of the Marine Environment from Land-based Activities” of the United Nations Environment Program. This was adopted by 108 Governments and the European Commission at an intergovernmental conference convened for this purpose in Washington, D.C., United States of America, in 1995 (See “Washington Declaration On Protection Of The Marine Environment From Land-Based Activities” of 1995, Rollo and Robin, 2010). The International Private Sewage Disposal Code (IPSDC) of 2009 was adopted in Puerto Rico in 2010 (PROGPe, 2012). The implementation of the IPSDC is particularly challenging in Puerto Rico given the unregulated construction of septic tanks over the years, limited size of many residential plots and poor quality of much of the soil where homes are located. The fractured jurisdiction of the issue, which also involves municipal governments, the Consumer Affairs Department (DACO by its Spanish acronym), the Permits & Endorsements Management Office (OGPe by its Spanish acronym) and the Aqueduct & Sewer Authority also doesn't help (Marino, 2012). All these obstacles must be overcome to protect our marine environment.

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