Environmental Impact of the Culebra Municipal Landfill on the Luis Peña Canal Reserve Coral Reef Population

Final Report

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by

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Abstract

This study was aimed at: (1) evaluating what are the physical and microbiological water quality conditions within and outside the Luis Peña Channel No-Take Natural Reserve (LPCNR); (2) determining if there was any significant water quality stress gradient associated to non-point source pollution (i.e., turbidity, sewage) that could pose any threat to coral reef communities; and (3) carry out a biological characterization of the actual status of shallow-water coral reef communities across a suspected water quality stress gradient. There was evidence of strong gradient impacts associated to sediment- and nutrient-laden runoff pulses, in combination with sewage pollution pulses in Culebra. Water quality (i.e., turbidity) showed both, significant spatial and temporal fluctuations, largely responding to heavy rainfall and subsequent runoff pulses. Lack of sewage treatment facilities in Culebra have resulted in a proliferation of poorly designed and constructed septic tanks, septic tanks constructed below the coastal water table, and numerous illegal raw sewage discharges to stormwater sewers and culverts. There is usually a strong relationship between sediment- and nutrient-laden runoff pulses and raw sewage pulses. Frequent violations to current microbiological water quality standards suggest that coastal waters near Culebra's population centers are chronically polluted by raw sewage pulses. Behavior of turbidity and sewage pulses largely followed to variable oceanographic and atmospheric dynamics associated to: (1) tidal flow; (2) turbidity pulses; (3) wind direction and velocity; (4) wind-driven circulation; (5) southeastern wave action; and (6) heavy rainfall. These fluctuations were suggested by the presence of higher concentrations of microbial indicators in samples collected during strong wind-driven circulation and sediment resuspension events, and ebbing tides followed by heavy rainfall runoff. Ebbing flow at Ensenada Honda causes a strong flow through largely-polluted Lobina Channel towards Bahía Sardinas, significantly increased the risks of pollution to adjacent waters. Eventually, such flows largely move towards the northwest throughout the Luis Peña Channel No-Take Natural Reserve, therefore carrying over all pollution towards significant coral reef and seagrass systems. Pollution pulses have created a strong gradient of impacts on shallow coral reef benthic communities. Variable sources may include (1) malfunctioning septic tanks, (2) illegal sewage discharges from private houses and businesses (i.e., restaurants), (3) raw sewage-polluted stormwater sewers; and (4) sediments from widespread deforestation for construction. Benthic communities at frequently disturbed sites were

dominated by macroalgae, dead coral surfaces, cyanobacteria, algal turfs, low coral species richness, low H’n and J’n, and the coral fauna was dominated by a few surviving patches of *Porites porites*. Old dead standing colonies of threatened *Acropora palmata*, as well as rodolites of former colonies of threatened *A. cervicornis*, were frequently observed. There is an imperative need to design integrated coastal management strategies to address marine, coastal-maritime zone, and watershed management needs in order to reduce fecal and runoff pollution impacts in the coast. Water quality management needs include identification of strategies to control most of the dispersed sources of fecal contamination in Culebra. In order to achieve this, surveys of sources of pollution need to be identified, geo-referenced and mapped. Similarly, mapping of areas subjected to strong erosion impacts, currently underway by DNER, needs to be completed. Such information may provide important tools to help prioritize management actions and land use in the near future. There is also a strong need to implement immediate erosion-control management practices and watershed reforestation. This is paramount to significantly reduce sedimentation impacts in sensitive habitats that serve as nursery and feeding grounds of commercially important species, as well as for the several endangered species. In face of expected recurrent environmental impacts associated to climate change in the near future, frequent non-point source pollution episodes can result in a further long-term decline of coastal ecological conditions. Such declines could further result in a major community phase shift, negatively affecting ecosystem functions, degrading essential fish habitats and losing their long-term economic value.

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I. Introduction and Rationale

This study was aimed at evaluating the physical and microbiological water quality conditions within and outside the LPCNR. Also, to determine if there was any significant water quality stress gradient associated to non-point source pollution (i.e., turbidity, sewage) that could pose any threat to coral reef communities. Our third objective was to carry out a biological characterization of the actual status of shallow-water coral reef communities across a suspected water quality stress gradient.

Human activities in Culebra Island, Puerto Rico, are one of the most significant environmental threats to marine communities located within the Luis Peña Channel No-Take Natural Reserve (LPCNR) (Hernández-Delgado, 1994, 2003b, 2004). Biological communities within the reserve support an outstanding biodiversity representative of the northeastern Caribbean region (Hernández-Delgado, 2000, 2003; Hernández-Delgado et al., 2000; Hernández-Delgado and Rosado-Matías, 2003). Biological communities located in Bahía Tamarindo and Punta Rompeanzuelo, are highly diverse and highly structured (Hernández-Delgado, 1994, 2003a). However, water quality degradation has been pointed out as one of the causes of coral reef and fish community declines in Culebra (Hernández-Delgado and Sabat, in review; Hernández-Delgado et al., in press). The Department of Natural and Environmental Resources (DNER) is highly concerned with the potential impacts of human activities on the local marine communities. But in spite of the ecological, economic, aesthetic and touristic significance of Bahía Tamarindo marine communities, there is no information regarding environmental impacts of human settlements.

Water quality study parameters have been changed to microbiological indicators of wastewater pollution. This is due to the fact that results of the study of heavy metal and toxic organic pollutants in the municipal landfill leachate show undetectable concentrations. It was decided to use fecal indicators to look for impacts of human settlements on coral reefs using the same methodology for coral sample evaluation and extraction. Results of analysis for heavy metals in leachate are included as an Appendix. Laboratory results for other parameters studied are presented as a separate file in PDF format.

II. Research Objectives

The objectives of this phase of the study were to:

1) test for the density of microbial indicators in the water column near the coral reef study sites
2) determine if there is any significant spatial pattern in the community structure of coral reefs resulting from human settlement-based pollution
3) determine if there is any significant spatial pattern in coral recruitment rates resulting from potential human settlement -based pollution
4) use a before-after-control-impact-paired-series (BACIPS) approach to document if there is any signal in coral annual growth bands that can detect spatial-temporal trends in human settlements -based pollution in coral reefs;

III. Methodology

Water quality sampling design.

The microbiological and physico-chemical water quality parameter sampling stations are shown in Figure 1. The sampling design includes three sampling stations located close to human population centers (Ensenada Honda, Canal de Lobina, Bahía Sardinas), and three located farther away, but downstream, within the Luis Peña Channel Natural Reserve (Punta Tamarindo Chico, Punta Rompeanzuelo, Playa Carlos Rosario). Punta Rompeanzuelo is presumably influenced by landfill operations.

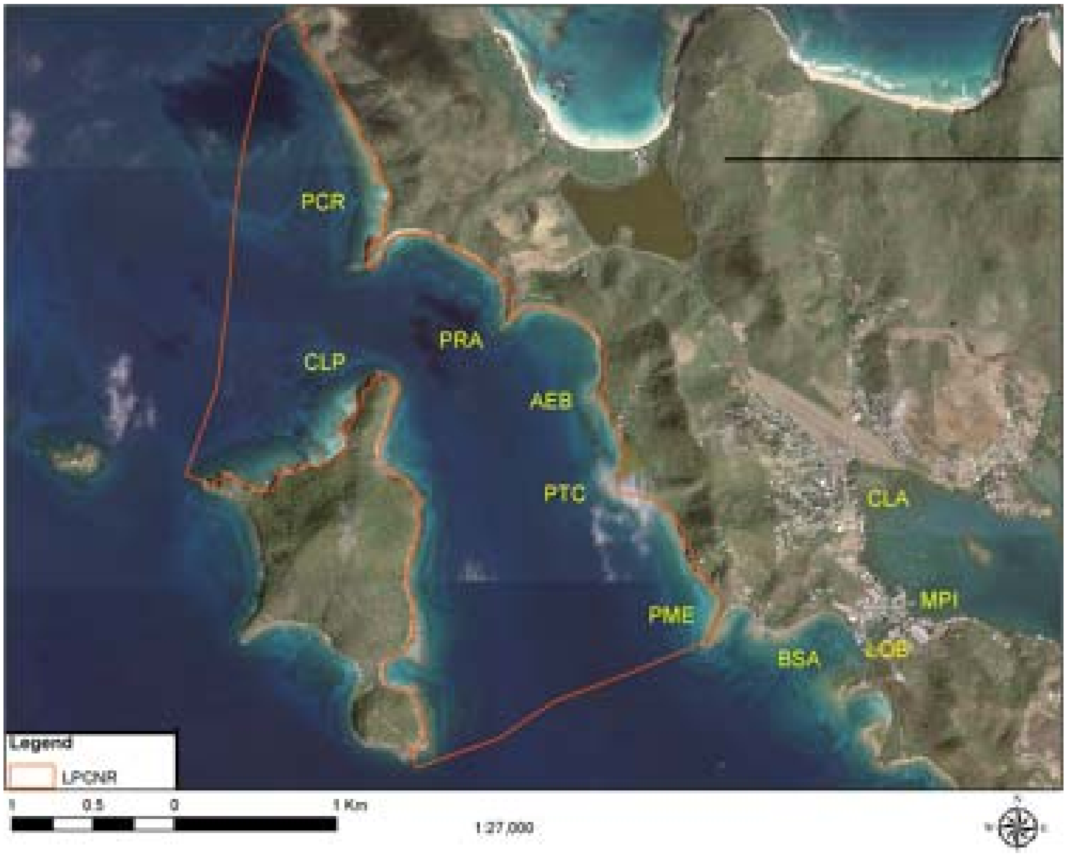

FIGURE 1. Study sites. CLA= Ensenada Honda at Barriada Clark; MPI= Ensenada Honda at Municipal Pier; LOB= Lobina Channel; BSA= Bahía Sardinas; PME= Punta Melones; PTC= Punta Tamarindo Chico; AEB= Arrecife El Banderote; PRA= Punta Rompe Anzuelo; CLP= Cayo Luis Peña-north; and PCR= Playa Carlos Rosario-south. Coral reef study sites included: BSA, PME, PTC, AEB, PRA, CLP, and PCR. Source: CRIM aerial image (2007).

There are no sewage treatment facilities operating in Culebra Island. Therefore raw sewage is emptied either at usually overloaded septic tanks or directly to the ocean. Human-influenced sites receive frequent raw sewage pulsed from downtown Dewey.

Water quality sampling design is based on triplicate samples from depth of 30 cm below surface. Samples will be collected once monthly for a period of one year beginning in May 16, 2008. Microbial analysis will include standard indicator microorganisms (fecal coliforms, enterococci) following standard methods, as well as the use of genetic probes designed to detect specific human-related microorganisms using QPCR techniques. Water physico-chemical parameters (i.e., temperature, salinity, conductivity, pH, dissolved oxygen concentration, turbidity) will also be assessed in triplicate measures during each sample collection. All materials for microbiological analyses were already ordered and will arrive within the next week or two before the first sampling effort.

Sampling sites

Sampling has been conducted periodically from May 2008 until November 2008. Representative water sampling sites were selected based on geographically related gradients from Clark neighborhood, Bahía Ensenada (MMU), Canal Lobina, Bahía Sardina (BSA), and through the Luis Peña Canal (CLP) up to Playa Carlos Rosario (PCR) representative coral reef/seagrass habitats. These included the following: Punta Melones (PME); Punta Rompe Anzuelo (PRA); Arrecife el Banderote (AEB); Punta Tamarindo Chico (PTC).

Site description

CLARK: high human impact expected, do to proximity to human settlements.

MMU: high human impact expected, do to proximity to human settlements and nautical operations.

LOBINA: high human impact expected, do to proximity to human settlements and nautical operations.

BSA: high human impact expected, do to proximity to human settlements and nautical operations.

PME: human impact expected, do to human settlements and nautical operations.

PRA: indirect human impact expected, do to landfill runoff during rainfall periods.

PTC: pristine area, no mayor human impact expected.

AEB: pristine area, no mayor human impact expected.

PCR: pristine area, no mayor human impact expected.

CLP: pristine area, no mayor human impact expected.

Water sample collection

Grab samples, were collected at each study site using autoclaved 1L wide mouth polypropylene Nalgene ® bottles (Nalgene Co.). Using sterilized gloves, samples were collected at 30 cm below surface with the bottle facing the current, and immediately placed on ice until analyzed within 6 hours.

Physicochemical Parameters

In situ analysis of physicochemical data such as temperature (°C), pH, salinity (%), conductivity (ms/cm), dissolved oxygen (mg/L) were measured using a Horiba Water Quality Checker U-10 portable instrument. These parameters were taken in triplicate to submit for statistical analysis. Water turbidity was documented using a Hach 2100P portable turbidimeter. Turbidity measurements were expressed in Nephelometric Turbidity Units (NTU). Results are presented in Appendix B.

Water quality analysis materials and methods

Samples were obtained of the water column for analysis of bacteriological water quality. Three parameters are being used to indicate presence of fecal matter of human origin. Fecal coliform and enteroccoci counts are being obtained from cultures using membrane filter techniques. QPCR technique will be used to capture DNA of the Human Bacteroides group and measure density or cell equivalents. The densities of the three parameters will be associated statistically with health of adjacent coral reef formations to determine the effect of wastewater on them.

Membrane Filtration

Samples were analyzed using standard membrane filtration techniques to quantify fecal coliforms and enterococci (APHA). Water samples were filtered using cellulose acetate membranes, which have a porosity of 0.45 µm, using Millipore filter funnels (Millipore, Co.). 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, while Fecal streptococci were quantified using m-Enterococcus media. Confirmatory tests for both were carried out using *Lauryl Triptose Broth* (LTB) and *Azide Dextrose Broth* (ADB) respectively. 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, 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, heated, agitated, mixed and poured on sterile plates. Plates were incubated at 35 °C for 48 hr. Intense red 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. Microorganisms that were positive for the m Enterecoccus media were transferred to the tubes containing ADB and incubating for 24 hours at 35 °C . 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:

$$\text{CFU (colony forming units)} = \frac{\text{(sum of colonies (both dishes) X 100 mL)}}{\text{total of filtered volume}}$$

Coral reef benthic sampling

Visits to the coral reef study areas began in May 2008 so as to collect and analyze water samples and evaluate coral reef conditions at the stations along the Luis Peña Canal, including the one closest to the municipal landfill. Dr. Hernández, along with graduate students, has been carrying out sampling of the water column near the coral reefs for quantitative and qualitative measurements of water quality, and measurements of species diversity and other parameters indicating coral reef health and conditions at the various stations in the Canal.

Coral reef benthic sampling of additional study sites was already completed for shallow reefs at Punta Tamarindo Grande (Playa Carlos Rosario), Bahía Tamarindo, and Bahía Sardinas.

Coral core collection.

Coral core collection sites were also selected. Sampling design includes three sampling stations located at the landfield impact site (Punta Rompeanzuelos), a presumably human-impacted control (Bahía Sardinas), and a presumably low-impacted control site (Playa Carlos Rosario). A verbal collaborative agreement was already completed with the Division of Marine Resources of the PR Department of Natural and Environmental Resources (PRDNER) to collect coral cores using their pneumatic hand-held driller. An approximately one meter long core will be collected from a single living colony of the mountainous star coral, *Montastraea faveolata*, from each site.

IV. Tasks Performed

A. Administrative Tasks

Dr. José Norat was in charge of administrative matters, study design, and report preparation. Dr. Hernando Mattei was in charge of acquiring Census data on human settlements in Tiger file format. Research Assistant Jeiger Medina helped to collect samples of the water column near the coral reefs at the study sites for analysis at the Environmental Health Department's lab at the Graduata School of Public Health. Molecular biology expert Dr. Mariel Pérez, from Universidad del Este, Carolina Campus, rendered services during the Summer of 2008 to set up the QPCR analysis process in the same laboratory, so as to filter the marine water samples and archive the filtered membranes at -80 degrees Centigrade for eventual QPCR analysis.

B. Field Work

Accomplished coral reef field work

Briefly, six replicate 30 m-long point-count line transects were randomly sampled using digital video imaging following the contour of the reefs at depths ranging from 1 to 4 m. Images are currently under analysis. This will complement our previous coral reef benthic community efforts during the first phase of the project and will complete our sampling design following an anthropogenic stress gradient across 7 reef sites, where 6 of them are located within the Luis Peña Channel Natural Reserve (LPCNR). The complete list of sampled reef sites following the anthropogenic stress gradient is as follows:

Name	**Location**	**Impacts**
Punta Melones	LPCNR	Occasional urban and rural runoff.
Punta Tamarindo Chico	LPCNR	Sporadic urban and rural runoff.
Arrecife El Banderote	LPCNR	Occasional rural runoff, possible runoff from landfield.
Punta Rompeanzuelos	LPCNR	Occasional rural runoff, possible runoff from landfield.
Punta Tamarindo Grande	LPCNR	Sporadic rural runoff.
Cayo Luis Peña	LPCNR	Rare to none rural runoff.

Biological characterization of coral reef communities adjacent to a municipal landfill, Luis Peña Channel Natural Reserve

Coral reef communities adjacent to the Culebra Island municipal landfill were assessed and quantitatively described for the first time to test for any potential landfill operation impact. Given the lack of long-term monitoring, and the resulting temporal and spatial constraints of this study, there were no signs of landfill impacts in adjacent coral reef benthic community structures. Coral reefs adjacent to the landfill still support a high biodiversity. However, reefs are showing signs of unequivocal decline associated with a combination of long-term regional (i.e. sea surface warming, coral bleaching, hurricanes, disease outbreaks) and local factors (i.e., sediment- and nutrient-laden runoff pulses, remote raw sewage impacts).

Sediment-laden runoff pulses are often occurring in the landfill after heavy rainfall. Plastic bags and other plastic debris are frequently blown by the wind and end up at Bahía Tamarindo, but impacts of these were not quantified in this study. There is a need to use coral proxy signals (i.e., annual growth bands, humic acids, heavy metal accumulation) to test for any spatial and/or temporal variation in patterns of impacts.

The municipal landfill of Culebra, PR is located at approximately 25 m from the shoreline of Bahía Tamarindo by the Luis Peña Channel Natural Reserve (LPCNR). It was established during 1984 in Flamenco Ward. Biological marine communities within the reserve support an outstanding biodiversity representative of the northeastern Caribbean region (Hernández-Delgado, 2000, 2003; Hernández-Delgado et al., 2000; Hernández-Delgado and Rosado-Matías, 2003).

Impacts to the reserve’s sea grass and coral reef communities due to sediment-laden runoff impacts from the landfill have never been quantified, but they have been documented. The objective of Phase I of this study was to produce a biological characterization of coral reef communities adjacent to the Culebra Island landfill, within the LCNR. The methodology for this part of the study consisted of the study of 4 coral reef locations within LPCNR: one impact site (Punta Rompeanzuelo), and 3 control sites (Arrecife El Banderote, Punta Tamarindo Chico, Cayo Luis Peña-north coast). Benthic surveys of coral reef communities were carried out to test for significant spatial pattern in the structure resulting from potential landfill-based pollution. The survey was performed by six replicate 30 m-long point-count transects randomly sampled using digital video imaging at the four study locations.

This approach provided baseline information regarding the actual condition of coral reefs. It also made possible a detailed biodiversity assessment of the species composition of the area. The coral reefs were assessed for any disease or adverse vitality conditions and sources of recent mortality were identified whenever possible. The differences among the sites were tested with a one way analysis of variance (ANOVA) and/or Kruskal-Wallis nonparametric ANOVA. Changes in community structure were tested by means of multivariate statistical tests. Community matrices were compiled and imported into PRIMER ecological statistics software package for multivariate analysis (Clarke and Warwick, 2001). Mean data from each site 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. Spatial variation patterns were tested using PRIMER's multivariate equivalent of an ANOVA called ANOSIM. Key taxa responsible for spatial variation in community structure between sites were determined using the SIMPER routine. Results showed a high biodiversity and importance of the coral reef community including critical habitat for the endangered green turtle, *Chelonia mydas*. Results demonstrated that no significant difference was found in coral reef species richness or colony abundance between areas. Percent partial colony mortality was not significant among sites (Figure 20), but percent recent colony mortality was significantly higher (p=0.0354) at AEB (8.5%) and the impacted site at PRA (7.4%).

A SIMPER test revealed that benthic community differences between impacted PRA and AEB were mostly the result of higher % cover of sponges at AEB. There was no evidence of direct impacts of landfill operations affecting the existing community structure of coral reefs adjacent to the landfill area in PRA. Existing differences among sites are largely the result of physical and oceanographic differences among sites. Actual conditions of coral reefs reflect basically similar patterns across sites, regardless of the distance from the landfill, suggesting that factors affecting corals are of larger geographical scales.

There was evidence of recent coral mortality during 2006 and 2007 at each site as a result of the 2005 unprecedented sea surface warming of the northeastern Caribbean that produced a mass coral bleaching event, and the subsequent mass coral mortality that occurred within the next year and a half. There are recurrent raw sewage pulses coming from Ensenada Honda downtown area through the Luis Peña Channel with almost every ebbing tide. Thus nutrient pulses are affecting all study sites, but particularly, PTC, AEB and PRA. This may explain their slightly higher % macroalgal cover, and % cyanobacterial cover.

The fact is that sediment-laden runoff pulses from the landfill site have been informed (Hernández-Delgado, 2003, 2004), but their impact on coral reef community structure, given the lack of long-term monitoring at adjacent sites, and the significant temporal and spatial constraints of this study, were not measured. For instance, during strong high pressure driven easterly winds, plastic bags often are blown by the wind and carried away to the water, ending up suffocating isolated coral colonies, or laying down on seagrass bottoms that constitute designated critical habitats for a resident endangered green turtle (*Cheloniamydas*) population. This will require stronger compliance with existing regulations toprevent plastic debris to be removed by wind.

Thus, determination of impacts will require further studies using coral proxy signals to address if there was any significant spatio-temporal pattern of landfill operation impacts on corals, part of this second phase of the study. Further work is to be carried out evaluating benthic conditions at the rest of the study sites.

Coral coring.

The coral core collection permitting process is still pending at DNER. The plan was to drill an approximately 50-60 cm core from *Montastraea faveolata* from a coral head near the landfield area, and three control colonies from Península Flamenco and Culebrita Island (two controls unblemished), and from Bahía Sardinas (control polluted). Core holes will be protected by placing a concrete cap to prevent bioerosion of the donor colony. Cores will be cut using a rock saw and slabs will be X-rayed, and analyzed for coral skeletal extension rates and skeletal density as a proxy for water quality impacts. Drilling will be carried out in direct collaboration with biologists Edwin Rodríguez and Maribel Rodríguez, from the Marine Resources Division of DNER. Contacts are being established with the USGS to conduct an additional analysis of accumulation of humic acids in coral cores to address temporal and spatial patterns in runoff impacts in coral reefs. Coral coring permits from PRDNER must be obtained before any collection activity. Coring dates will be coordinated with PRDNER personnel following permit approval.

X. Results

Laboratory evaluation for the determination of leachate constituents at the Culebra solid waste landfill

During Phase 1 of this project a contract was signed with the geotechnical engineering firms of Víctor Ortiz, P.E., GEOCOM, and Alchem Laboratories to carry out well monitoring within the landfill. Integrity tests of existing wells were carried out. Sample analysis for general organic and heavy metal pollutants of well samples were carried out by the firms. Substances that were detected were to be part of the water quality parameters tested in phase II of the coral reef study. A formal request was approved by the Mayor of Culebra Municipality granting a permit to sample leachate from the Culebra landfill.

In samples taken at the Culebra landfill's monitoring wells, the contaminants detected were Barium (0.043 ppm), Florurides (2.13 ppm), and Nitrates (7.4 ppm). Results shown for each parameter are averages of all readings. There was no detection of heavy metals, pesticides, PCB's, or volatile organics. Copy of the Laboratory results are presented as Appendix A to this Progress Report. The absence of the pollutants from the landfill's leachate led us to change the water quality parameter used to measure anthropogenic contamination of the water column near the coral reefs.

Coral reef characterization

Benthic surveys consisted of haphazard sampling of six replicate 30m-long point-count transects with the use of digital video imaging at each site. This method supplied data on the actual conditions of the coral reefs by quantifying coral species richness, % coral cover, % algae cover (functional groups: macroalgae, filamentous algae, Halimeda, crustose coralline algae), % sponge cover, and % cyanobacterial cover. Data was used to calculate coral species diversity index (H'n) and evenness (J'n). In addition, the coral reefs vitality condition was reported as recent or partial mortality. Whenever possible, the source of mortality was identified, whether it was sediment deposition, bio-erosion, effect of adjacent benthic algae, and/or size reduction.

<u>Bahía Tamarindo-North (BTN)</u>: This zone extends from Punta Tamarindo Chico down to Punta Rompeanzuelo. It covers an approximate area of 32.55 ha, and extends from the shoreline to

depths of approximately 20 m. Predominant benthic habitats include continuous seagrass communities, colonized bedrock, and linear reefs bordered by sandy bottoms. A total of 47 coral species have been identified, including 14 octocorals, 4 hydrocorals, and 29 scleractinians. Its shallow reefs support a relatively low % coral cover (<20%), and constitute important nursery grounds for a myriad of reef fish and invertebrate species, while their seagrass communities constitute a nursery ground for Queen Conch, Strombus gigas. They are also part of the designated critical habitat for endangered green turtle, Chelonia mydas.

Punta Rompeanzuelo (PRA): Most of this zone is composed by a shallow (0-7 m) coralline community with a combination of colonized bedrock and linear reef, with limited % coral cover (<15%). However, its shallow grounds right in front of the rocky headland supports an extensive population of zoanthid Palythoa caribbaeorum, with % cover exceeding 80% in some areas. There is also a high abundance of juvenile corals, including elkhorn coral (Acropora palmata), brain corals (Diploria strigosa, D. clivosa), as well as mustard hill coral (Porites astreoides), finger coral (P. porites), starlet coral (Siderastrea radians), golfball coral (Favia fragum), and fire coral (Millepora complanata). This reef also supports an abundant community of juvenile reef fishes and occasional large piscivorous fishes. PRA and BTN constitute the two closest coral reef communities to the Culebra Island municipal landfill. Colonized bedrock and linear reefs end up in a sandy bottom fringe that separates them from continuous seagrasses. Deeper habitats (15-20 m) are characterized by a combination of sandy or low-density seagrass bottoms, intermingled with colonized pavements and algal plains.

Bahía Tamarindo-South (BTS): This zone extends from Punta Rompeanzuelo south to Punta Tamarindo Chico, and reach depths of 12 to 18 m. Predominant benthic habitats include continuous seagrasses, colonized bedrock, sandy bottoms, and limited linear reefs. A total of 42 coral species have been documented, including 11 octocorals, 3 hydrocorals, and 28 scleractinians. There are usually high Queen Conch (Strombus gigas) densities, as well as a resident population of endangered green turtle (Chelonia mydas). These seagrasses were designated as critical habitat for this species. The narrow shallow fringing reef system (<2 m), and Arrecife El Banderote (< 4 m) function as a critical nursery ground of a large amount of fish species, including many that are commercially important, such as snappers (Lutjanus analis, L.

griseus, L. mahogoni, Ocyurus chrysurus), grunts (Haemulon spp.), groupers (Epinephelus striatus, E. guttatus, E. ascencions, Cephalopholis fulva), and parrotfishes (Scaridae spp.).

Arrecife El Banderote (AEB) also supports a highly abundant juvenile coral population, as well as a surviving population of elkhorn coral (Acropora palmate), and a recovering population of staghorn coral (A. cervicornis). There is also a current staghorn coral farming and restocking project conducted by the University of Puerto Rico's Coral Reef Research Group, in collaboration with NGOs Sociedad Ambiente Marino, Coralations, and the Culebra Island Fishers Association. El Banderote also supports an impressive dense population of the long-spine sea urchin (Diadema antillarum).

Punta Tamarindo Chico (PTC): This area separates Bahía Tamarindo from Bahía Tarja. Depths range from the shoreline down to approximately 12 m, with predominant habitats that include colonized bedrock, continuous seagrasses and sandy bottoms. A total of 58 coral species have been documented at PTC, including 16 octocorals, 4 hydrocorals, and 38 scleractinians. PTC shares a lot of characteristics with previously described locations, with the difference that it sustains higher habitat heterogeneity, thus supporting high density juvenile coral stands, as well as high fish densities.

Cayo Luis Peña-northern coast (CLPN): This area is largely composed of colonized pavements, often with channels, and colonized bedrock. These habitats function as a hard ground dominated by soft corals and sponges. There is also a small linear reef, dominated by massive star corals, Montastraea annularis, as well as some isolated patches of elkhorn coral, Acropora palmata, surrounded by sand bottoms. Depths range from shoreline to approximately 18 m. A total of 63 coral species have been identified at CLPN, including 27 octocorals, 3 hydrocorals, and 33 scleractinians.

Results of water quality analysis

Appendix B presents results obtained from the laboratory analysis of microbiological water quality of sampling sites using culture techniques. Membrane filtration of samples taken at all study sites has been performed for seven field visits. Polycarbonate membranes have been stored at -80ºC for further analysis using QPCR techniques. These results will eventually give information on Bacteroides DNA densities (cell equivalents) at study sites to allow for an alternate water quality indicator and to statistically associate with benthic evaluation results at coral reefs. Results are summarized in the following Tables and Figures.

TABLE 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	9,150	25.69	<0.0001
Temperature	ºC	9,150	65.03	<0.0001
pH	pH units	9,150	13.42	<0.0001
Dissolved oxygen	mg/L	9,135	8.87	<0.0001
Salinity	Ppt	9,150	2.09	0.0336
Conductivity	ms/cm	9,150	2.12	0.0313

TABLE 2. Water turbidity (NTU) standard violations across sites.

Sample	May	Jun	Jun	Jun	Jul	Aug	Sep	Oct	Nov	Mean	% Frq
CLA	8.69	6.23	15.9	10.6	19.0	7.49	19.9	7.39	21.0	12.36	56%
MPI	NS	NS	4.43	1.64	1.30	1.25	9.99	1.59	2.78	3.28	0%
LOB	7.65	2.13	9.13	3.87	6.99	2.38	20.05	2.24	1.60	6.09	11%
BSA	1.27	1.59	5.68	1.28	1.02	0.58	3.16	0.89	0.49	1.74	0%
PME	1.61	0.75	1.25	1.37	0.48	0.51	1.39	0.52	0.28	0.93	0%
PTC	NS	NS	NS	NS	0.51	0.54	1.54	1.05	0.38	0.80	0%
AEB	NS	NS	NS	NS	0.98	1.00	1.71	1.01	0.38	1.02	0%
PRA	1.17	1.50	4.64	0.66	0.56	0.40	1.21	0.53	0.34	1.23	0%
CLP	NS	NS	1.13	0.37	0.53	0.45	0.88	0.67	0.29	0.61	0%
PCR	2.20	0.89	2.20	0.84	0.51	0.38	1.63	0.74	0.28	1.12	0%

NS= Site not sampled for that time period. Red color= violation to EQB class SB water quality classification turbidity standard (10 NTU).

TABLE 3. Microbiological water quality standard violations for fecal coliforms across sites.

Sample	May	Jun	Jun	Jun	Jul	Aug	Sep	Oct	Nov	Mean	% Frq
CLA	40	168	23	26	1000	673	550	ND	ND	357	33%
MPI	NS	NS	ND	537	389	18	20	70	113	191	29%
LOB	95	194	39	850	1000	564.8	230	1975	228	555	67%
BSA	32	5.3	0	1	95	29	546	0	0	79	11%
PME	0	0	0	0	1	2	0	0	0	0.2	0%
PTC	NS	NS	NS	NS	0	0	0	0	0	0	0%
AEB	NS	NS	NS	NS	0	0	10	0	0	1	0%
PRA	350	3	0	102	3	1	2	0	2	51	11%
CLP	NS	NS	2	0	2	0	0	0	0	0.3	0%
PCR	0	0.5	0	0	2	0	5	0	0	0.7	0%

NS= Site not sampled for that time period. ND= Not detected due to interference overgrowth by other microbes. Red color= violation to EQB class SB water quality classification FC standard (200 cfu/100 mL).

TABLE 4. Microbiological water quality standard violations for enterococci across sites.

Sample	May	Jun	Jun	Jun	Jul	Aug	Sep	Oct	Nov	Mean	% Frq
CLA	15	119.5	190	19	1123	76	133	300	10	221	67%
MPI	NS	NS	25	134	125	5	157	100	20	80	57%
LOB	80	65	240	245	2095	1570	70	1150	45	590	100%
BSA	84	77	10	1	57	76	8126	30	0	948	56%
PME	0	0.5	10	1	2	3	239	0	0	26	11%
PTC	NS	NS	NS	NS	2	9	20	0	0	6	0%
AEB	NS	NS	NS	NS	0	6	833	10	0	88	20%
PRA	130	10	28	5	6	13	290	60	1	61	33%
CLP	NS	NS	54	0	0	3	77	0	2	19	29%
PCR	0	0.5	47	0.5	0.5	6	607	0	0	66	22%

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

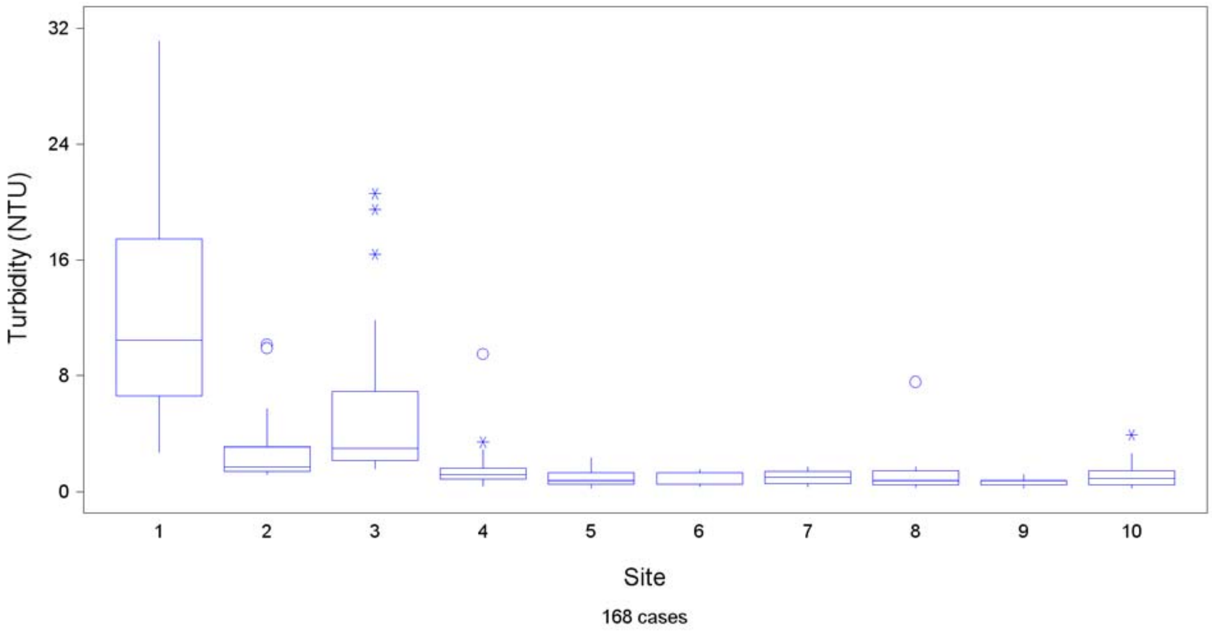

FIGURE 2. Box and whisker plot of turbidity data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). 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 upper whiskers and outlier points suggest presence of non-point turbid runoff pulses at locations close to population centers (CLA, MPI, LOB, BSA, PME), as well as in PRA (close to the landfill) and at PCR.

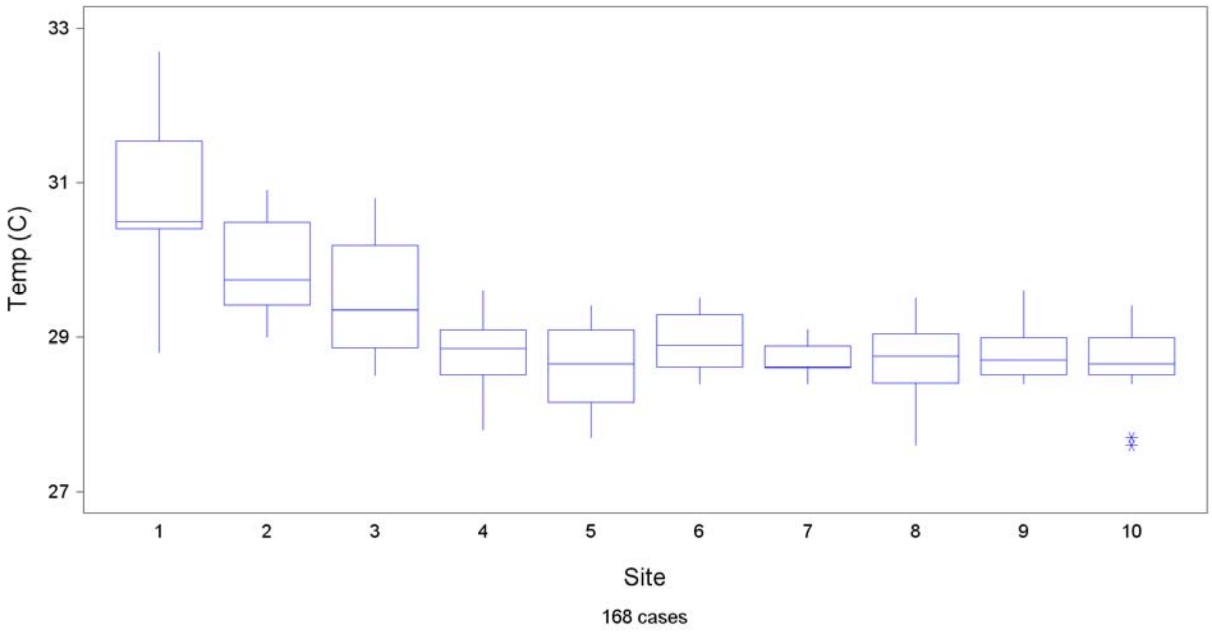

FIGURE 3. Box and whisker plot of sea surface temperature data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). All mean temperatures exceeded the mean monthly maximum sea surface temperature for the PR-USVI area (28.5°C). Significantly higher temperatures were detected within shallow approaches at Ensenada Honda bay (CLA, MPI) and at LOB. Other open water areas showed fairly similar values and fluctuations.

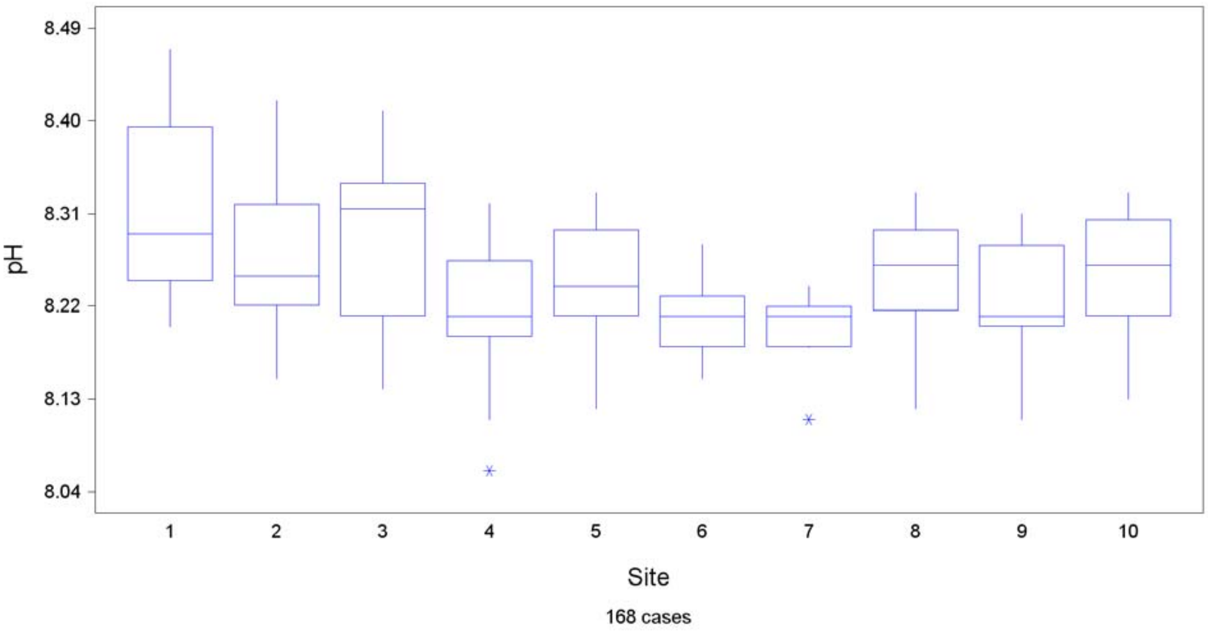

FIGURE 4. Box and whisker plot of pH data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). Lower whiskers and outlier points reflect non-point source runoff pulse impacts across all sites.

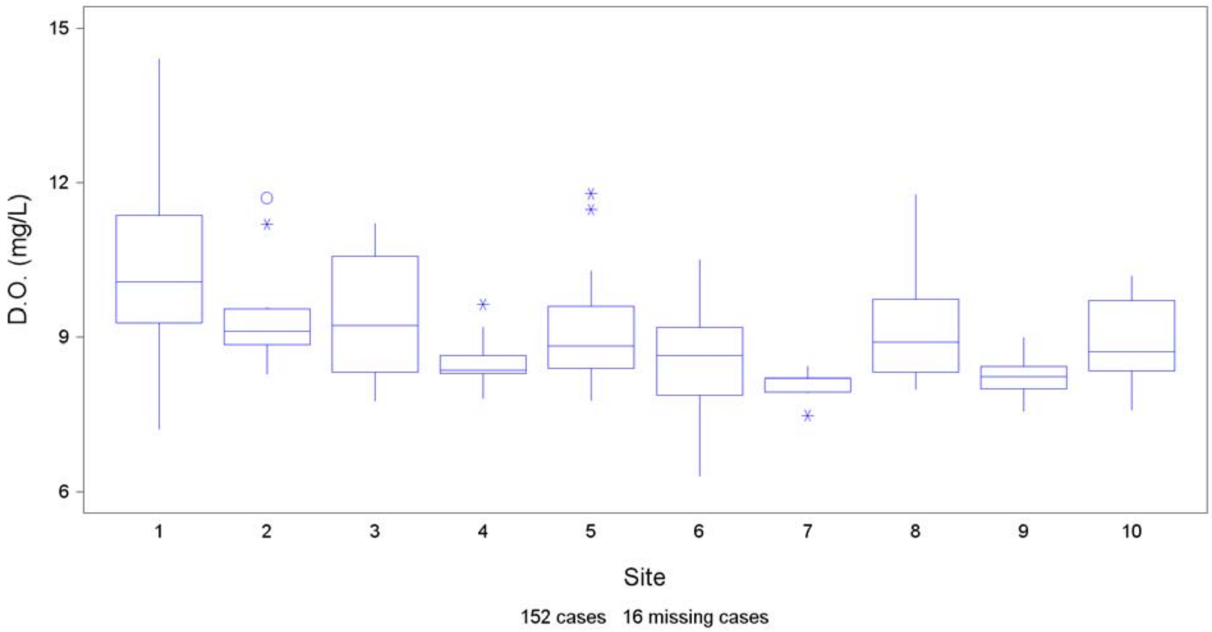

FIGURE 5. Box and whisker plot of dissolved oxygen data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). Presence of lower long whiskers and outlier points suggest presence of lower-dissolved oxygen water pulses from non-point source runoff, mostly at CLA, PTC, and AEB. Upper long whiskers and outlier points represent stronger oxygen-rich water mixing due to incoming tides and strong winds, mostly at CLA, MPI, BSA, PME, PTC, and PRA.

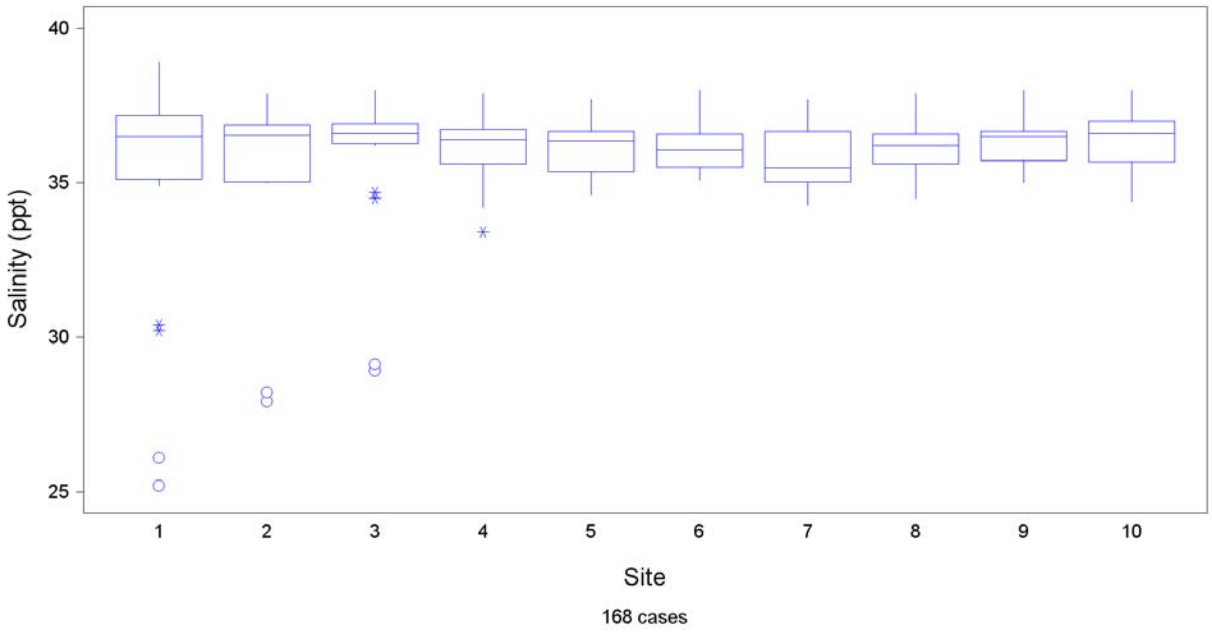

FIGURE 6. Box and whisker plot of salinity data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). Presence of lower long whiskers and outlier points suggest presence of lower-salinity water pulses from non-point source runoff, mostly at sampling sites located close to population centers.

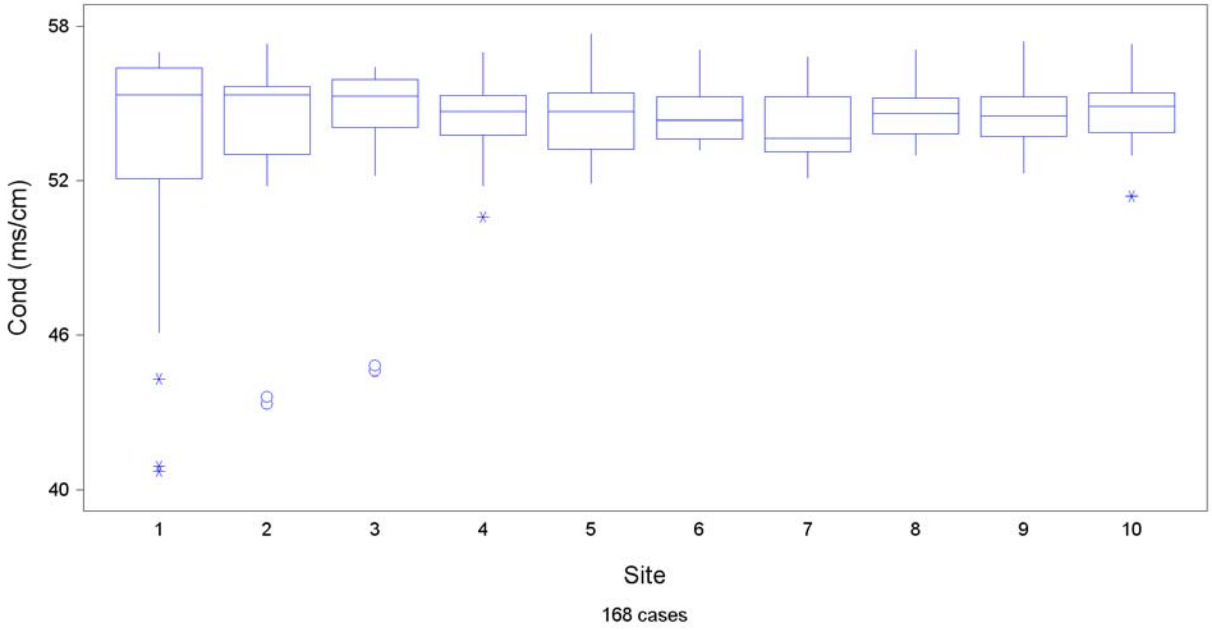

FIGURE 7. Box and whisker plot of conductivity data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). Presence of lower long whiskers and outlier points suggest presence of lower-conductivity water pulses from non-point source runoff pulses associated to heavy rainfall events. Sampling sites around population centers showed the most significant impacts by runoff pulses, as well as PCR, regardless of the fact that it was the most remote site.

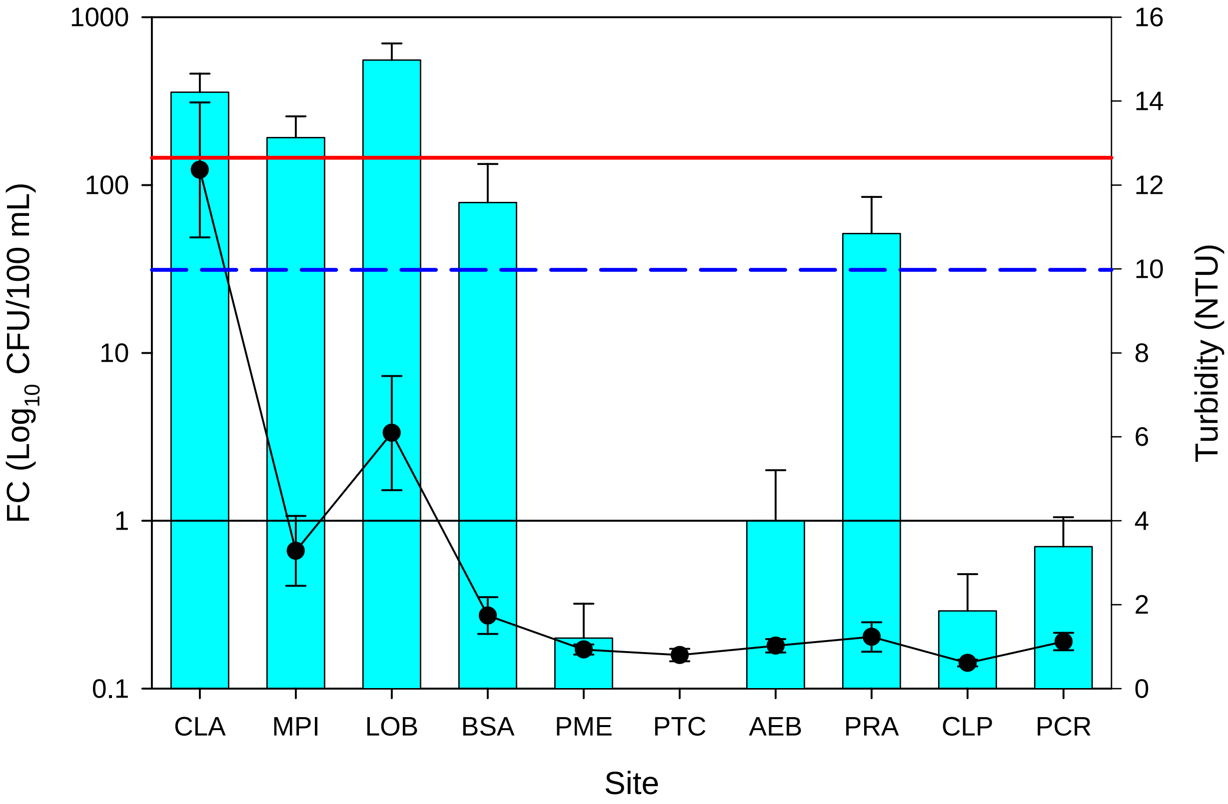

FIGURE 8. Fecal coliform counts (turquoise bars) as a function of water turbidity by site (mean±one standard error). Red solid line represents FC standard (200 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

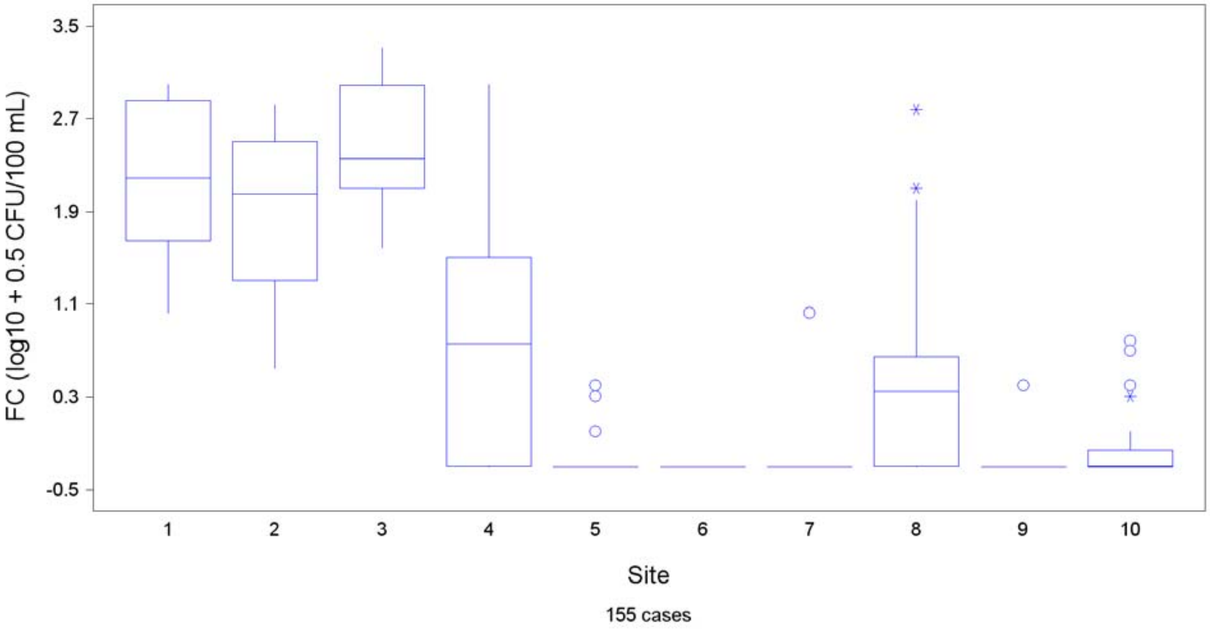

FIGURE 9. Box and whisker plot of fecal coliform data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR).

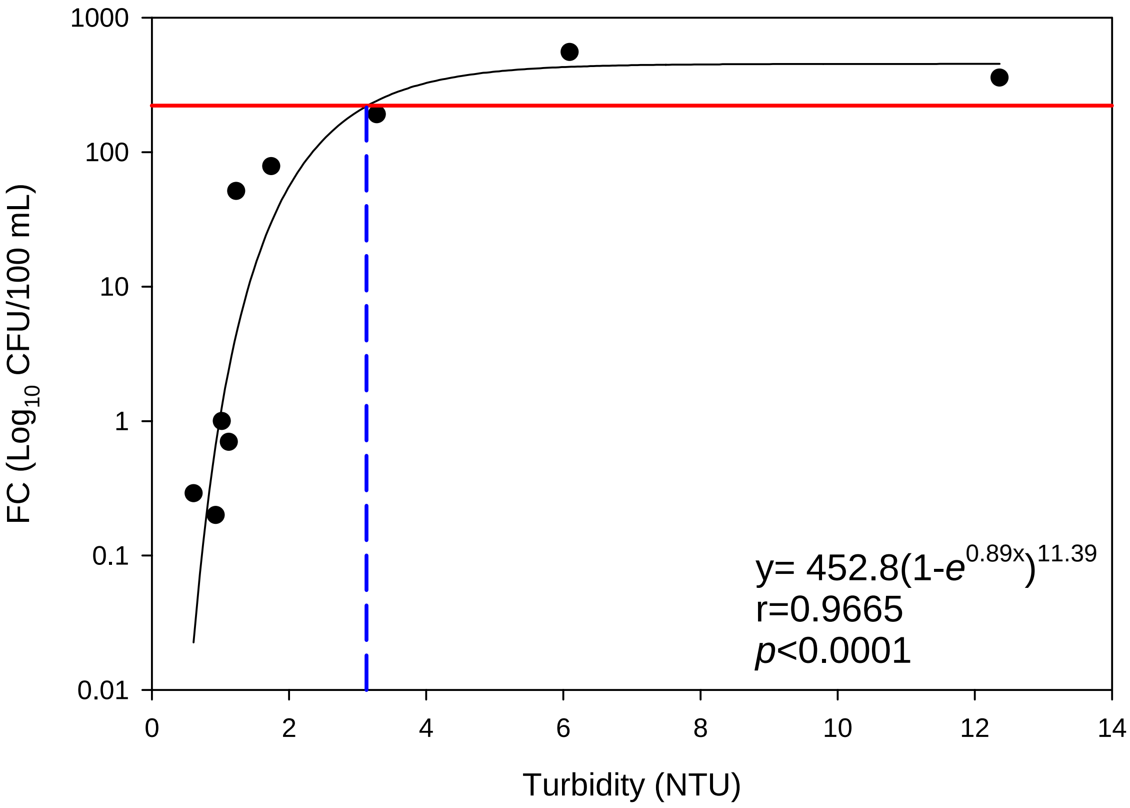

FIGURE 10. Chapman non-linear regression for fecal coliform (FC) counts and water turbidity by site. Red solid line represents FC standard (200 cfu/100 mL). Blue dashed line represents approximate turbidity level at which FC counts may exceed current standards. Water turbidity values approximately above 3.2 NTU suggest FC counts above current legal standards for coastal waters. Significantly higher turbidity values did not result in further increases in FC counts probably as a result of growth interference by other opportunistic microbes in culture media.

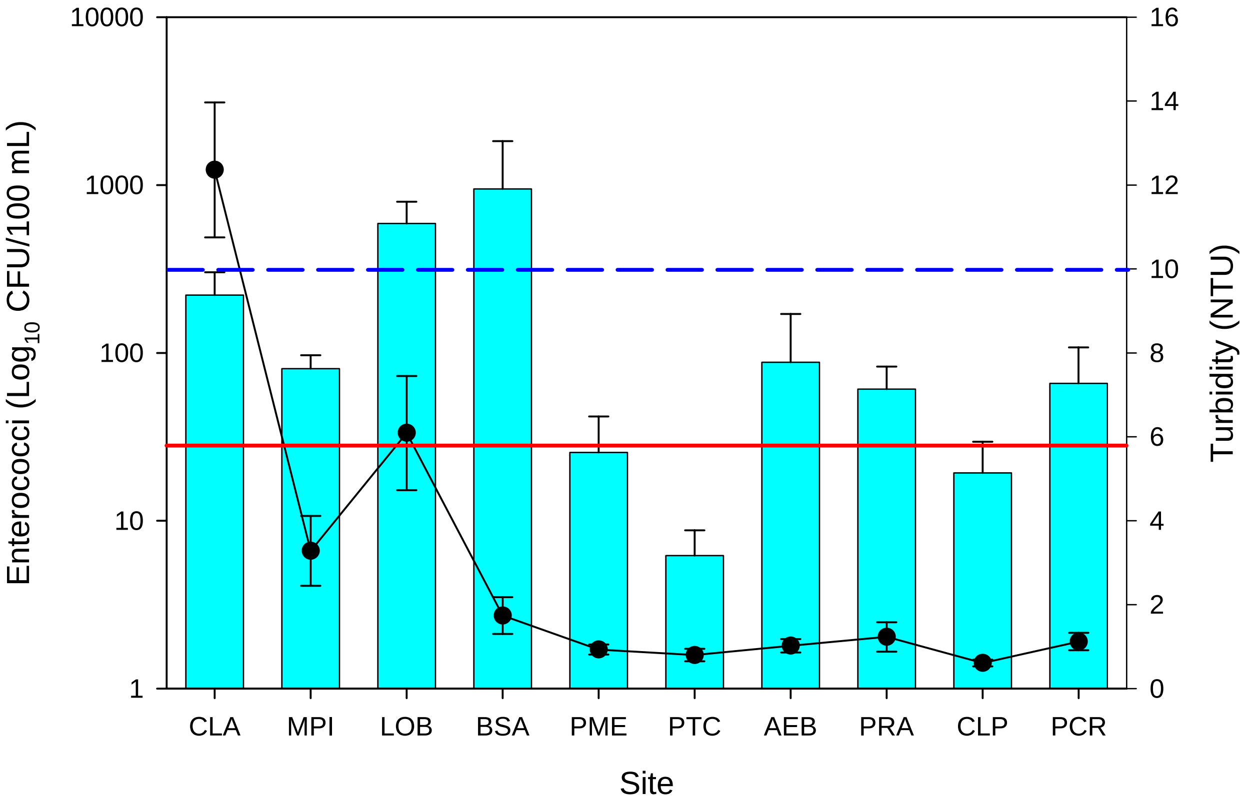

FIGURE 11. ENT counts (turquoise bars) as a function of water turbidity by site (mean±one standard error). Red solid line represents FC standard (35 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

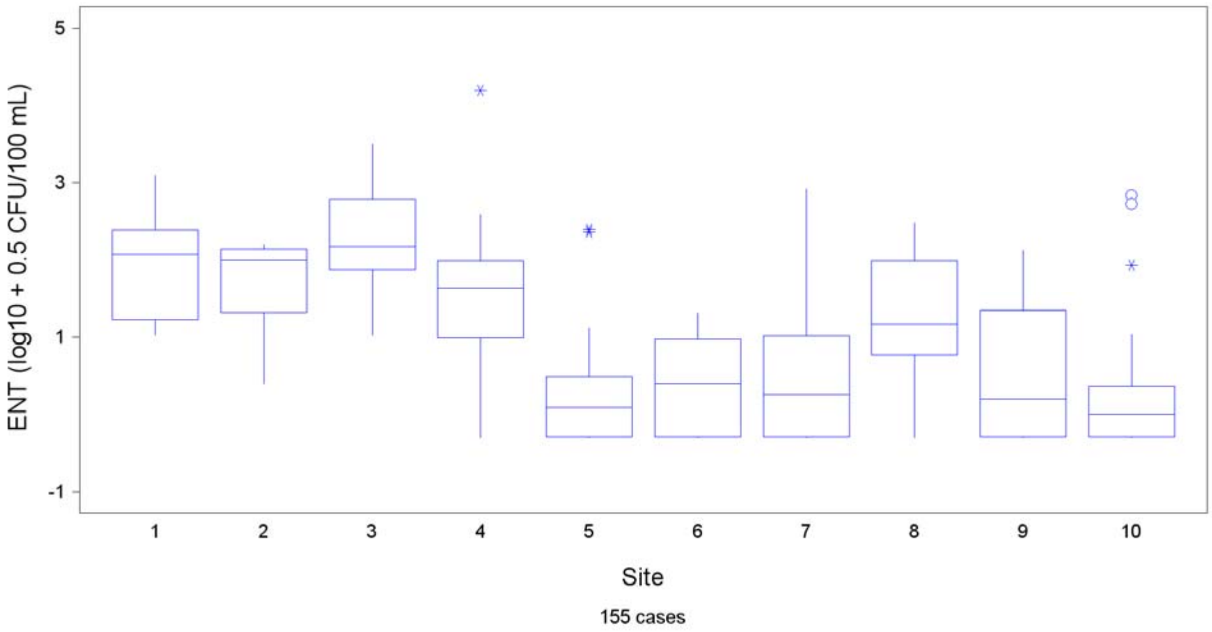

FIGURE 12. Box and whisker plot of enterococci data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR).

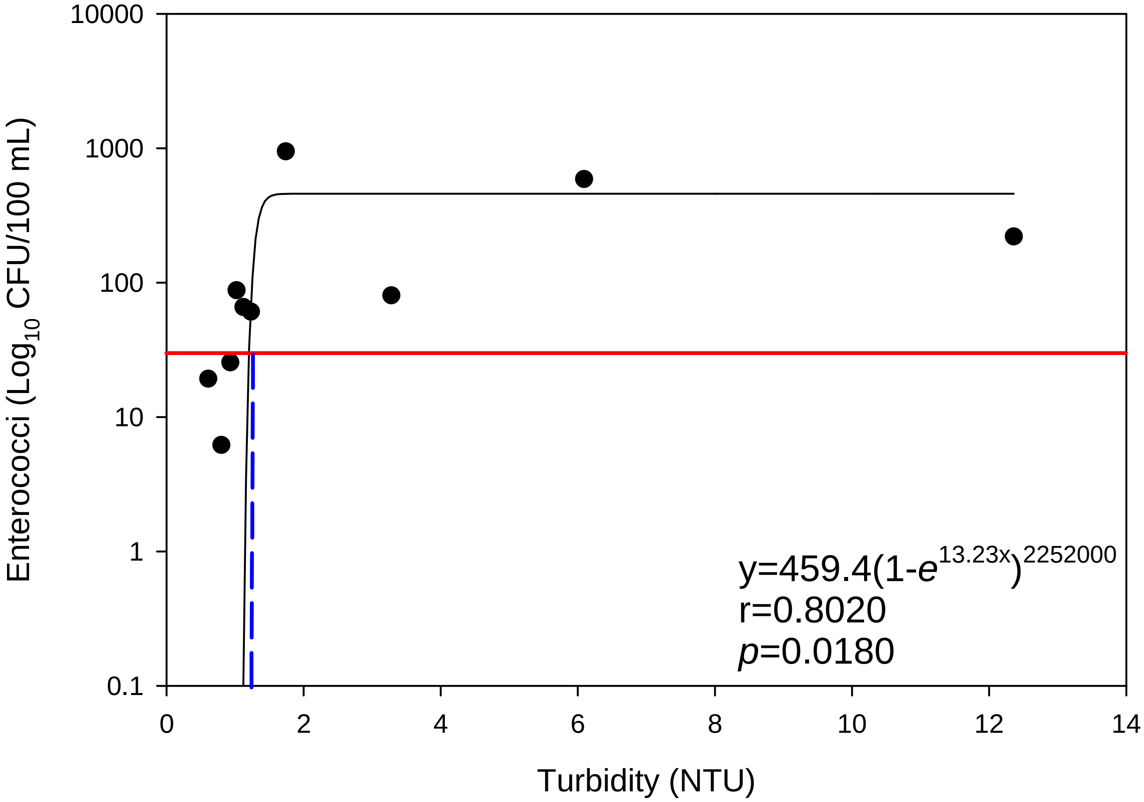

FIGURE 13. Chapman non-linear regression for enterococci counts and water turbidity by site. Red solid line represents FC standard (35 cfu/100 mL). Blue dashed line represents approximate turbidity level at which FC counts may exceed current standards. Water turbidity values approximately above 1.5 NTU suggest enterococci counts above current legal standards for coastal waters. Significantly higher turbidity values did not result in further increases in enterococci counts probably as a result of growth interference by other opportunistic microbes in culture media.

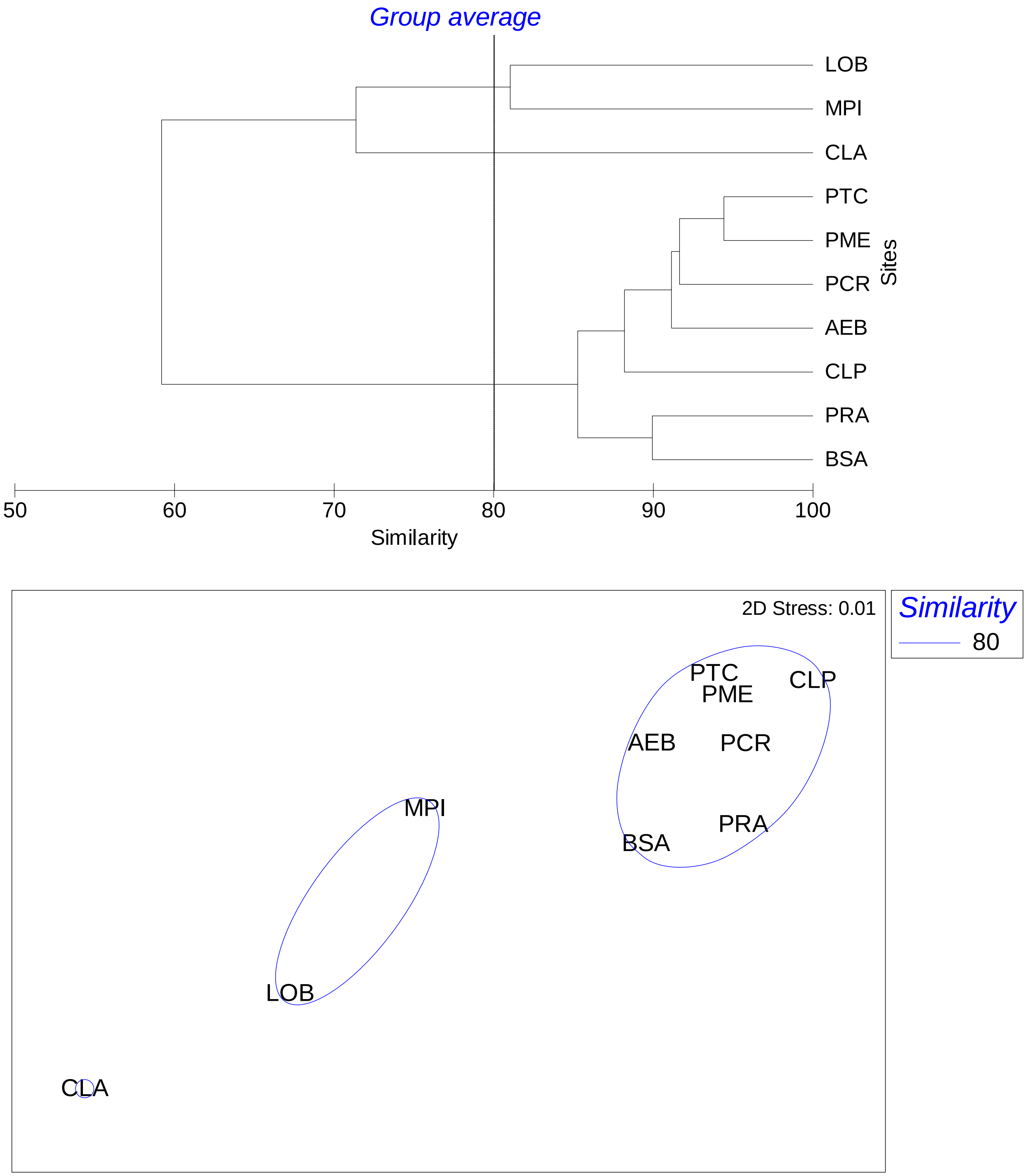

FIGURE 14. Cluster analysis and multi-dimensional scaling (MDS) plot based on water turbidity. Vertical bar in cluster chart and blue contour line in MDS plot represent the 80% similarity cutoff level. There were three clusters that evidenced a strong turbidity gradient: one highly turbid site (CLA), two moderately turbid sites (LOB, MPI), and a third low turbidity cluster.

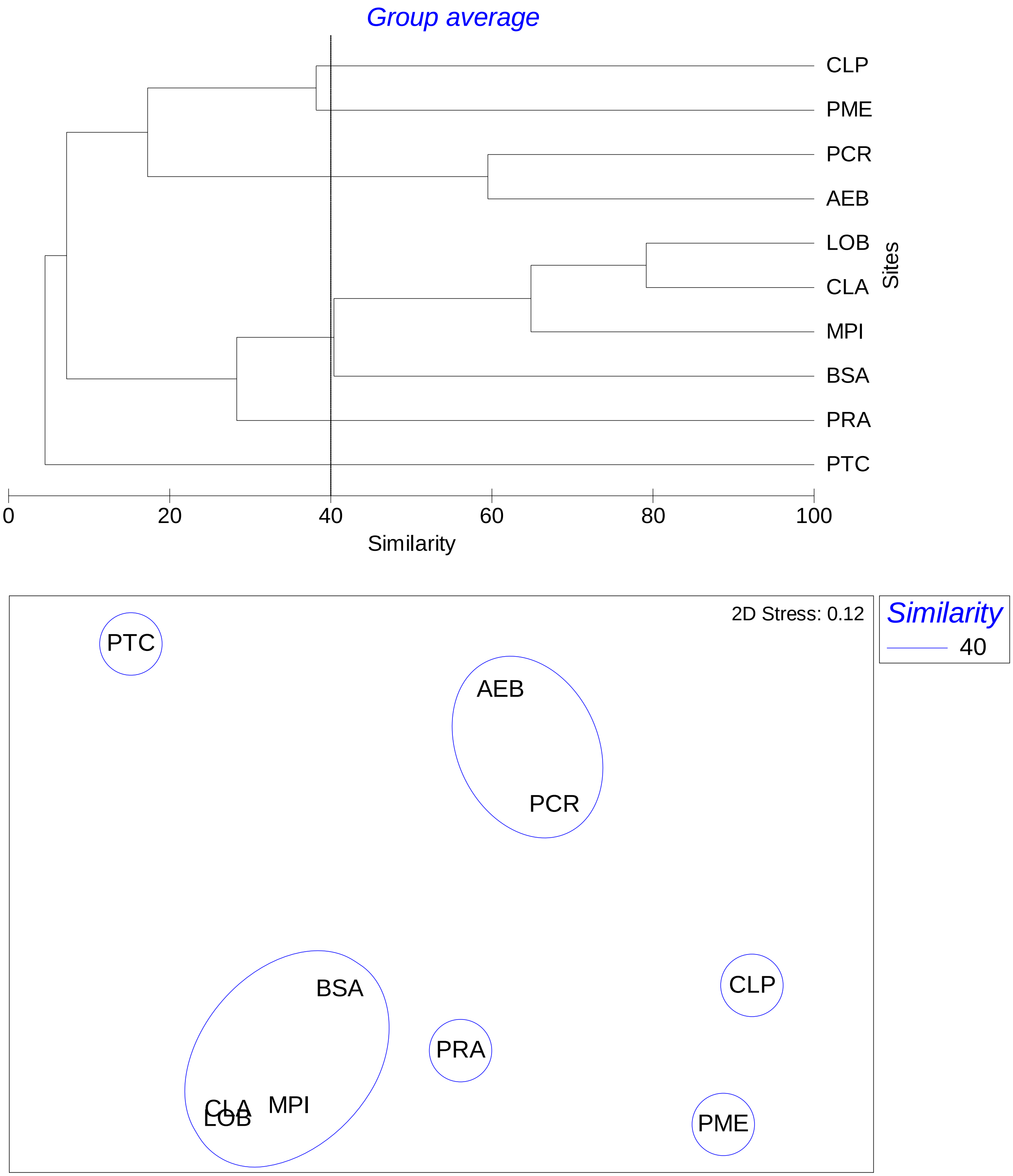

FIGURE 15. Cluster analysis and multi-dimensional scaling (MDS) plot based on FC counts. Vertical bar in cluster chart and blue contour line in MDS plot represent the 40% similarity cutoff level. There were six clusters that evidenced a moderate water quality gradient, with known polluted sites clustering together (CLA, LOB, MPI, BSA), and several isolated cluster of the remaining sites.

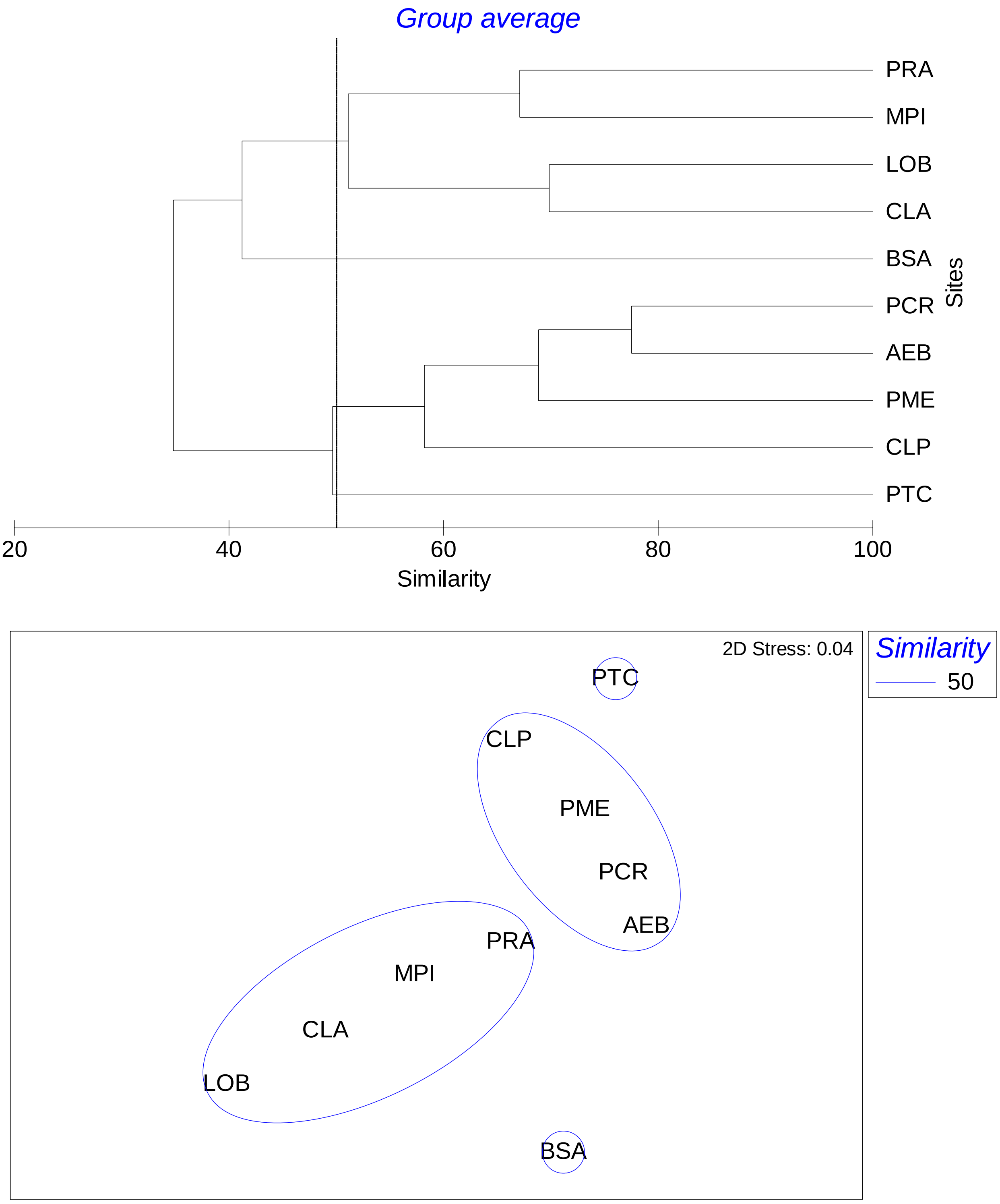

FIGURE 16. Cluster analysis and multi-dimensional scaling (MDS) plot based on enterococci counts. Vertical bar in cluster chart and blue contour line in MDS plot represent the 50% similarity cutoff level. There were four clusters that evidenced a strong water quality gradient, with known polluted sites clustering together (CLA, LOB, MPI, PRA), and BSA separately.

XI. Discussion

There was evidence of strong gradient impacts associated to sediment- and nutrient-laden runoff pulses, in combination with sewage pollution pulses. Water quality showed both, significant spatial and temporal fluctuations, largely responding to heavy rainfall and subsequent runoff pulses. This was particularly reflected in water turbidity pulses as previously evidenced by Bonkosky et al. (2009) in the western coast of Puerto Rico. Microbiological water quality also showed significant spatial and temporal fluctuations as a combined result of runoff pulses and sewage pulses during ebbing tides, closely reflecting patterns described by Bonkosky et al. (2009). Chronic high fecal indicator counts at some sampling sites revealed stronger influences of various non-point sewage pollution sources. Lack of sewage treatment facilities in Culebra Island have resulted in a proliferation of poorly designed and constructed septic tanks, septic tanks constructed below the coastal water table, and numerous illegal raw sewage discharges to storm water sewers and culverts. Thus, there is usually a strong relationship between sediment- and nutrient-laden runoff pulses and raw sewage pulses. In several sampling sites, mostly from inner Ensenada Honda bay locations, LOB and BSA, fecal indicators exceeded recognized standards for coastal waters of 200 cfu/100mL for FC, and 35 cfu/100mL for enterococci. Frequent violations to current standard suggest that coastal waters near Culebra’s population centers are chronically polluted by raw sewage pulses and that heavy rainfall exacerbates the problem.

Behavior of turbidity and sewage pulses largely followed oceanographic and atmospheric dynamics associated to: (1) tidal flow; (2) turbidity; (3) wind direction and velocity; (4) wind-driven circulation; (5) southeastern wave action; and (6) heavy rainfall. These fluctuations were suggested by the presence of higher concentrations of microbial indicators in samples collected during strong wind-driven circulation and sediment resuspension, and during ebbing tides, particularly after heavy rainfall runoff. Ebbing flow at Ensenada Honda causes a strong flow through largely-polluted LOB towards BSA. Eventually, such flows move towards the northwest through LPCNR (Capella, 2004), therefore carrying over all pollution towards significant coral reef and seagrass systems.

Benthic communities at sediments frequently disturbed by sediment- and nutrient-laden runoff were dominated by macroalgae, dead coral surfaces, cyanobacteria, algal turfs, low coral species richness, low H’n and J’n, and the coral fauna was dominated by surviving patches of *Porites porites*. Old dead standing colonies of threatened *A. palmata*, as well as rodolites of former colonies of threatened *A. cervicornis*, were frequently observed. Coral recruits were very limited at these sites. Also, sites such as PCR-S, where sediment bedload was a significant benthic community structuring factor, were dominated by thin sand veneers moving atop pavements, and dominated also by algal turfs, dead colonies, or small colonies of ephemeral taxa such as *P. astreoides*. In contrast, sites under strong water circulation that prevents sediment deposition, are largely dominated by a larger coral species richness, H’n, J’n, and by a higher abundance of juvenile corals and sponges. These patterns reflected variation associated to gradient impacts and were consistent with previous observations in the Caribbean (Tomascik and Sander 1987a; Tomascik 1991).

Turbid waters and raw sewage pollution have been pointed out as one of the causes of coral reef and fish community declines in Culebra (Hernández-Delgado et al., 2006). Increasing chronic non-point source sewage pollution may result in permanent water quality degradation 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. Chronic water quality degradation associated to non-point source runoff pulses and 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). Therefore, it is a paramount problem that deserves immediate attention and rapid solutions.

Pastorok and Byliard (1985), McComb (1995), Cloern (2001), Livingston (2001), Kennish (2002), and Szmant (2002) reviewed the effects of coastal water quality degradation associated to non-point hypertrophic pollution sources. Impacts may typically result in hypereutrophication, increasing biological oxygen demand, and causing hypoxia and anoxia (Kennish, 2002), as well as increased levels of organic carbon, nitrogen, concentration of chlorophyll *a* and phospholipids, and hydrolytic enzymatic activities (Köster et al., 1997). Also, it may result in a chronic increase in water turbidity, declining sunlight penetration and in a chronic deterioration of sediment quality (Livingston, 2001). Further, it may result in fostering phytoplankton blooms (McComb, 1995; Arhonditsis et al., 2003), rapid macroalgal growth (Naim, 1993), and a long-term general decline of fisheries (Hodgkiss and Yim, 1995), and decline in seagrass communities (Duarte, 1995) and coral reefs (Hernández-Delgado, 2000, 2005; Cloern, 2001). Corals are particularly susceptible to eutrophication as a result of declining growth rates (Tomascik and Sander, 1985; Tomascik, 1990), reproductive output (Tomascik and Sander, 1987b), larval settlement rates (Tomascik, 1991), increased incidence of diseases (Kaczmarsky, et al., 2005), mortality (Pastorok and Byliard, 1985), often impacting benthic community structure (Tomascik and Sander, 1987a). These impacts are often confounded with sedimentation impacts (Rogers, 1990; Meesters et al., 1998).

Water quality degradation is a concern in shallow coastal habitats due to the accumulation of sediments, nutrients and contaminants (McComb, 1995), that can foster microbial proliferation, potentially becoming an enterotoxigenic pathogen reservoir, particularly if there are recurrent impacts of sewage pulses. Sediment resuspension can fluctuate as a function of wind-or wave-driven factors, but also as a function of navigation activities on shallow soft bottoms (i.e., BSA), and can be a major factor in nutrient accumulation and loading processes (de Jong, 1995; de Jong and van Raaphorst, 1995), as well as in microbe resuspension. 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.

There was no evidence of direct impacts of landfill operations affecting the existing community structure of coral reefs adjacent to the landfill area in PRA. Existing differences among sites are largely the result of physical and oceanographical differences among sites and strong influences by remote runoff and sewage pulses. Actual conditions of coral reefs located close to the landfill reflect basically similar patterns across sites, regardless of the distance from the landfill, suggesting that factors affecting corals are of larger geographical scales. These could be separated into regional factors and large-scale local factors. Regional factors included: sea surface warming, coral bleaching, hurricanes, and coral disease outbreaks. There was evidence of recent coral mortality during 2006 and 2007 at each site as a result of the 2005 unprecedented sea surface warming of the northeastern Caribbean that produced a mass coral bleaching event, and the subsequent mass coral mortality that occurred within the next year and a half. Most of the corals at each study site suffered massive bleaching (Hernández-Delgado, unpub. data) and many colonies of several taxa suffered significant mortality. There was also old evidence of coral disease outbreaks with the presence of dead standing colonies of elkhorn coral (*Acropora palmata*). Further, there was some old fragmentation of coral colonies as a result of past hurricanes.

Local factors impacting reefs near the landfill included recurrent sediment-laden runoff pulses that may come from the landfill area, adjacent roads, and other adjacent land cleared sites. Also, there are recurrent raw sewage pulses coming from Ensenada Honda downtown area through the Luis Peña Channel with almost every ebbing tide. Thus nutrient pulses are affecting all study sites located in the eastern quadrant of LPCNR. However, sediment-laden runoff pulses from the landfill site have been informed to occur (Hernández-Delgado, 2003a, 2004), but their impact in coral reef community structure, given the lack of long-term monitoring at adjacent sites, and the significant temporal and spatial constraints of this study, did not allow us to measure it. This will require the use of proxy methods (i.e., detection of heavy metals accumulated in coral annual growth bands, detection of changes in coral annual growth rates, concentration of humic acids in annual growth bands) to determine if there have been spatio-temporal variations in landfill impacts. But certainly, landfill operations do affect adjacent coral reefs in other ways. For instance, during strong high pressure-driven easterly winds, plastic bags often are blown by the wind and carried away to the water, ending up suffocating isolated coral colonies, or laying down on seagrass bottoms that constitute designated critical habitats for a resident endangered green turtle (*Chelonia mydas*) population. This will require stronger compliance with existing regulations to prevent plastic debris to be removed by wind.

It is paramount to implement and strongly enforce existing regulations regarding sediment-erosion controls, and regarding coastal water quality. Coastal seagrass communities were designated by the U.S. Federal government in 1994 as Resource Category 1, and in 1998 as Designated Critical Habitat (DCH) for the endangered green turtle (*Chelonia mydas*). Coral reef habitats were designated in 2008 as DCH for threatened Acroporid corals.

VI. Conclusions

Coastal waters in Culebra Island were polluted by non-point fecal pollution and sediment- and nutrient-laden runoff, creating a strong gradient of impacts on shallow coral reef benthic communities. Variable sources may include (1) malfunctioning septic tanks, (2) illegal sewage discharges from private houses and businesses (i.e., restaurants), (3) raw sewage-polluted stormwater sewers; and (4) widespread deforestation for construction. Non-point source fecal pollution and high turbidity pulses are impacting widespread coral reef and seagrass areas, even within LPCNR. There is a need to expand the microbial water quality monitoring efforts to other areas in Culebra, in combination with the simultaneous monitoring of other physico-chemical factors to address spatio-temporal variation patterns. There is also a need to study the impact of runoff pulses on microbial indicators, physico-chemical factors and short-term impact in coral reefs. There is a particular need to experimentally test runoff impacts on corals along a sewage-sediment stress gradient.

There is an imperative need to design integrated coastal management strategies to address marine, coastal-maritime zone, and watershed management needs in order to reduce fecal and runoff pollution impacts in the coast. Water quality management needs include identification of strategies to control most of the dispersed sources of fecal contamination in Culebra. In order to achieve this, surveys of sources of pollution need to be identified, geo-referenced and mapped. Similarly, mapping of areas subjected to strong erosion impacts, currently underway by DNER, needs to be completed. Such information may provide important tools to help prioritize management actions and land use in the near future.

There is also a strong need to implement immediate erosion-control management practices and watershed reforestation. This could be accomplished throughout the implementation of a “green certification” program for construction workers and heavy machinery operators, in combination with steep dirt road repair, and a massive community-based restoration program. This is paramount to significantly reduce sedimentation impacts in sensitive habitats that serve as nursery and feeding grounds of commercially important species, as well as for the endangered Caribbean manatee (*Trichechus manatus*), hawksbill turtle (*Eretmochelys imbricata*), brown pelican (*Pelecannus occidentalis occidentalis*), and threatened Acroporid corals. They also harbor nursery grounds of a myriad of fish and macroivertebrate species of commercial significance.

Finally, there is a need to address coral microbial community dynamics. Coral microbes can be excellent sentinels or an early warning sign of stress in coral reef ecosystems (Stewart et al., 2008). In addition, there is a need to experimentally test the resilience of several coral species to recurrent runoff conditions. It would be important to address coral survival threshold points in the context of viability of coral reef restoration efforts. In face of expected recurrent environmental impacts associated to climate change in the near future, frequent non-point source pollution episodes can result in a dramatic long-term decline of coastal environmental 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.

VII. Acknowledgements

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VIII. Cited literature

Arhonditsis, G., M. Karydis, & G. Tsirtsis. 2003. Analyisis of phytoplankton community structure using similarity indices: A new methodology for discriminating among eutrophication levels in coastal marine ecosystems. *Environ. Mgmt*. 31:619-632.

Ballantine, D.L., R.S. Appeldoorn, P. Yoshioka, E. Weil, R. Armstrong, J.R. García, E. Otero, F. Pagán, C. Sherman, E.A. Hernández-Delgado, A. Bruckner, & C. Lilyestrom. 2008. Biology and ecology of Puerto Rican coral reefs. 375-406. In, B.M. Riegl & R.E. Dodge (eds.), Coral Reefs of the World, Vol. I. Coral Reefs of the USA, Springer-Science + Business Media B.V. 803pp.

Bonkosky, M., E.A. Hernández-Delgado, B. Sandoz, I.E. Robledo, J. Norat-Ramírez, & H. Mattei. 2009. Detection of spatial fluctuations of non-point source fecal pollution in coral reef surrounding waters in southwestern Puerto Rico using PCR-based assays. *Mar. Poll. Bull*. 58:45-54.

Capella, J.A. 2004. Culebra final current meter deployment report. Culebra OOCA Studies, Dept. Marine Science, UPR-Mayaguez. 14 pp. + App.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication model. *Mar. Ecol. Prog. Ser*. 210:223-253.

de Jong, V.N. 1995. The Ems estuary, the Netherlands. 81-108. In, A.J. McComb (ed.), *Eutrophic Shallow Estuaries and Lagoons*. CRC Press, Boca Raton, FL. 240 pp.

de Jong, V.N. & W. van Raaphorst. 1995. Eutrophication of the Dutch Wadden Sea (Western Europe), an estuarine area controlled by the River Rhine. 81-108. In, A.J. McComb (ed.), *Eutrophic Shallow Estuaries and Lagoons*. CRC Press, Boca Raton, FL. 240 pp.

Duarte, C.M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41:87-112.

Fabricius, K.E., & E. Wolanski. 2000. Rapid smothering of coral reef organisms by muddy marine snow. *Est. Coast. Mar. Sci*. 50:115-120.

Fabricius, K.E., & D. McCorry. 2006. Changes in octocoral communities and benthic cover along a water quality gradient in the reefs of Hong Kong. *Mar. Poll. Bull*. 52:22-33.

García, J.R., J. Morelock, R. Castro, C. Goenaga, & E. Hernández. 2003. Puerto Rican reefs: Research synthesis, present threats and management perspectives. 111-130. In, J. Cortés (ed.), Latin American Coral Reefs. Elsevier Publ., Amsterdam, Holland. 497 pp.

García-Sais, J.R., R. Appeldoorn, T. Batista, L. Bauer, A. Bruckner, C. Caldow, L.M. Carruba, J. Corredor, E. Díaz, C. Lyliestrom, G. García-Moliner, E. Hernández-Delgado, C. Menza, J. Morell, A. Pait, J. Sabater, E. Weil, E. Williams, & S. Williams. 2008. The state of coral reef ecosystems in Puerto Rico. Pp. 75-116. In, J.E. Waddell & A.M. Clarke (eds.), The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States: 2008. NOAA Tech. Mem. NOS NCCOS 73. NOAA/NCCOS Center for Coastal Monitoring and Assessment's Biogeography Team. Silver Spring, MD. 569 pp.

Goenaga, C., & G. Cintrón. 1979. Inventory of the Puerto Rican Coral Reefs. Report submitted to the Coastal Zone Management of the Department of Natural Resources, San Juan, P.R. 190 pp.

Hernández-Delgado, E.A. 2000. Effects of anthropogenic stress gradients in the structure of coral reef epibenthic and fish communities. Ph.D. Dissertation, Department of Biology, University of Puerto Rico, Río Piedras, P.R. 330 pp.

Hernández-Delgado, E.A. 2003a. Suplemento técnico al Plan de Manejo para la Reserva Natural del Canal Luis Peña, Culebra, Puerto Rico. I. Caracterización de habitáculos. Informe Técnico sometido al Programa de Manejo de la Zona Costanera, DRNA. San Juan, PR. 109 pp.

Hernández-Delgado, E.A. 2003b. Suplemento técnico al Plan de Manejo para la Reserva Natural del Canal Luis Peña, Culebra, Puerto Rico. IV. Alternativas de demarcación y de zonificación. Informe Técnico sometido al Programa de Manejo de la Zona Costanera, DRNA. San Juan, PR. 48 pp.

Hernández-Delgado, E.A. 2004. Análisis del estado de los recursos y de la situación ambiental actual de la Reserva Natural del Canal Luis Peña, Culebra, P.R. Informe Técnico sometido a la Autoridad de Conservación y Desarrollo de Culebra, Culebra, PR. 133 pp.

Hernández-Delgado, E.A. 2005. Historia natural, caracterización, distribución y estado actual de los arrecifes de coral Puerto Rico. 281-356. In, R.L. Joglar (Ed.), Biodiversidad de Puerto Rico: Vertebrados Terrestres y Ecosistemas. Serie Historia Natural. Editorial Instituto de Cultura Puertorriqueña, San Juan, PR. 563 pp.

Hernández-Delgado, E.A., & A.M. Sabat. 2000. Ecological status of essential fish habitats through an anthropogenic environmental stress gradient in Puerto Rican coral reefs. *Proc. Gulf Caribb. Fish. Inst*. 51:457-470.

Hernández-Delgado, E.A., L. Alicea-Rodríguez, C.G. Toledo-Hernández, & A.M. Sabat. 2000. Baseline characterization of coral reef epibenthic and fish communities within the proposed Culebra Island Marine Fishery Reserve, Puerto Rico. *Proc. Gulf Caribb. Fish. Inst*. 51:537-556.

Hernández-Delgado, E.A. y B.J. Rosado-Matías. 2003. Suplemento técnico al Plan de Manejo para la Reserva Natural del Canal Luis Peña, Culebra, Puerto Rico. II. Inventario biológico. Informe Técnico sometido al Programa de Manejo de la Zona Costanera, DRNA. San Juan, PR. 60 pp.

Hernández-Delgado, E.A., B.J. Rosado-Matías, & A.M. Sabat. 2006. Management failures and coral decline threatens fish functional groups recovery patterns in the Luis Peña Channel No-Take Natural Reserve, Culebra Island, PR. *Proc. Gulf Caribb. Fish. Inst*. 57:577-605.

Hodgkiss, I.J., W.S. Yim. 1995. A case study of Tolo Harbour, Hong Kong. 41-58. In, A.J. McComb (ed.), *Eutrophic Shallow Estuaries and Lagoons*. CRC Press, Boca Raton, FL. 240 pp.

Kaczmarsky, L.T., M. Draud, & E.H. Williams. 2005. Is there a relationship between proximity to sewage effluent and the prevalence of coral disease? *Caribb. J. Sci*. 41:124-137.

Kennish, M.J. 2002. Environmental threats and environmental future of estuaries. *Environ. Conserv*. 29:78-107.

Köster, M, S. Dahlke, L.A. Meyer-Reil. 1997. Microbiological studies along a gradient of eutrophication in a shallow coastal inlet in the southern Baltic Sea (Nordrügensche Bodden). *Mar. Ecol. Prog. Ser*. 152:27-39.

Livingston, R.J. 2001. Eutrophication Processes in Coastal Systems. Origin and Succession of Plankton Blooms and Effects on Secondary Production in Gulf Coast Estuaries. CRC Press, Boca Raton, FL 327 pp.

McComb, A.J. 1995. Eutrophic Shallow Estuaries and Lagoons. pp. 1-240, CRC Press, Boca Raton, FL.

Meesters, E.H., R.P.M. Bak, S. Westmacott, M. Ridgley, & S. Dollar. 1998. A fuzzy logic model to predict coral reef development under nutrient and sediment stress. *Conserv. Biol*. 12:957-965.

Naim, O. 1993. Seasonal responses of a fringing reef community to eutrophication (Reunion Island, Western Indian Ocean

Nugues, M.M., & C.M. Roberts. 2003. Coral mortality and interaction with algae in relation to sedimentation. *Coral Reefs* 22:507-516.

Pastorok, R.A., & G.R. Bilyard. 1985. Effects of sewage pollution on coral-reef communities. *Mar. Ecol. Prog. Ser*. 175-189.

Rogers, C.S. 1990. Responses of coral reefs and reef organisms to sedimentation. *Mar. Ecol. Progr. Ser*. 62:185-202.

Scott, T.M., J.B. Rose, T.M. Jenkins, S.M. Farrah, & J. Lukasik. 2002. Microbial source tracking: current methodology and future directions. *Appl. Environ. Microbiol*. 68:5796-5803.

Smith, T.B., R.S. Nemeth, J. Blondeau, J.M. Calnan, E. Kadison, & S. Herzlieb. 2008. Assessing coral reef health across onshore to offshore gradients in the US Virgin Islands. 56:1983-1991.

Stewart, J.R., R.J. Gast, R.S. Fujioka, H.M. Solo-Gabriele, J.S. Mschke, L.A. Amaral-Zettler, E. del Castillo, M.F. Polz, T.K. Collier, M.S. Strom, C.D. Sinigalliano, P.D.R. Moeller, & A.F. Holland. 2008. The coastal environment and human health: microbial indicators, pathogens, sentinels and reservoirs. *Environ. Health* 7(Suppl 2):S3-S16.

Szmant,.A.M. 2002. Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries*. 25:743-766.

Tomascik, T., & F. Sander. 1985. Effects of eutrophication on reef-building corals. I. Growth rate of the reef-building coral *Montastrea annularis*. *Mar. Biol*. 87:132-155.

Tomascik, T., & F. Sander. 1987a. Effects of eutrophication on reef-building corals. II. Structure of scleractinian coral communities on fringing reefs, Barbados, West Indies. *Mar. Biol*. 94:53-75.

Tomascik, T., & F. Sander. 1987b. Effects of eutrophication on reef-building corals. III. Reproduction of the reef-building coral *Porites porites*. *Mar. Biol*. 94:53-75.

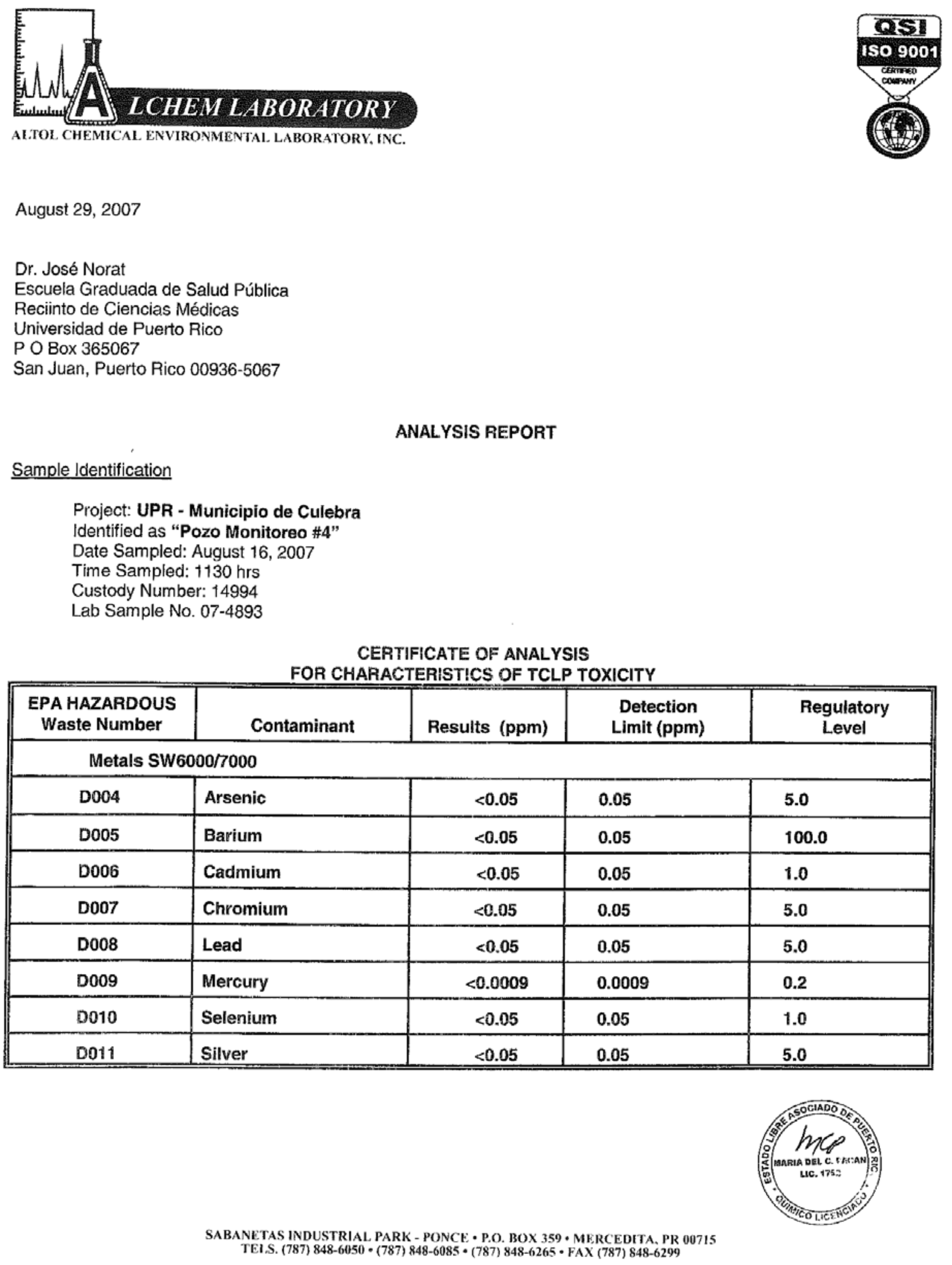
Tomascik, T. 1990. Growth rates of two morphotypes of *Montastrea annularis* along a eutrophication gradient, Barbados, W.I. *Mar. Poll. Bull*. 21:376-381.

Tomascik, T. 1991. Settlement patterns of Caribbean scleractinian corals on artificial substrata along a eutrophication gradient, Barbados, West Indies. *Mar. Ecol. Progr. Ser*. 77:261-269.

Zar, J.H. 1984. Biostatistical Analysis, 2nd Ed. Prentice-Hall, Inc., Englewood Cliffs, N.J. 718 pp.

Appendix A

Results of laboratory evaluation of heavy metals in leachate at the Culebra sanitary landfill



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August 29, 2007

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ANALYSIS REPORT

Sample Identification

Project: **UPR - Municipio de Culebra**
Identified as **"Pozo Monitoreo #4"**
Date Sampled: August 16, 2007
Time Sampled: 1130 hrs
Custody Number: 14994
Lab Sample No. 07-4893

CERTIFICATE OF ANALYSIS
FOR CHARACTERISTICS OF TCLP TOXICITY

EPA HAZARDOUS Waste Number	Contaminant	Results (ppm)	Detection Limit (ppm)	Regulatory Level
Metals SW6000/7000				
D004	Arsenic	<0.05	0.05	5.0
D005	Barium	<0.05	0.05	100.0
D006	Cadmium	<0.05	0.05	1.0
D007	Chromium	<0.05	0.05	5.0
D008	Lead	<0.05	0.05	5.0
D009	Mercury	<0.0009	0.0009	0.2
D010	Selenium	<0.05	0.05	1.0
D011	Silver	<0.05	0.05	5.0

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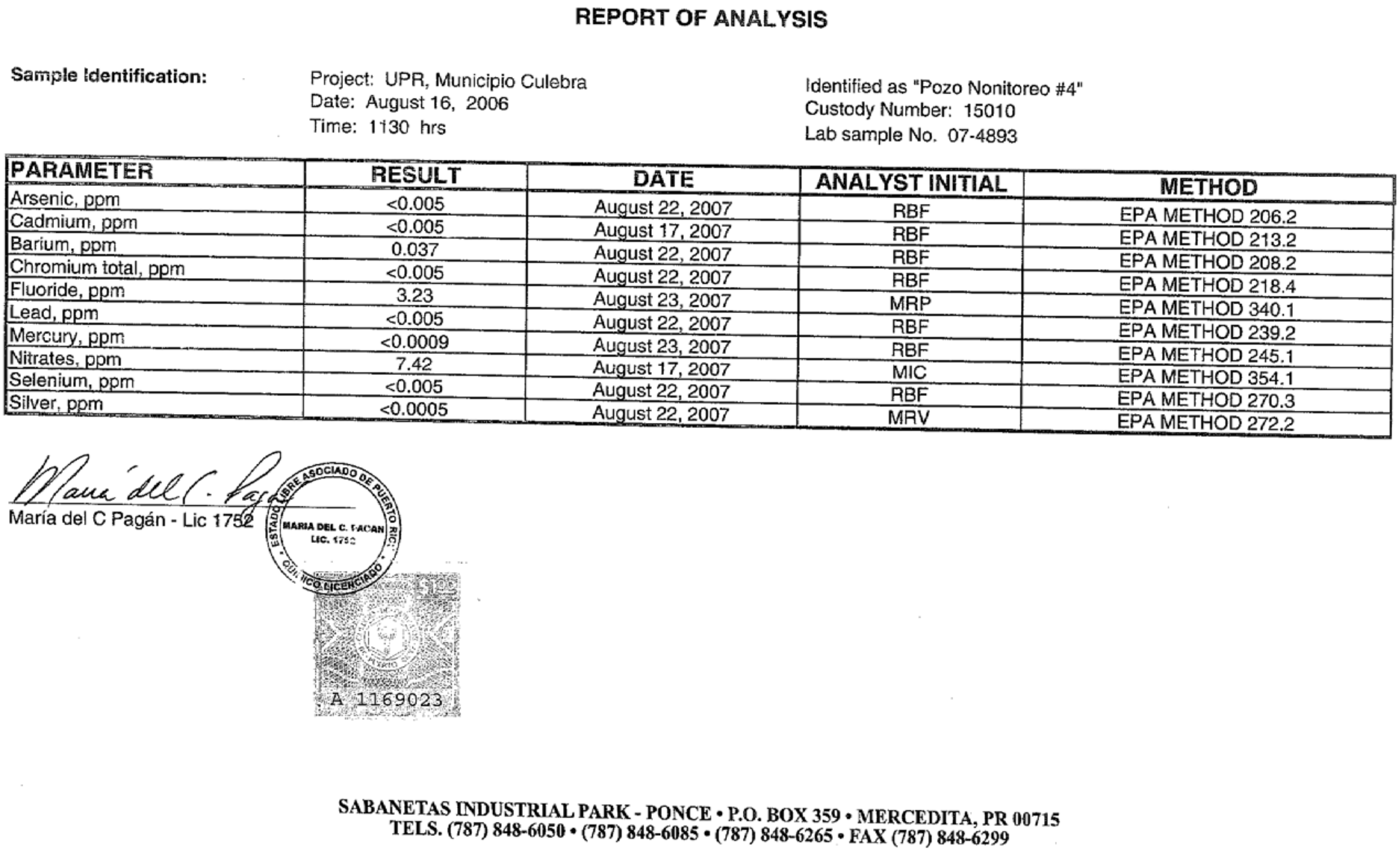

REPORT OF ANALYSIS

Sample Identification:

Project: UPR, Municipio Culebra
Date: August 16, 2006
Time: 1130 hrs

Identified as "Pozo Nonitoreo #4"
Custody Number: 15010
Lab sample No. 07-4893

PARAMETER	RESULT	DATE	ANALYST INITIAL	METHOD
Arsenic, ppm	<0.005	August 22, 2007	RBF	EPA METHOD 206.2
Cadmium, ppm	<0.005	August 17, 2007	RBF	EPA METHOD 213.2
Barium, ppm	0.037	August 22, 2007	RBF	EPA METHOD 208.2
Chromium total, ppm	<0.005	August 22, 2007	RBF	EPA METHOD 218.4
Fluoride, ppm	3.23	August 23, 2007	MRP	EPA METHOD 340.1
Lead, ppm	<0.005	August 22, 2007	RBF	EPA METHOD 239.2
Mercury, ppm	<0.0009	August 23, 2007	RBF	EPA METHOD 245.1
Nitrates, ppm	7.42	August 17, 2007	MIC	EPA METHOD 354.1
Selenium, ppm	<0.005	August 22, 2007	RBF	EPA METHOD 270.3
Silver, ppm	<0.0005	August 22, 2007	MRV	EPA METHOD 272.2

María del C. Pagán

María del C Pagán - Lic 1752

MARIA DEL C. PAGAN
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A 1169023

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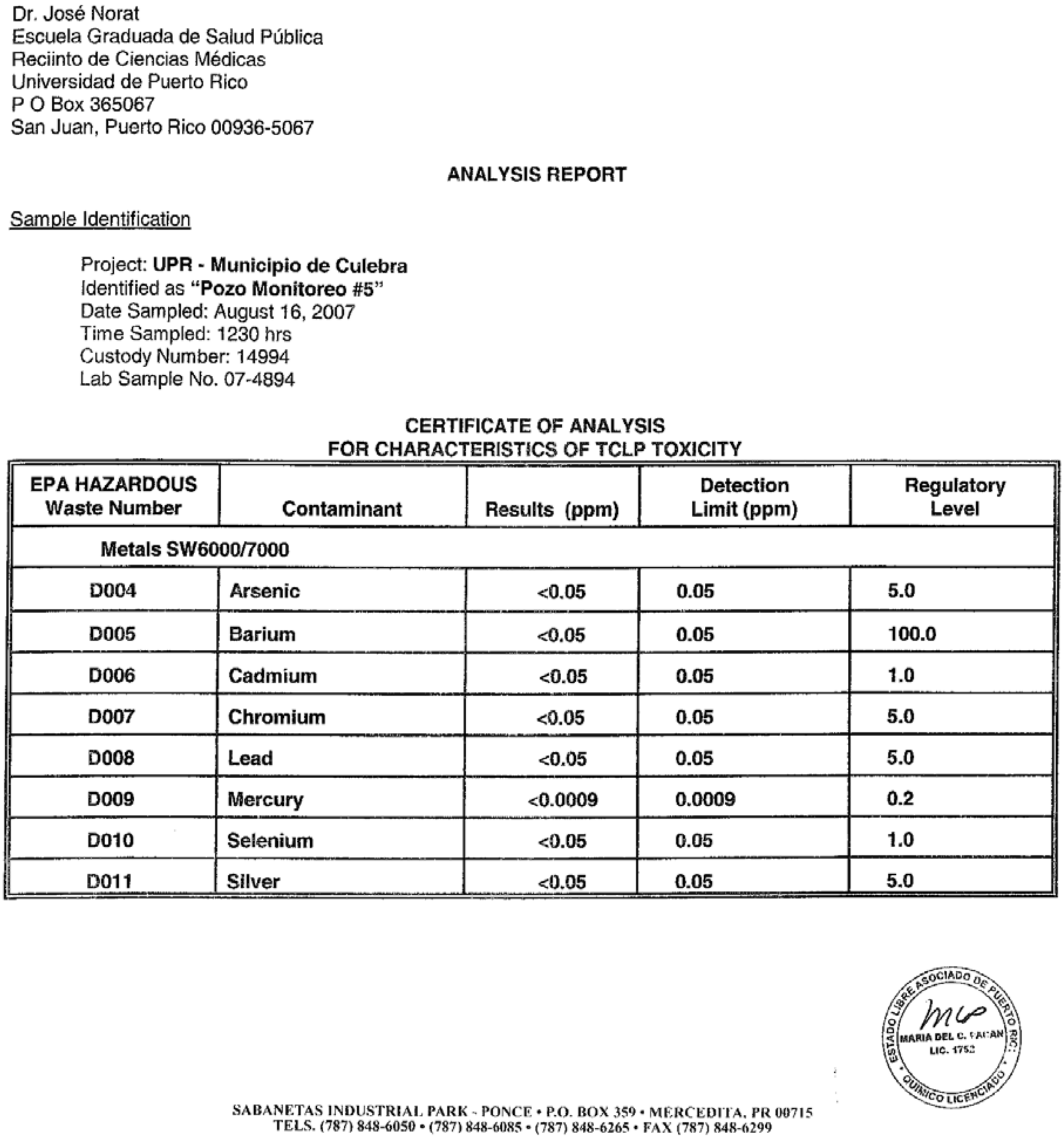

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Universidad de Puerto Rico
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San Juan, Puerto Rico 00936-5067

ANALYSIS REPORT

Sample Identification

Project: **UPR - Municipio de Culebra**
Identified as **"Pozo Monitoreo #5"**
Date Sampled: August 16, 2007
Time Sampled: 1230 hrs
Custody Number: 14994
Lab Sample No. 07-4894

CERTIFICATE OF ANALYSIS FOR CHARACTERISTICS OF TCLP TOXICITY

EPA HAZARDOUS Waste Number	Contaminant	Results (ppm)	Detection Limit (ppm)	Regulatory Level
Metals SW6000/7000				
D004	Arsenic	<0.05	0.05	5.0
D005	Barium	<0.05	0.05	100.0
D006	Cadmium	<0.05	0.05	1.0
D007	Chromium	<0.05	0.05	5.0
D008	Lead	<0.05	0.05	5.0
D009	Mercury	<0.0009	0.0009	0.2
D010	Selenium	<0.05	0.05	1.0
D011	Silver	<0.05	0.05	5.0

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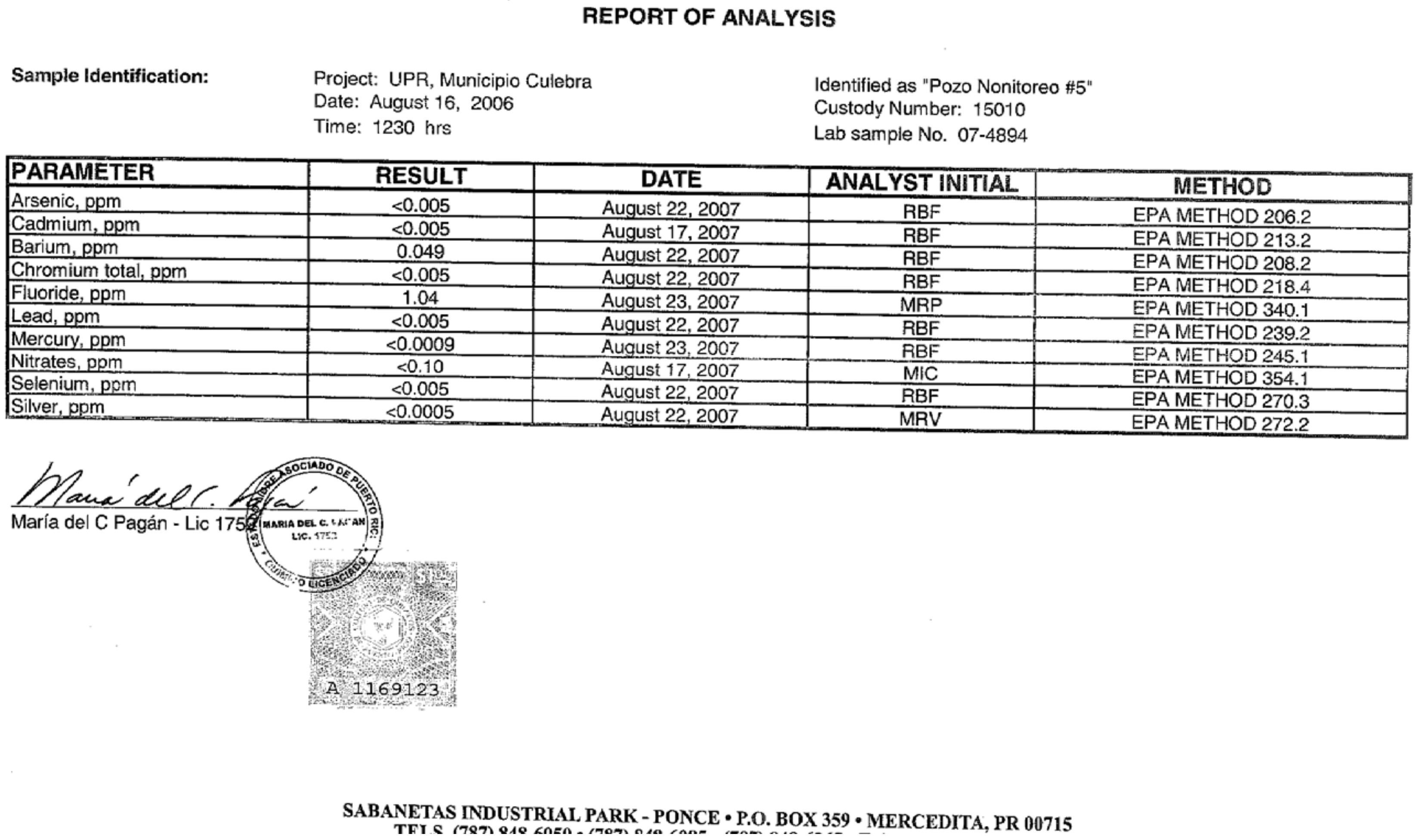

REPORT OF ANALYSIS

Sample Identification:

Project: UPR, Municipio Culebra
Date: August 16, 2006
Time: 1230 hrs

Identified as "Pozo Nonitoreo #5"
Custody Number: 15010
Lab sample No. 07-4894

PARAMETER	RESULT	DATE	ANALYST INITIAL	METHOD
Arsenic, ppm	<0.005	August 22, 2007	RBF	EPA METHOD 206.2
Cadmium, ppm	<0.005	August 17, 2007	RBF	EPA METHOD 213.2
Barium, ppm	0.049	August 22, 2007	RBF	EPA METHOD 208.2
Chromium total, ppm	<0.005	August 22, 2007	RBF	EPA METHOD 218.4
Fluoride, ppm	1.04	August 23, 2007	MRP	EPA METHOD 340.1
Lead, ppm	<0.005	August 22, 2007	RBF	EPA METHOD 239.2
Mercury, ppm	<0.0009	August 23, 2007	RBF	EPA METHOD 245.1
Nitrates, ppm	<0.10	August 17, 2007	MIC	EPA METHOD 354.1
Selenium, ppm	<0.005	August 22, 2007	RBF	EPA METHOD 270.3
Silver, ppm	<0.0005	August 22, 2007	MRV	EPA METHOD 272.2

María del C. Pagán
María del C Pagán - Lic 175

MARIA DEL C. PAGAN

A 1169123

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Appendix B

Analysis of Variance for Water Quality Among Stations

ANOVA for Water Quality parameters Culebra 2008

```
Analysis of Variance Table for TC

Source    DF        SS        MS       F        P
Site       9   91.0044   10.1116   65.03   0.0000
Time       8   43.2074    5.4009   34.74   0.0000
Error    150   23.3227    0.1555
Total    167

Tukey HSD All-Pairwise Comparisons Test of TC for Site

Site    Mean  Homogeneous Groups
   1  30.874  A
   2  29.667   B
   3  29.564   B
   4  28.824    C
   8  28.749    C
  10  28.709    C
   6  28.662    C
   5  28.659    C
   9  28.638    C
   7  28.432    C

Tukey HSD All-Pairwise Comparisons Test of TC for Time

Time    Mean  Homogeneous Groups
   6  29.865  A
   7  29.790  A
   8  29.245   B
   3  29.180   BC
   9  29.065   BC
   5  28.865   BC
   2  28.853   BC
   4  28.817    C
   1  28.020     D

Analysis of Variance Table for pH

Source    DF        SS        MS       F        P
Site       9   0.14842   0.01649   13.42   0.0000
Time       8   0.54995   0.06874   55.95   0.0000
Error    150   0.18430   0.00123
Total    167

Tukey HSD All-Pairwise Comparisons Test of pH for Site

Site    Mean  Homogeneous Groups
   1  8.3103  A
   2  8.2814  AB
   3  8.2798  AB
   8  8.2478   BC
   6  8.2450   BCD
  10  8.2433   BCD
```

```
   5  8.2393    CD
   9  8.2350    CD
   7  8.2280    CD
   4  8.2083     D

Tukey HSD All-Pairwise Comparisons Test of pH for Time

Time    Mean  Homogeneous Groups
   4  8.3287  A
   2  8.3148  AB
   7  8.2985  AB
   1  8.2837   BC
   3  8.2599    CD
   5  8.2275     DE
   9  8.2160      E
   8  8.2000      E
   6  8.1370       F

Analysis of Variance Table for Cond

Source    DF        SS        MS       F        P
Site       9    80.475    8.9417    2.12   0.0313
Time       8   612.437   76.5547   18.13   0.0000
Error    150   633.369    4.2225
Total    167

Tukey HSD All-Pairwise Comparisons Test of Cond for Site

Site    Mean  Homogeneous Groups
   6  55.348  A
   9  54.938  AB
   7  54.918  AB
  10  54.564  AB
   8  54.539  AB
   5  54.429  AB
   4  54.384  AB
   3  53.959  AB
   2  53.709  AB
   1  52.684   B

Tukey HSD All-Pairwise Comparisons Test of Cond for Time

Time    Mean  Homogeneous Groups
   5  56.850  A
   2  55.943  AB
   4  55.409  AB
   1  55.265  AB
   6  54.995  AB
   9  54.080   BC
   3  53.815   BC
   8  52.565    C
   7  50.205     D

Analysis of Variance Table for DO

Source    DF        SS        MS       F        P
```

```
Site       9   50.4165    5.6018    8.87   0.0000
Time       7   77.7402   11.1057   17.59   0.0000
Error    135   85.2519    0.6315
Total    151
```

Tukey HSD All-Pairwise Comparisons Test of DO for Site

```
Site    Mean        1        2        3        4        5        6        7
   1  10.284
   2   9.688   0.596
   3   9.297   0.987*   0.391
   4   8.381   1.903*   1.307*   0.916*
   5   8.999   1.285*   0.689    0.298    0.618
   6   8.823   1.461*   0.865    0.474    0.442    0.176
   7   8.364   1.920*   1.324*   0.933    0.017    0.635    0.459
   8   9.062   1.222*   0.626    0.235    0.681    0.063    0.239    0.698
   9   8.526   1.758*   1.163*   0.772    0.144    0.473    0.298    0.161
  10   8.829   1.455*   0.859    0.468    0.448    0.170    0.006    0.465
Site    Mean        8        9
   8   9.062
   9   8.526   0.537
  10   8.829   0.233    0.303
```

Tukey HSD All-Pairwise Comparisons Test of DO for Time

```
Time    Mean  Homogeneous Groups
   2  10.757  A
   7   9.394   B
   6   9.039   BC
   1   9.013   BC
   4   8.673   BCD
   8   8.646   BCD
   9   8.561    CD
   5   8.122     D
```

Analysis of Variance Table for Sal

```
Source    DF        SS        MS       F        P
Site       9    38.507    4.2785    2.09   0.0336
Time       8   328.433   41.0541   20.07   0.0000
Error    150   306.890    2.0459
Total    167
```

Tukey HSD All-Pairwise Comparisons Test of Sal for Site

```
Site    Mean        1        2        3        4        5        6        7
   1  34.954
   2  35.546   0.592
   3  35.769   0.815    0.223
   4  36.064   1.110    0.518    0.295
   5  36.109   1.155    0.563    0.340    0.045
   6  36.696   1.741    1.150    0.926    0.631    0.586
   7  36.376   1.421    0.830    0.606    0.311    0.266    0.320
   8  36.099   1.145    0.553    0.330    0.035    0.010    0.596    0.276
   9  36.574   1.620*   1.029    0.805    0.510    0.465    0.121    0.199
```

```
  10  36.329   1.375    0.783    0.560    0.265    0.220    0.366    0.046
Site    Mean       8        9
   8  36.099
   9  36.574   0.475
  10  36.329   0.230    0.245

Alpha              0.05     Standard Error for Comparison  VARIES
Critical Q Value  4.470     Critical Value for Comparison  VARIES
Error term used: Error, 150 DF
The homogeneous group format can't be used
because of the pattern of significant differences.

Tukey HSD All-Pairwise Comparisons Test of Sal for Time

Time    Mean  Homogeneous Groups
   5  37.735  A
   2  37.286  AB
   4  36.827  ABC
   6  36.615  ABC
   1  36.536  ABC
   9  36.120   BC
   3  35.684    CD
   8  34.705     D
   7  32.955      E

Analysis of Variance Table for NTU

Source    DF         SS         MS        F        P
Site       9   2374.71   263.857   25.69   0.0000
Time       8    423.27    52.909    5.15   0.0000
Error    150   1540.54    10.270
Total    167

Tukey HSD All-Pairwise Comparisons Test of NTU for Site

Site    Mean  Homogeneous Groups
   1  12.459  A
   3   6.197   B
   2   2.985   BC
   4   1.841    C
   8   1.336    C
  10   1.225    C
   5   1.037    C
   7   0.852    C
   6   0.641    C
   9   0.317    C

Tukey HSD All-Pairwise Comparisons Test of NTU for Time

Time    Mean  Homogeneous Groups
   7  6.1400  A
   3  5.0081  AB
   5  3.1850  ABC
   9  2.7800   BC
   1  2.6397   BC
   4  2.0362   BC
```

```
   8  1.6605    C
   6  1.4950    C
   2  1.0547    C
```

Analysis of Variance Table for LogFC

```
Source    DF        SS        MS       F         P
Site       9   160.198   17.7997   51.29   0.0000
Time       8     9.966    1.2457    3.59   0.0008
Error    137    47.542    0.3470
Total    154
```

Tukey HSD All-Pairwise Comparisons Test of LogFC for Site

```
Site    Mean  Homogeneous Groups
   3  2.4529  A
   1  2.1157  A
   2  1.8027  A
   4  0.8281   B
   8  0.5907   B
  10 -0.1196    C
   9 -0.1938    C
   5 -0.2235    C
   7 -0.2274    C
   6 -0.3596    C
```

Tukey HSD All-Pairwise Comparisons Test of LogFC for Time

```
Time    Mean  Homogeneous Groups
   5  1.1038  A
   7  0.8981  AB
   4  0.8015  ABC
   1  0.7933  ABC
   6  0.6878  ABC
   2  0.5920  ABC
   9  0.4691   BC
   8  0.4673   BC
   3  0.1868    C
```

Analysis of Variance Table for LogEnt

```
Source    DF        SS        MS       F         P
Site       9   83.3867   9.26519   20.65   0.0000
Time       8   46.4589   5.80736   12.94   0.0000
Error    143   64.1560   0.44864
Total    160
```

Tukey HSD All-Pairwise Comparisons Test of LogEnt for Site

```
Site    Mean  Homogeneous Groups
   3  2.2925  A
   1  1.8800  AB
   2  1.5770  AB
   4  1.4883   B
```

```
   8  1.2601   BC
   7  0.4546    CD
   9  0.3826     D
  10  0.3558     D
   5  0.3438     D
   6  0.3178     D

Tukey HSD All-Pairwise Comparisons Test of LogEnt for Time

Time    Mean  Homogeneous Groups
   7  2.2004  A
   3  1.4130   B
   6  1.1652   B
   5  1.1077   B
   8  0.9718   B
   2  0.7772   BC
   1  0.7391   BC
   4  0.7152   BC
   9  0.2277    C
```

CORALES

Sites 1=BSA, 2=PME, 3=PTC, 4=AEB, 5=PRA, 6=CLP, 7=PCR

```
One-Way AOV for LogTSpp by SITE

Source   DF        SS        MS        F        P
SITE      6   0.61007   0.10168     3.95   0.0046
Error    32   0.82411   0.02575
Total    38   1.43418

Tukey HSD All-Pairwise Comparisons Test of LogTSpp by SITE

SITE    Mean  Homogeneous Groups
   3  0.9324  A
   2  0.8493  A
   5  0.8428  A
   4  0.8389  AB
   6  0.7521  AB
   7  0.7174  AB
   1  0.5293   B

One-Way AOV for LogTAbun by SITE

Source   DF        SS        MS        F        P
SITE      6   0.54795   0.09133     2.23   0.0656
Error    32   1.31054   0.04095
Total    38   1.85849

One-Way AOV for LogHSpp by SITE

Source   DF        SS        MS        F        P
SITE      6   0.37820   0.06303     2.93   0.0217
Error    32   0.68937   0.02154
```

```
Total    38   1.06757
```

Tukey HSD All-Pairwise Comparisons Test of LogHSpp by SITE

```
SITE    Mean  Homogeneous Groups
   4  0.8024  A
   3  0.7893  A
   2  0.6977  AB
   5  0.6221  AB
   7  0.5766  AB
   6  0.5715  AB
   1  0.5293   B
```

One-Way AOV for LogHAbun by SITE

```
Source   DF        SS        MS       F        P
SITE      6   0.47311   0.07885    2.70   0.0309
Error    32   0.93401   0.02919
Total    38   1.40712
```

Tukey HSD All-Pairwise Comparisons Test of LogHAbun by SITE

```
SITE    Mean  Homogeneous Groups
   2  1.2151  A
   4  1.1325  AB
   1  1.0705  AB
   3  1.0585  AB
   5  0.9757  AB
   7  0.9683  AB
   6  0.8442   B
```

One-Way AOV for ArcCoral by SITE

```
Source   DF        SS        MS       F        P
SITE      6   0.30387   0.05064    2.32   0.0569
Error    32   0.69918   0.02185
Total    38   1.00305
```

One-Way AOV for ArcScler by SITE

```
Source   DF        SS        MS       F        P
SITE      6   1.85678   0.30946    4.83   0.0013
Error    32   2.04894   0.06403
Total    38   3.90572
```

Tukey HSD All-Pairwise Comparisons Test of ArcScler by SITE

```
SITE    Mean  Homogeneous Groups
   1  1.4900  A
   4  1.1024  AB
```

```
   2  0.9607   B
   3  0.9536   B
   5  0.9024   B
   7  0.8521   B
   6  0.8162   B
```

```
One-Way AOV for ArcHydro by SITE

Source    DF         SS         MS       F         P
SITE       6   0.88401   0.14733    3.90   0.0049
Error     32   1.20923   0.03779
Total     38   2.09324

Tukey HSD All-Pairwise Comparisons Test of ArcHydro by SITE

SITE    Mean  Homogeneous Groups
   4  0.4265  A
   6  0.3997  A
   3  0.3001  AB
   2  0.1028  AB
   1  0.0808  AB
   5  0.0566   B
   7  0.0343   B

One-Way AOV for ArcOcto by SITE

Source    DF         SS         MS       F         P
SITE       6   2.46924   0.41154    7.07   0.0001
Error     32   1.86293   0.05822
Total     38   4.33217
```

```
One-Way AOV for ArcOcto by SITE

Source    DF         SS         MS       F         P
SITE       6   2.46924   0.41154    7.07   0.0001
Error     32   1.86293   0.05822
Total     38   4.33217
```

```
Tukey HSD All-Pairwise Comparisons Test of ArcOcto by SITE

SITE    Mean  Homogeneous Groups
   7  0.7115  A
   5  0.6499  A
   2  0.5751  AB
   6  0.5330  AB
   3  0.4598  AB
   4  0.1051   BC
   1  0.0000    C

One-Way AOV for ArcMac by SITE

Source    DF         SS         MS       F         P
SITE       6   0.52296   0.08716    5.73   0.0004
```

```
Error    32   0.48717   0.01522
Total    38   1.01013
```

Tukey HSD All-Pairwise Comparisons Test of ArcMac by SITE

```
SITE    Mean  Homogeneous Groups
   1  0.4109  A
   3  0.3884  A
   2  0.3874  A
   5  0.3069  A
   4  0.2699  AB
   6  0.2013  AB
   7  0.0746   B
```

One-Way AOV for ArcTurf by SITE

```
Source   DF        SS        MS        F        P
SITE      6   2.45310   0.40885    16.7   0.0000
Error    32   0.78330   0.02448
Total    38   3.23640

Grand Mean 0.2772    CV 56.45
```

Tukey HSD All-Pairwise Comparisons Test of ArcTurf by SITE

```
SITE    Mean  Homogeneous Groups
   6  0.7717  A
   3  0.5004  AB
   4  0.3220   BC
   5  0.2721   BCD
   1  0.1285    CD
   2  0.0428    CD
   7  0.0000     D
```

One-Way AOV for ArcHal by SITE

```
Source   DF        SS        MS        F        P
SITE      6   0.10463   0.01744    4.76   0.0014
Error    32   0.11730   0.00367
Total    38   0.22194
```

Tukey HSD All-Pairwise Comparisons Test of ArcHal by SITE

```
SITE    Mean  Homogeneous Groups
   1  0.1519  A
   2  0.0642  AB
   6  0.0514  AB
   3  0.0303   B
   4  0.0000   B
```

```
   5  0.0000   B
   7  0.0000   B

One-Way AOV for ArcCCA by SITE

Source   DF        SS        MS       F        P
SITE      6   3.01892   0.50315    36.2   0.0000
Error    32   0.44420   0.01388
Total    38   3.46312


Tukey HSD All-Pairwise Comparisons Test of ArcCCA by SITE

SITE    Mean  Homogeneous Groups
   4  0.7428  A
   5  0.5946  A
   3  0.5129  A
   6  0.4955  A
   2  0.0428   B
   7  0.0373   B
   1  0.0214   B

One-Way AOV for ArcCya by SITE

Source   DF        SS        MS       F        P
SITE      6   0.20839   0.03473    5.58   0.0005
Error    32   0.19905   0.00622
Total    38   0.40744

Tukey HSD All-Pairwise Comparisons Test of ArcCya by SITE

SITE    Mean  Homogeneous Groups
   4  0.2780  A
   3  0.1338  AB
   7  0.0945   B
   6  0.0878   B
   5  0.0587   B
   1  0.0303   B
   2  0.0214   B

One-Way AOV for ArcRock by SITE

Source   DF        SS        MS       F        P
SITE      6   2.61303   0.43550    38.2   0.0000
Error    32   0.36516   0.01141
Total    38   2.97819

Tukey HSD All-Pairwise Comparisons Test of ArcRock by SITE

SITE    Mean  Homogeneous Groups
   1  0.7211  A
   2  0.4678   B
   7  0.4669   B
   5  0.0890    C
   3  0.0731    C
   6  0.0621    C
```

```
   4  0.0000    C
```

One-Way AOV for ArcRub by SITE

```
Source   DF        SS        MS       F        P
SITE      6   0.40219   0.06703    6.95   0.0001
Error    32   0.30873   0.00965
Total    38   0.71092
```

Tukey HSD All-Pairwise Comparisons Test of ArcRub by SITE

```
SITE    Mean  Homogeneous Groups
   1  0.3048  A
   2  0.2141  AB
   7  0.1765  ABC
   4  0.1014   BC
   3  0.0428   BC
   6  0.0364   BC
   5  0.0214    C
```

One-Way AOV for ArcSand by SITE

```
Source   DF        SS        MS       F        P
SITE      6   2.21993   0.36999    28.3   0.0000
Error    32   0.41793   0.01306
Total    38   2.63786
```

Tukey HSD All-Pairwise Comparisons Test of ArcSand by SITE

```
SITE    Mean  Homogeneous Groups
   7  0.6891  A
   2  0.3414   B
   1  0.1232    C
   5  0.0857    C
   6  0.0257    C
   3  0.0000    C
   4  0.0000    C
```

One-Way AOV for ArcPmort by SITE

```
Source   DF        SS        MS       F        P
SITE      6   1.99475   0.33246    4.85   0.0013
Error    32   2.19288   0.06853
Total    38   4.18764
```

Tukey HSD All-Pairwise Comparisons Test of ArcPmort by SITE

```
SITE    Mean  Homogeneous Groups
   1  0.8091  A
   7  0.5964  AB
   2  0.4948  AB
   5  0.2951   B
   3  0.2138   B
```

```
   4  0.1979   B
   6  0.1660   B

One-Way AOV for ArcRmort by SITE

Source   DF        SS        MS       F        P
SITE      6   2.73279   0.45547    8.64   0.0000
Error    32   1.68716   0.05272
Total    38   4.41995



Tukey HSD All-Pairwise Comparisons Test of ArcRmort by SITE

SITE    Mean  Homogeneous Groups
   1  0.6836  A
   7  0.6646  A
   2  0.5061  AB
   4  0.2431  ABC
   5  0.2221   BC
   3  0.0421    C
   6  0.0000    C

One-Way AOV for HnT by SITE

Source   DF        SS        MS       F        P
SITE      6   4.73764   0.78961    5.57   0.0005
Error    32   4.53623   0.14176
Total    38   9.27388

Tukey HSD All-Pairwise Comparisons Test of HnT by SITE

SITE    Mean  Homogeneous Groups
   3  2.0230  A
   4  1.7917  AB
   5  1.7205  AB
   2  1.6667  AB
   6  1.5915  ABC
   7  1.3202   BC
   1  0.8937    C

One-Way AOV for JnT by SITE

Source   DF        SS        MS       F        P
SITE      6   0.22246   0.03708    5.09   0.0009
Error    32   0.23321   0.00729
Total    38   0.45567

Tukey HSD All-Pairwise Comparisons Test of JnT by SITE

SITE    Mean  Homogeneous Groups
   3  0.9377  A
   4  0.9294  A
   6  0.9126  A
   5  0.8863  A
```

```
   2  0.8491  AB
   7  0.8000  AB
   1  0.7145   B
```

One-Way AOV for HnH by SITE

```
Source   DF        SS        MS       F        P
SITE      6   2.83705   0.47284    3.42   0.0101
Error    32   4.42446   0.13826
Total    38   7.26151
```

Tukey HSD All-Pairwise Comparisons Test of HnH by SITE

```
SITE    Mean  Homogeneous Groups
   4  1.7101  A
   3  1.6969  A
   2  1.3462  AB
   6  1.1957  AB
   5  1.1867  AB
   7  1.1812  AB
   1  0.8937   B
```

One-Way AOV for JnH by SITE

```
Source   DF        SS        MS       F        P
SITE      6   0.19351   0.03225    3.04   0.0180
Error    32   0.33899   0.01059
Total    38   0.53251
```

Tukey HSD All-Pairwise Comparisons Test of JnH by SITE

```
SITE    Mean  Homogeneous Groups
   3  0.9281  A
   4  0.9252  A
   6  0.8943  AB
   7  0.8818  AB
   2  0.8320  AB
   5  0.8175  AB
   1  0.7145   B
```

Appendix C

Report of Coral Reef Consultants

Biological characterization of shallow-water coral reef communities across a water quality gradient within the Luis Peña Channel Natural Reserve, Culebra Island, Puerto Rico

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Final Technical Report

Submitted to the Department of Environmental Health, Public Health Graduate School,
University of Puerto Rico-Medical Sciences Campus, and
Department of Natural and Environmental Resources
San Juan, Puerto Rico

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Summary

1. Coral reef communities adjacent to the Culebra Island municipal landfill were assessed and quantitatively described for the first time to test for any potential landfill operation impact.
2. Given the lack of long-term monitoring, and the resulting temporal and spatial constraints of this study, there were no signs of landfill impacts in adjacent coral reef benthic community structures.
3. Coral reefs adjacent to the landfill still support a high biodiversity.
4. However, reefs are showing signs of unequivocal decline associated to a combination of long-term regional (i.e. sea surface warming, coral bleaching, hurricanes, disease outbreaks) and local factors (i.e., sediment- and nutrient-laden runoff pulses, remote raw sewage impacts).
5. Sediment-laden runoff pulses are often occurring in the landfill after heavy rainfall. Plastic bags and other plastic debris is frequently blown by the wind and ends up at Bahía Tamarindo, but impacts of these were not quantified in this study.
6. There is a need to use coral proxy signals (i.e., annual growth bands, humic acids, heavy metal accumulation) to test for any spatial and/or temporal variation in patterns of impacts.

Introduction

Long-term degradation of coastal water quality in Puerto Rico has been implied in long-term coral reef decline (Goenaga and Cintrón, 1979; Hernández-Delgado and Sabat, 2000; García-Saís et al., 2003, 2008; Hernández-Delgado, 2005; Ballantine et al., 2008). Major threats to water quality include high sediment loads (Rogers, 1990), excess nutrient inputs (Tomascik, 1990), marine snow associated to turbid water pulses (Fabricius and Wolanski, 2000), sewage and a sort of non-point source pollutants (Bonkosky et al., 2008). Some of the factors negatively impacting coral reefs act over a broad geographic area (e.g., climate change, coral bleaching, inputs of Sahara dust, occurrence of disease). However, most of the factors are due to local anthropogenic activities and their impact will depend on factors such as the spatial distribution of human population centers, river mouths, sewage outfalls, non-point pollution sources, and coastal currents, as well as factors variable in time, such as storm-induced damage and runoff, and continued coastal development. These factors often create variable chronic anthropogenic stress gradients, usually negatively affecting water quality (Tomascik and Sander, 1985), and affecting coral growth (Tomascik, 1990), coral disease prevalence (Smith et al., 2008), partial colony mortality (Nugues and Roberts, 2003), and coral reef benthic community structure (Fabricius and McCorry, 2006). As a consequence, the condition of coral reefs around Puerto Rico should show a large degree of spatial variability, as well as temporal variability over the long term. But such variability has been poorly documented due to significant spatial limitation of previous long-term monitoring efforts.

Sediment-laden runoff, raw sewage, landfill and pier operations in Culebra Island, Puerto Rico, have been identified among the most significant environmental threats to marine communities located within the Luis Peña Channel No-Take Natural Reserve (LPCNR) (Hernández-Delgado, 2003b, 2004). However, such impacts, although documented, have never been quantified in the past. Further, although highly potential, there is no quantitative evidence yet of chemical pollution to nearby seashore, coral reef, seagrass or soft bottom communities as a result of landfill operations. Sewage impacts have neither been recorded in Culebra Island. Thus, there is a paramount need to quantify such impacts, with particular emphasis in the LPCNR.

Biological communities within the LPCNR support an outstanding biodiversity representative of the northeastern Caribbean region (Hernández-Delgado, 2000, 2003a; Hernández-Delgado et al., 2000; Hernández-Delgado and Rosado-Matías, 2003). NOAA and the Department of Natural and Environmental Resources (DNER) are highly concerned with the potential polluting impacts of the Culebra landfill operations and of non-point sources on local marine communities, with particular interest in the LPCNR. Studies in progress (Hernández-Delgado and Sabat, in preparation) have shown dramatic changes in coral reef benthic community composition in Culebra Island during the last decade or so. Some of those changes have been attributed to long-term impacts of water quality decline. Another significant factor has been recurrent coral disease outbreaks, and more recently the 2006 massive coral mortality event that followed the record breaking 2005 sea surface warming event and massive coral bleaching.

This study was aimed at evaluating what are the physical and microbiological water quality conditions within and outside the LPCNR. Also, to determine if there was any significant water quality stress gradient associated to non-point source pollution (i.e., tudbidity, sewage) that could pose any threat to coral reef communities. Our third objective was to carry out a biological characterization of the actual status of shallow-water coral reef communities across a suspected water quality stress gradient.

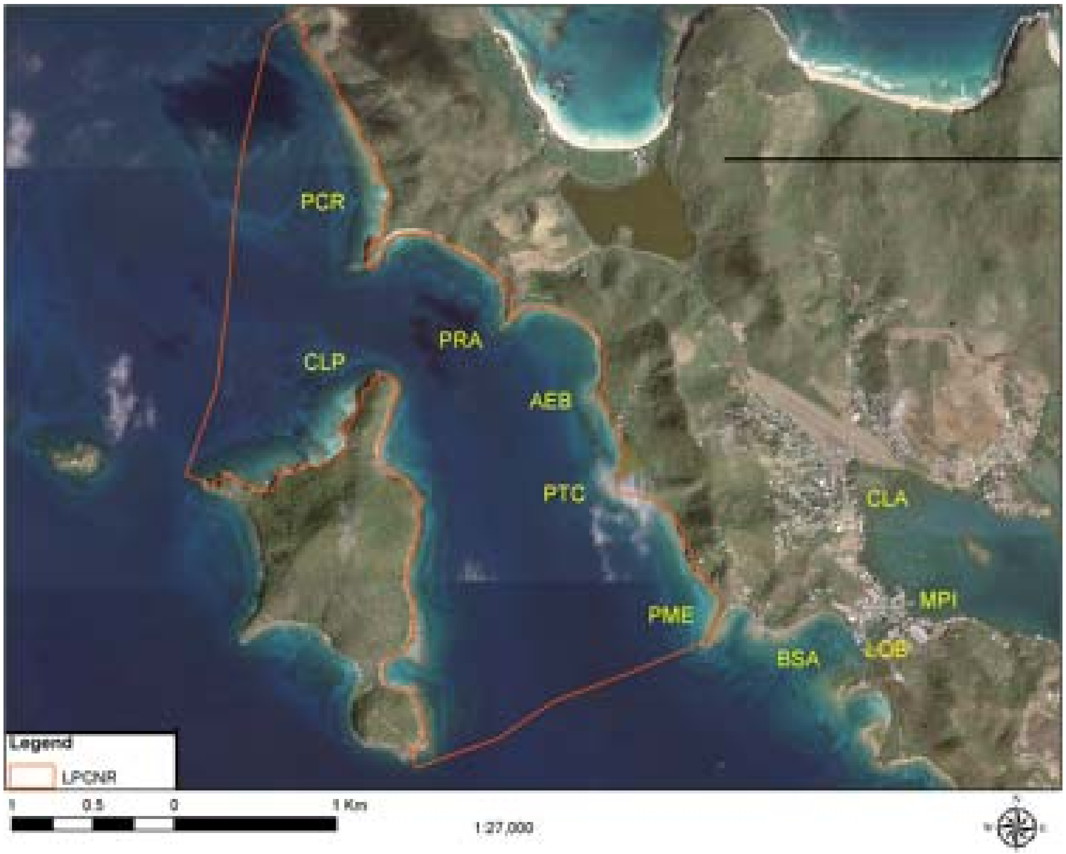

FIGURE 1. Study sites. CLA= Ensenada Honda at Barriada Clark; MPI= Ensenada Honda at Municipal Pier; LOB= Lobina Channel; BSA= Bahía Sardinas; PME= Punta Melones; PTC= Punta Tamarindo Chico; AEB= Arrecife El Banderote; PRA= Punta Rompe Anzuelo; CLP= Cayo Luis Peña-north; and PCR= Playa Carlos Rosario-south. Coral reef study sites included: BSA, PME, PTC, AEB, PRA, CLP, and PCR. Source: CRIM aerial image (2007).

Methodology

Water quality sampling sites

Studies were carried out at 10 sampling sites located along a suspected water quality gradient, ranging in an east-west-northwest direction, from Ensenada Honda Bay towards Luis Peña Channel, in Culebra Island (Figure 1). Six out of ten water quality sampling sites were located within LPCNR. According to Capella (2004), predominant sea surface currents around Culebra have along-isobath flow along the axis 300° ↔ 120° true. Further, there are strong semidiurnal (two cycles per day), and weaker diurnal (one cycle per day) tidal components, with maximum amplitudes of 20-30 cm/s, but that can occasionally reach as much as 40-50 cm/s. Also, northwestward flow (towards 300°-320° true) occurs during the flooding tide (as the sea surface elevation is increasing), whereas the ebbing tide coincides with southeastward flow (120°-140° true). Strongest currents were detected during flooding tide, which suggests that poor water quality pulses flowing from Ensenada Honda and Lobina Channel during ebbing tide towards Bahía Sardinas are subsequently carried out through the Luis Peña Channel towards LPCNR during the next flooding tidal cycle. Therefore, sampling sites were selected following the suspected impact of recurrent pulses of non-point source pollution through this along-isobath flow within LPCNR.

Predominant benthic communities at each water quality sampling site were as follows, following a non-point source pollution gradient: CLA= muddy bottom, sporadic seagrass, algal plain; MPI= muddy bottom, sporadic seagrass; LOB= muddy bottom; BSA= muddy bottom, seagrass, coral reefs; PME, PTC, AEB, PRA= coral reefs, colonized pavement, seagrass, sand bottom; and CLP, PCR= coral reefs, colonized pavement, sand bottom. CLA, MPI, and LOB receive recurrent impacts of raw sewage, as well as sediment- and nutrient-laden runoff pulses from urbanized areas during heavy rainfall events. BSA receives occasional non-point source pollution from similar sources, as well as recurrent pulses of polluted waters from Ensenada Honda and LOB during ebbing tide. PME receives occasional nutrient- and sediment-laden runoff pulses from illegal deforestation and culvert deviation towards the shore. AEB receives frequent nutrient- and sediment-laden runoff pulses from a nearby road and poorly managed developed areas nearby. PRA receives occasional nutrient- and sediment-laden runoff pulses from a nearby road and poorly managed landfill surfaces. PCR receives similar pulses from an unpaved and highly eroded abandoned dirt road, as well as from a sort of small ephemeral creeks that empty into the shoreline. PTC and CLP are indirectly impacted by pollution pulses carried out from population centers by surface currents during tidal cycles.

Water quality parameters

Bimonthly and then 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 using portable instrumentation (Horiba, Co.) during the period of May to November, 2008. Triplicate lectures of water turbidity were obtained during each visit to study sites using a Hach portable turbidimeter. Turbidity data was expressed in nephelometric turbidity units (NTU).

Microbial water quality analysis

Grab water samples were collected at each study site using autoclaved Nalgene 1 L plastic bottles (Nalgene Co.). Samples were collected in duplicates at 30 cm below surface using sterilized gloves and immediately placed on ice until analyzed within 12-24 hr. Samples were analyzed using standard membrane filtration techniques to quantify fecal coliforms (FC) and enterococci (ENT). 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 source and 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, resulted inconvenient for highly turbid water samples due to mechanical obstruction of membrane surface area by sediment particles interfering with microbial growth, and due to excess overgrowth of interfering opportunist microorganisms after heavy rainfall and turbid runoff pulse events.

Culture media and confirmatory tests

FC 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 ENT. 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:

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

Hypothesis testing

We tested the null hypothesis of no significant difference in water quality parameters and microbial indicators among sites. Microbial data were $\log_{10}$-transformed to standardize variances (Zar, 1984). 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* and *Time* were used as main variable and replicate samples as error term. No site x time interactions was analyzed due to unbalanced nature of data matrix. Chapman non-linear regression was carried out to test the relationship between water turbidity and the concentration of indicator microorganisms (fecal coliforms and enterococci).

Coral reef study sites

Coral reef benthic community characterization studies were conducted at seven coral reef locations within or adjacent to the LPCNR (Figure 1). These included six sites located within

LPCNR, namely: 1) Punta Melones (PME); 2) Punta Tamarindo Chico (PTC); 3) Arrecife El Banderote (AEB); 4) Punta Rompeanzuelo (PRA), 5) Cayo Luis Peña-north coast (CLP); and 6) Playa Carlos Rosario-south (PCR); and one site located outside of LPCR, close to Dewey downtown area at Bahía Sardinas (BSA). The Culebra Island Municipal Landfill is located adjacent to PRA at the LPCNR (Figure 2). LPCNR covers an area of approximately 636 ha, with depths reaching approximately 24 m. Patchy macroalgal plains cover approximately 30% of the bottom, closely followed by continuous seagrass communities with 28% (Hernández-Delgado, 2003). Colonized pavements cover nearly 20% of the bottom. Other benthic categories are listed in Table 1.

TABLE 1. Benthic habitat categories within the LPCNR*.

Benthic categories	%
Linear reefs	5.59
Colonized bedrock	6.67
Colonized pavement	19.75
Scattered coral rock	0.65
Colonized pavement with channels	3.30
Patch reefs	0.86
Seagrass (continuous)	28.36
Seagrass (70-90%)	1.20
Seagrass (50-70%)	0.0
Seagrass (30-50%)	0.78
Patchy macroalgal plain (10-50%)	29.89
Sand	2.95

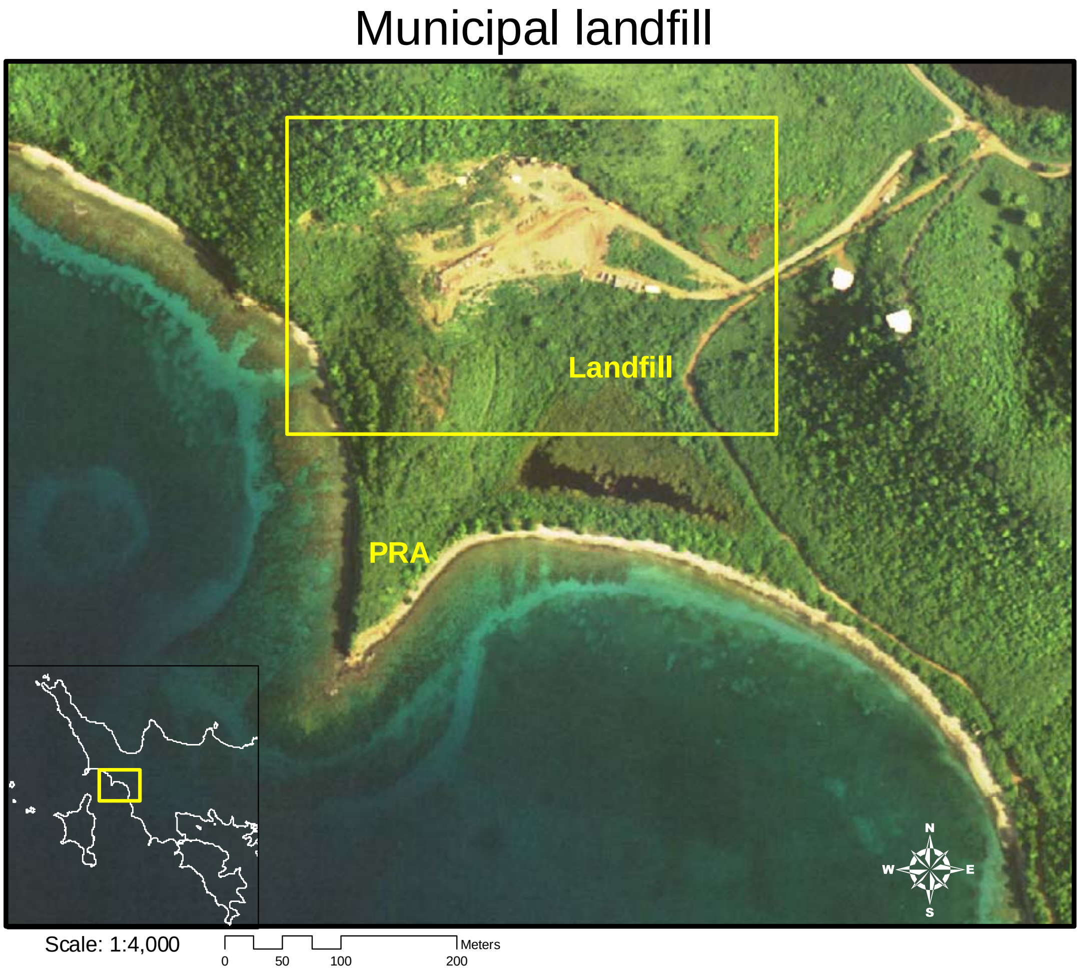

FIGURE 2. Detail of Culebra Island municipal landfill and its adjacent marine communities at Punta Rompeanzuelo (PRA). Note the presence of linear reefs, colonized pavements, colonized bedrock, continuous seagrass, sandy bottoms, rocky shores and sandy beaches. Also, note the wetland area between the landfill and the shoreline.

Coral reef characterization

Benthic surveys consisted of haphazard sampling of six replicate 30m-long point-count transects with the use of digital video imaging at each site. This method supplied data on the actual conditions of the coral reefs by quantifying coral species richness, % coral cover, % algae cover (functional groups: macroalgae, filamentous algae, *Halimeda*, crustose coralline algae), % sponge cover, and % cyanobacterial cover. Data was used to calculate coral species diversity index (H'n) and evenness (J'n). In addition, the coral reefs vitality condition was reported as recent or partial mortality. Whenever possible, the source of mortality was identified whether it was sediment deposition, bio-erosion, effect of adjacent benthic algae, and/or size reduction.

Statistical analysis

A one-way ANOVA test or Kruskal-Wallis non-parametric ANOVA test was performed to examine the differences among sites using Minitab 15 statistical software. Change in community structure was assessed by means of multivariate statistical tests using PRIMER 6.0 ecological statistical software (Clarke and Warwick 2001). Mean data from each site was classified for hierarchical clustering using the Bray-Curtis group average linkage method (Bray and Curtis 1957) and then ordinated using non-parametric multidimensional scaling (MDS) plot. PRIMER's multivariate equivalence of ANOVA called ANOSIM (analysis of similarities) was performed to evaluate the spatial variation patterns. A SIMPER routine was done to determine key taxa responsible for spatial variation in community structure between sites.

Benthic surveys of coral reef communities were carried out to test for any significant spatial pattern in the community structure of shallow coral reef zones resulting from potential landfill-based pollution. Briefly, six replicate 30 m-long point-count transects were randomly sampled using digital video imaging at the presumed *impact* site (Punta Rompeanzuelo) and at each one of three *control* sites (Arrecife El Banderote, Punta Tamarindo Chico, Cayo Luis Peña-north coast). This approach provided baseline information regarding the actual condition of coral reefs (i.e., coral species richness, % coral cover, % algal cover (functional groups: macroalgae, filamentous algae, *Halimeda*, crustose coralline algae), % sponge cover, % zoanthid cover, % cyanobacterial cover, and other components, coral species diversity index, coral species evenness). Corals were also assessed for any disease, syndrome, bleaching or other adverse vitality conditions. Whenever possible, sources of recent mortality were identified. Possibilities included sediment deposition, storm damage, parrotfish bites, damselfish bites and/or algal gardens, predation on the soft tissues by snails like *Coralliophila abbreviata* or the bristle worm *Hermodice carunculata*, various effects of adjacent benthic algae, and any other spatial competitors (e.g., *Erythropodium caribaeorum*, other stony corals).

Differences among sites were tested with a one way analysis of variance (ANOVA) and/or Kruskal-Wallis non-parametric ANOVA where indicated. Changes in community structure were tested by means of multivariate statistical tests. Community matrices were compiled and imported into PRIMER ecological statistics software package for multivariate analysis (Clarke and Warwick, 2001). Mean data from each site 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. Spatial variation patterns were tested using PRIMER's multivariate equivalent of an ANOVA called ANOSIM. Key taxa responsible for spatial variation in community structure between sites were determined using the SIMPER routine.

Results

Water quality characterization

There was a highly significant difference in water turbidity among sites (Table 2) and among sampling times (Table 3). Higher turbidity values were documented in sites located close to known urban runoff pollution sources, including CLA, MPI, and LOB (Figure 3). The highest mean value was documented at CLA (12.36 NTU), followed by LOB (6.09 NTU) (Table 4). The lowest mean value was observed at CLP (0.61 NTU). Collectively, turbidity values exceeded current standard of 10 NTU for coastal waters only 7.3% of the time. But there were violations 56% of the time at CLA, with peak values of up to 21.0 NTU following heavy runoff pulses. LOB also exceeded current water turbidity standard 11% of the time, with a peak value of 20.05 NTU. Further, CLA, MPI, and LOB, as well as PRA and PCR-S, showed impacts by different water turbidity pulses following heavy rainfall as suggested by high turbidity outlier values.

Sea surface temperature (SST) was also significantly higher at CLA, MPI, and LOB (Table 2, Figure 4). These sites were shallow grounds located in the inner part of Ensenada Honda Bay (Figure 1). Also, SST was significantly higher during late summer months (Table 3). pH values were significantly higher at the inner bay sites as well (Figure 5), and showed significant declines during major runoff events. Dissolved oxygen was also significantly higher in shallow

TABLE 2. 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	9,150	25.69	**<0.0001**
Temperature	ºC	9,150	65.03	**<0.0001**
pH	pH units	9,150	13.42	**<0.0001**
Dissolved oxygen	mg/L	9,135	8.87	**<0.0001**
Salinity	ppt	9,150	2.09	**0.0336**
Conductivity	ms/cm	9,150	2.12	**0.0313**

TABLE 3. Summary results of one-way ANOVA analysis of water quality parameters among sampling times.

Parameter	**Unit**	**D.F. (within, between)**	**F statistic**	***P* value**
Turbidity	NTU	8,150	5.15	**<0.0001**
Temperature	ºC	8,150	34.74	**<0.0001**
pH	pH units	8,150	55.95	**<0.0001**
Dissolved oxygen	mg/L	7,135	17.59	**<0.0001**
Salinity	ppt	8,150	20.07	**<0.0001**
Conductivity	ms/cm	8,150	18.13	**<0.0001**

TABLE 4. Water turbidity (NTU) standard violations across sites.

Sample	**May**	**Jun**	**Jun**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Mean**	**% Frq**
CLA	8.69	6.23	15.9	10.6	19.0	7.49	19.9	7.39	21.0	12.36	56%
MPI	NS	NS	4.43	1.64	1.30	1.25	9.99	1.59	2.78	3.28	0%
LOB	7.65	2.13	9.13	3.87	6.99	2.38	20.05	2.24	1.60	6.09	11%
BSA	1.27	1.59	5.68	1.28	1.02	0.58	3.16	0.89	0.49	1.74	0%
PME	1.61	0.75	1.25	1.37	0.48	0.51	1.39	0.52	0.28	0.93	0%
PTC	NS	NS	NS	NS	0.51	0.54	1.54	1.05	0.38	0.80	0%
AEB	NS	NS	NS	NS	0.98	1.00	1.71	1.01	0.38	1.02	0%
PRA	1.17	1.50	4.64	0.66	0.56	0.40	1.21	0.53	0.34	1.23	0%
CLP	NS	NS	1.13	0.37	0.53	0.45	0.88	0.67	0.29	0.61	0%

PCR	2.20	0.89	2.20	0.84	0.51	0.38	1.63	0.74	0.28		1.12	0%

NS= Site not sampled for that time period. Red color= violation to EQB class SB water quality classification turbidity standard (10 NTU).

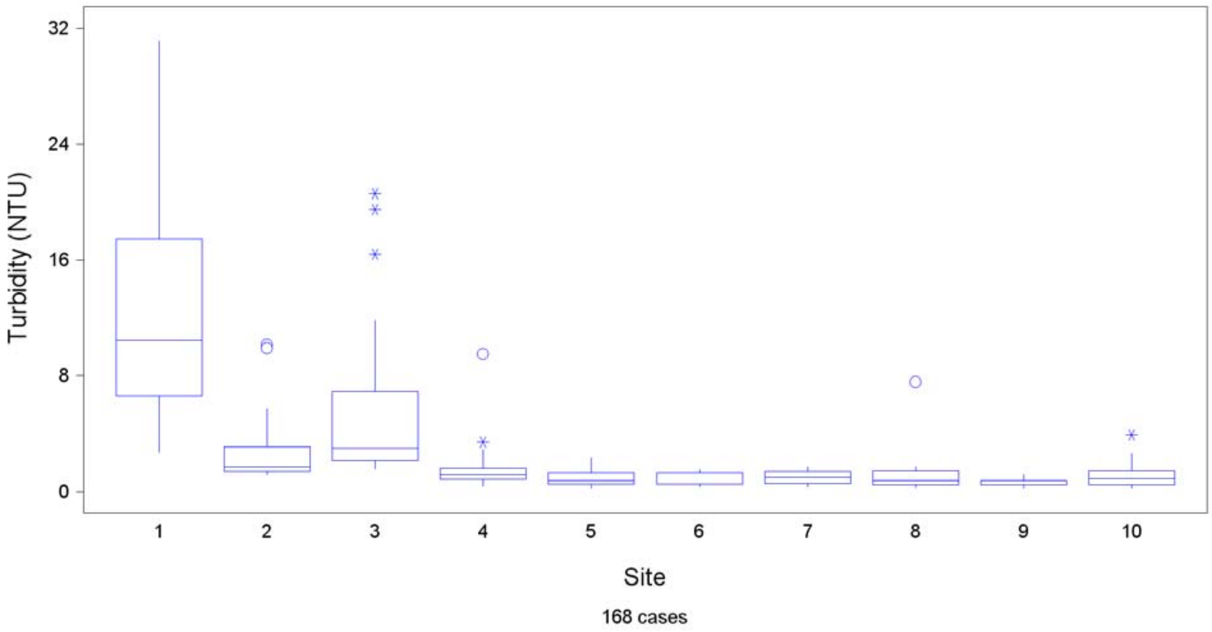

FIGURE 3. Box and whisker plot of turbidity data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario-South (PCR-S). 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 upper whiskers and outlier points suggest presence of non-point turbid runoff pulses at locations close to population centers (CLA, MPI, LOB, BSA, PME), as well as in PRA (close to the landfill) and at PCR.

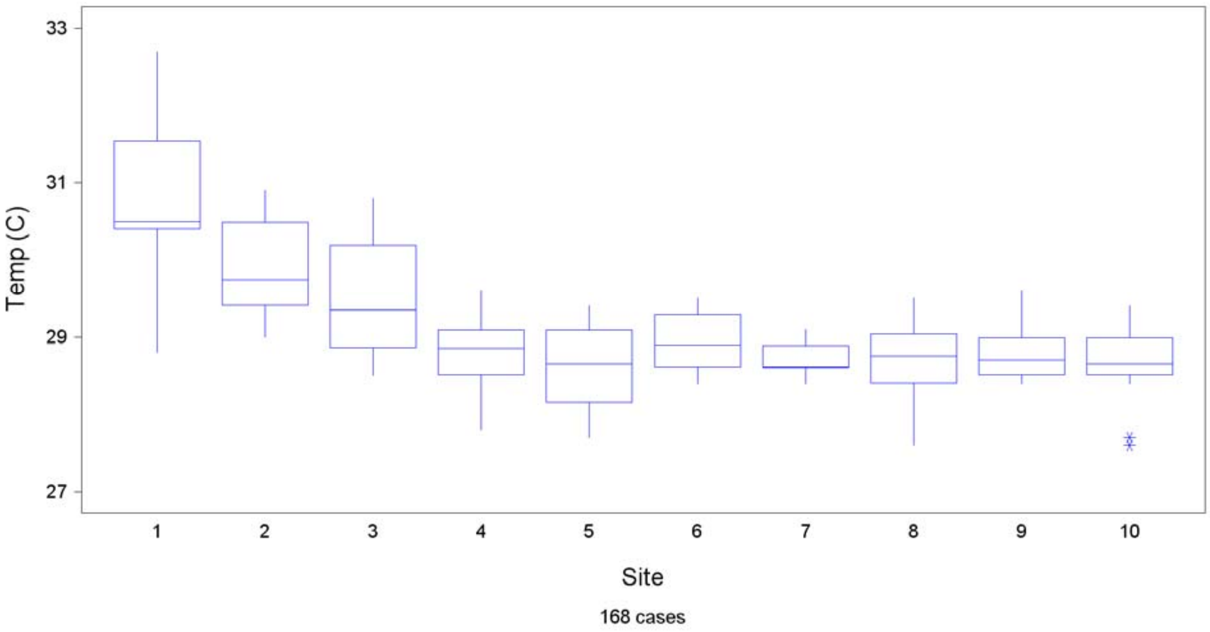

FIGURE 4. Box and whisker plot of sea surface temperature data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). All mean temperatures exceeded the mean monthly maximum sea surface temperature for the PR-USVI area (28.5°C). Significantly higher temperatures were detected within shallow approaches at Ensenada Honda bay (CLA, MPI) and at LOB. Other open water areas showed fairly similar values and fluctuations.

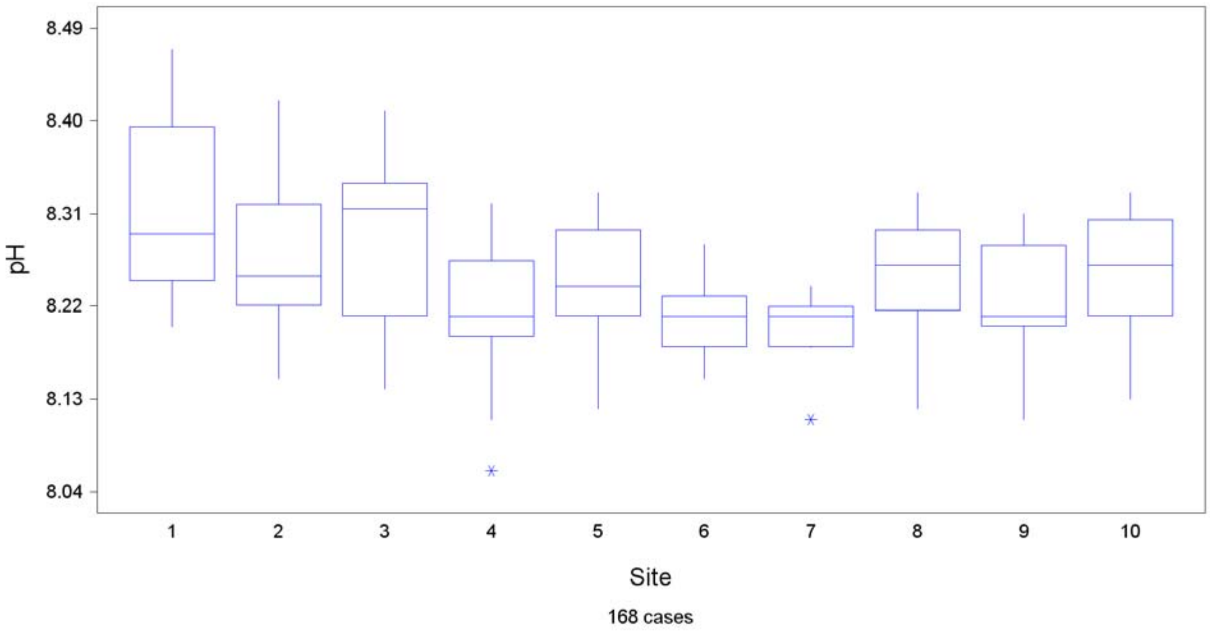

FIGURE 5. Box and whisker plot of pH data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). Lower whiskers and outlier points reflect non-point source runoff pulse impacts across all sites.

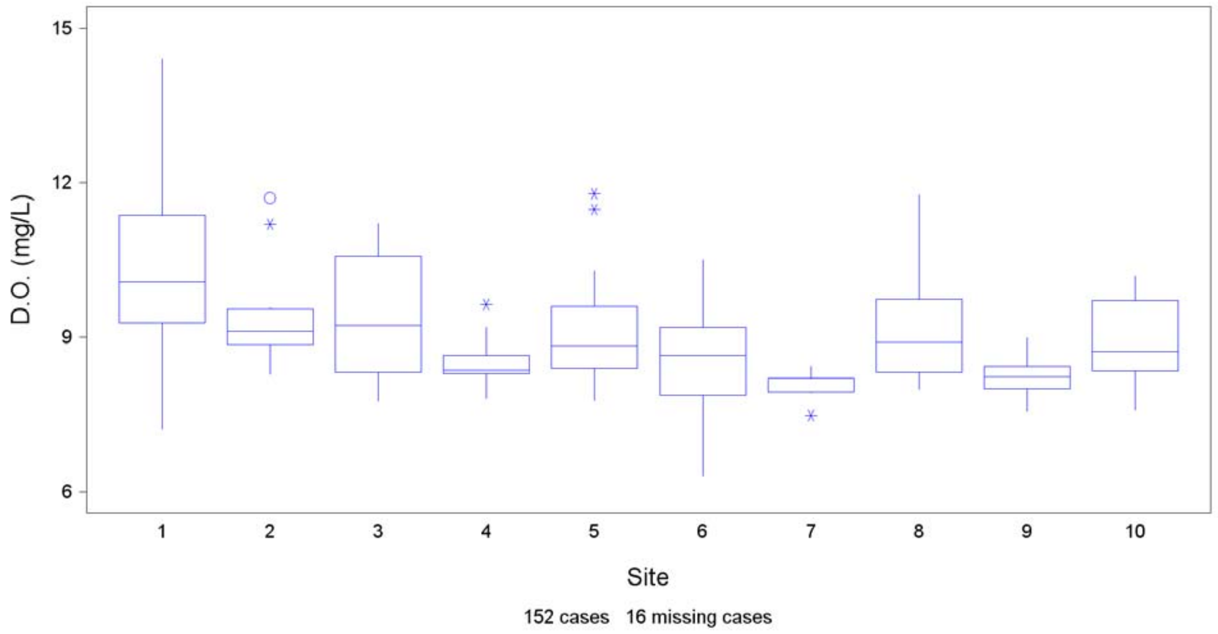

FIGURE 6. Box and whisker plot of dissolved oxygen data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). Presence of lower long whiskers and outlier points suggest presence of lower-dissolved oxygen water pulses from non-point source runoff, mostly at CLA, PTC, and AEB. Upper long whiskers and outlier points represent stronger oxygen-rich water mixing due to incoming tides and strong winds, mostly at CLA, MPI, BSA, PME, PTC, and PRA.

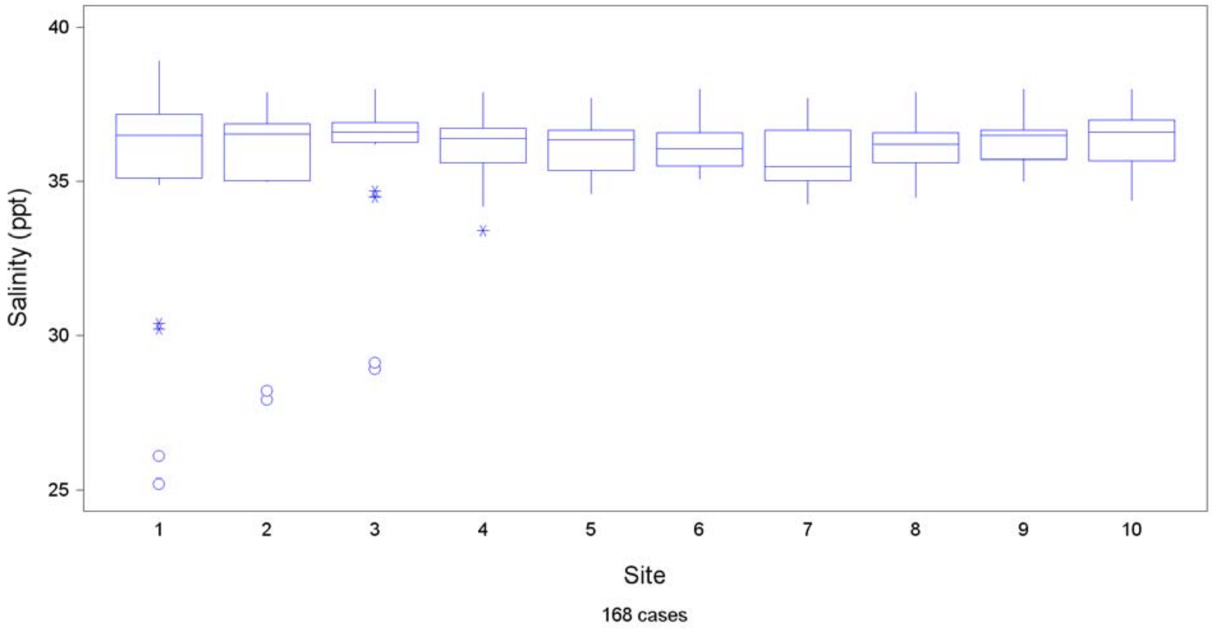

FIGURE 7. Box and whisker plot of salinity data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). Presence of lower long whiskers and outlier points suggest presence of lower-salinity water pulses from non-point source runoff, mostly at sampling sites located close to population centers.

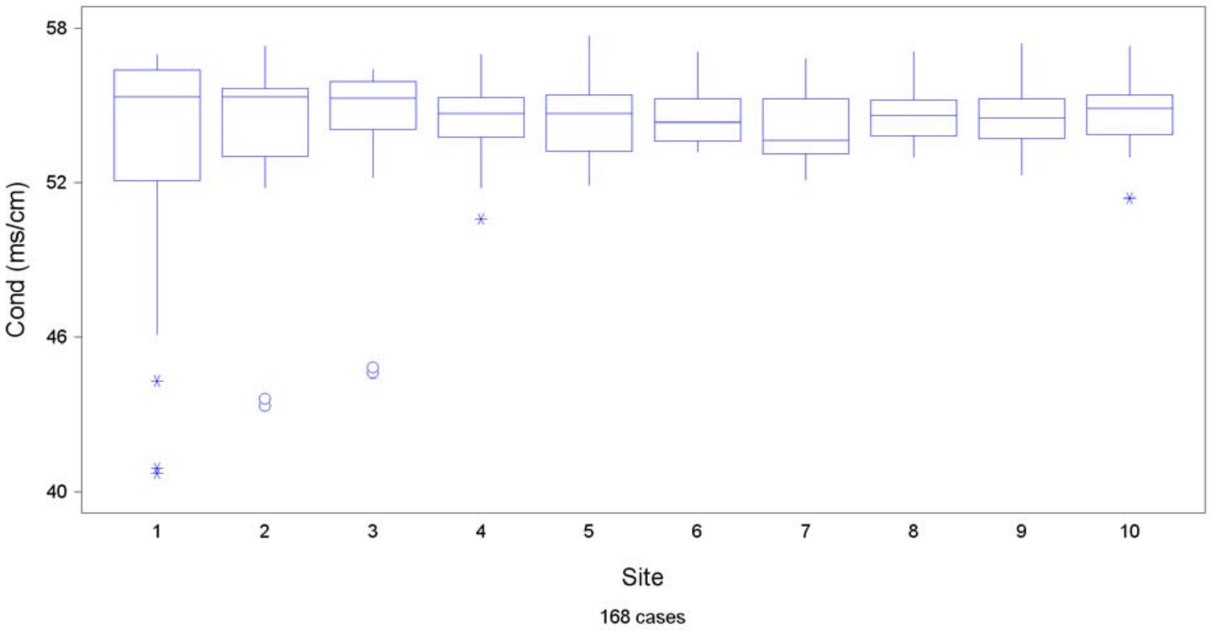

FIGURE 8. Box and whisker plot of conductivity data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR). Presence of lower long whiskers and outlier points suggest presence of lower-conductivity water pulses from non-point source runoff pulses associated to heavy rainfall events. Sampling sites around population centers showed the most significant impacts by runoff pulses, as well as PCR, regardless of the fact that it was the most remote site.

TABLE 5. Summary results of one-way ANOVA analysis of fecal coliform and enterococci concentrations among sites and time intervals.

Parameter	**D.F. (within, between)**	**F statistic**	***P* value**
Fecal coliforms			
- Site	9,137	51.29	**<0.0001**
- Time	8,137	3.59	**0.0008**
Enterococci			
- Site	9,143	20.65	**<0.0001**
- Time	8,143	12.94	**<0.0001**

inner bay sites (Figure 6), in spite of receiving recurrent runoff impacts. These areas usually east faced trade winds which contributed to strong mixing. There was also significant temporal variation associated to either, wind and runoff impacts. Salinity (Figure 7) and conductivity (Figure 8) were significantly lower at the inner bay sites CLA, MPI, and LOB as a result of recurrent runoff. These sites showed significant temporal declines as a result of runoff pulses. BSA also showed significant fluctuations following runoff.

Microbial water quality.

There were significant differences in the concentration of fecal coliforms (FC) and enterococci (ENT) among sites and between temporal sampling (Table 5). FC was significantly higher at CLA, MPI, LOB, BSA, which are known to receive sewage and sediment-laden turbid runoff (Figure 9). FC was also significantly higher at PRA, which is located in front of the Culebra Island landfill. FC also showed strong temporal fluctuations associated to the recurrence of runoff events (Figure 10). Microbial concentrations showed particularly significant increases at sites within LPCNR.

Further, FC showed a significant non-linear increase in concentration in function of water turbidity, with an exponential rise above a threshold value of 3 NTU (Figure 11). The highest mean value was documented at CLA (555 CFU/100 mL), followed by LOB (357 CFU/100 mL) (Table 6). The lowest mean value was observed at PME (0.2 CFU). No FC colonies were ever detected at PTC. Collectively, FC concentrations exceeded current standard of 135 CFU/100 mL for coastal waters only 16% of the time. However, mean FC concentrations violated current standards 30% of the time. But there were violations 67% of the time at CLA, with mean peak values of up to 1,000 CFU/100 mL following heavy runoff pulses. LOB also exceeded current FC standard 33% of the time, with a peak value of 1,975 CFU/100 mL. Further, MPI, BSA, and PRA showed significant FC pulses that violated current standards following runoff or sewage pulse events.

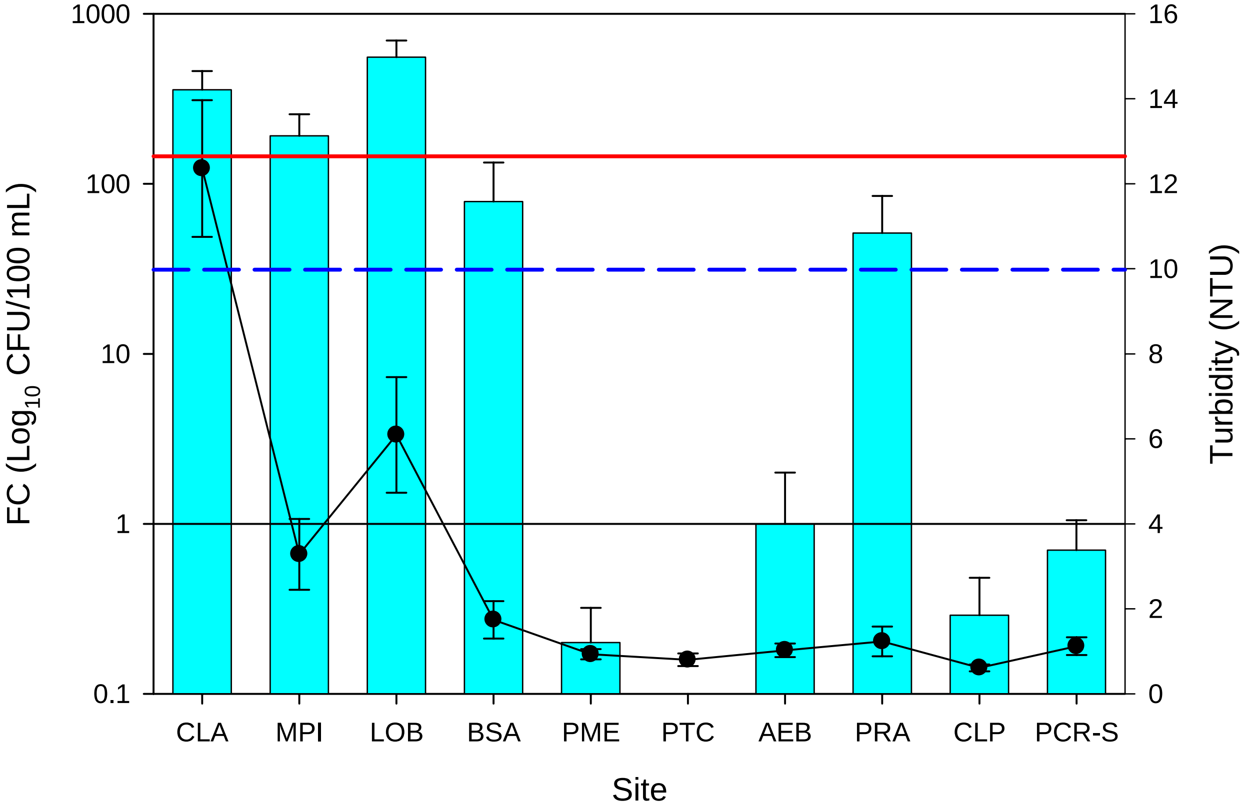

FIGURE 9. Fecal coliform counts (turquoise bars) as a function of water turbidity by site (mean±one standard error). Red solid line represents FC standard (200 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

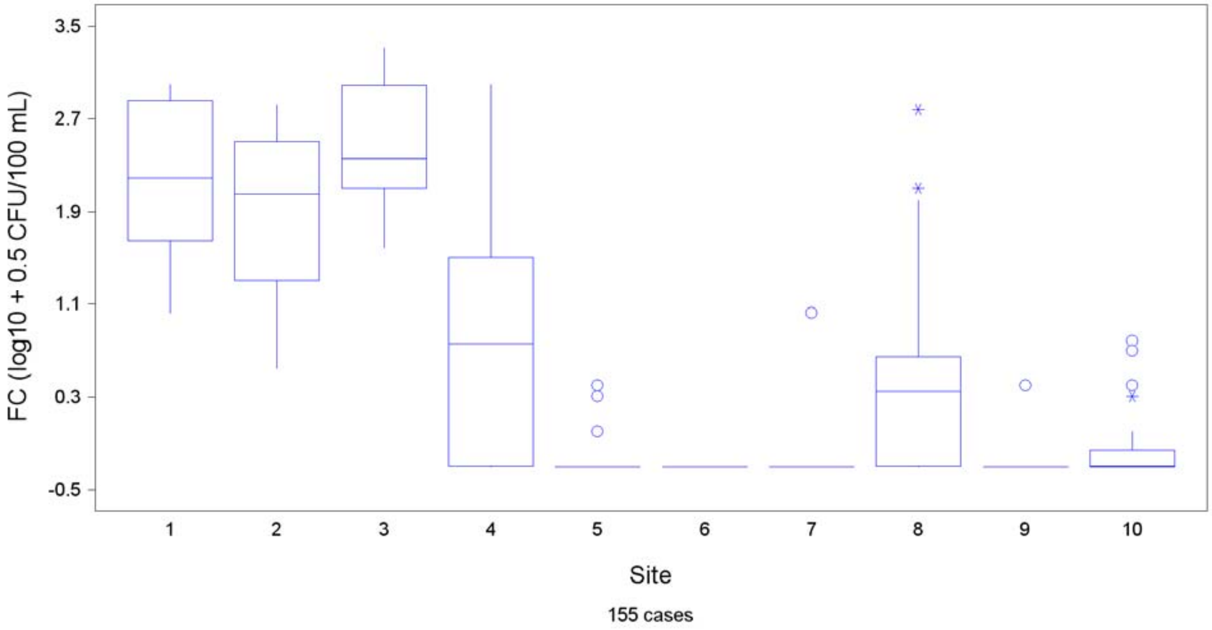

FIGURE 10. Box and whisker plot of fecal coliform data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR).

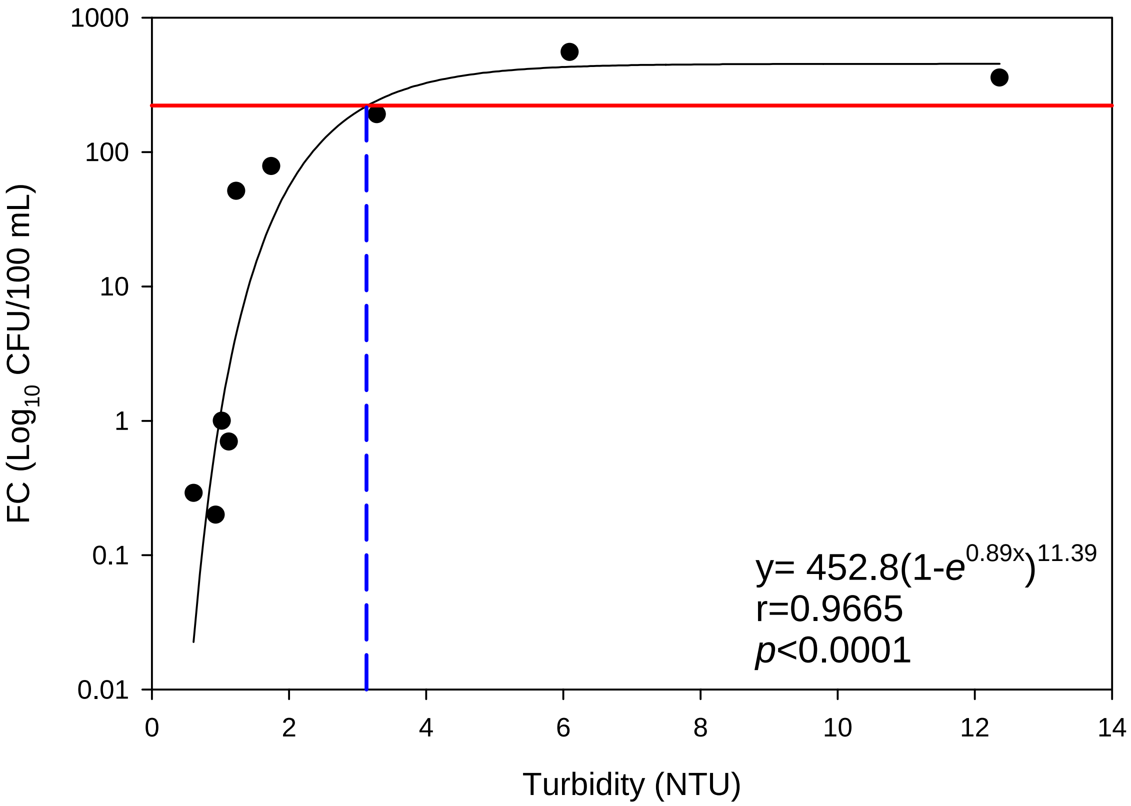

FIGURE 11. Chapman non-linear regression for fecal coliform (FC) counts and water turbidity by site. Red solid line represents FC standard (200 cfu/100 mL). Blue dashed line represents approximate turbidity level at which FC counts may exceed current standards. Water turbidity values approximately above 3.2 NTU suggest FC counts above current legal standards for coastal waters. Significantly higher turbidity values did not result in further increases in FC counts probably as a result of growth interference by other opportunistic microbes in culture media.

TABLE 6. Microbiological water quality standard violations for fecal coliforms across sites.

Sample	May	Jun	Jun	Jun	Jul	Aug	Sep	Oct	Nov	Mean	% Frq
CLA	40	168	23	26	1000	673	550	ND	ND	357	33%
MPI	NS	NS	ND	537	389	18	20	70	113	191	29%
LOB	95	194	39	850	1000	564.8	230	1975	228	555	67%
BSA	32	5.3	0	1	95	29	546	0	0	79	11%
PME	0	0	0	0	1	2	0	0	0	0.2	0%
PTC	NS	NS	NS	NS	0	0	0	0	0	0	0%
AEB	NS	NS	NS	NS	0	0	10	0	0	1	0%
PRA	350	3	0	102	3	1	2	0	2	51	11%
CLP	NS	NS	2	0	2	0	0	0	0	0.3	0%
PCR	0	0.5	0	0	2	0	5	0	0	0.7	0%

NS= Site not sampled for that time period. ND= Not detected due to interference overgrowth by other microbes. Red color= violation to EQB class SB water quality classification FC standard (200 cfu/100 mL).

TABLE 7. Microbiological water quality standard violations for enterococci across sites.

Sample	May	Jun	Jun	Jun	Jul	Aug	Sep	Oct	Nov	Mean	% Frq
CLA	15	119.5	190	19	1123	76	133	300	10	221	67%
MPI	NS	NS	25	134	125	5	157	100	20	80	57%
LOB	80	65	240	245	2095	1570	70	1150	45	590	100%
BSA	84	77	10	1	57	76	8126	30	0	948	56%
PME	0	0.5	10	1	2	3	239	0	0	26	11%
PTC	NS	NS	NS	NS	2	9	20	0	0	6	0%
AEB	NS	NS	NS	NS	0	6	833	10	0	88	20%
PRA	130	10	28	5	6	13	290	60	1	61	33%
CLP	NS	NS	54	0	0	3	77	0	2	19	29%
PCR	0	0.5	47	0.5	0.5	6	607	0	0	66	22%

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

ENT was significantly higher at CLA, MPI, LOB, BSA, which are known to receive sewage and sediment-laden runoff (Figure 12). ENT also showed strong temporal fluctuations associated to the recurrence of turbid runoff events (Figure 13). Microbial concentrations showed particularly significant increases at sites within LPCNR. Further, ENT showed a significant non-linear increase in concentration in function of water turbidity, with an exponential rise above a threshold value of 1.6 NTU (Figure 14). The highest mean value was documented at BSA (948 CFU/100 mL), followed by LOB (590 CFU/100 mL) (Table 7). The lowest mean value was observed at PTC (6 CFU/100 mL). Collectively, ENT concentrations exceeded current standard of 135 CFU/100 mL for coastal waters 40% of the time. However, mean ENT concentrations violated current standards 70% of the time. But there were violations 100% of the time at LOB, with mean peak values of up to 2,095 CFU/100 mL following heavy runoff pulses. CLA exceeded current ENT standard 67% of the time, with a peak value of 1,123 CFU/100 mL. BSA also exceeded current ENT standard 56% of the time, with a peak value of 8,126 CFU/100 mL. Further, all sites, with the exception of PTC, showed at least one significant ENT pulse that violated current standards following incidental runoff or sewage pulse events.

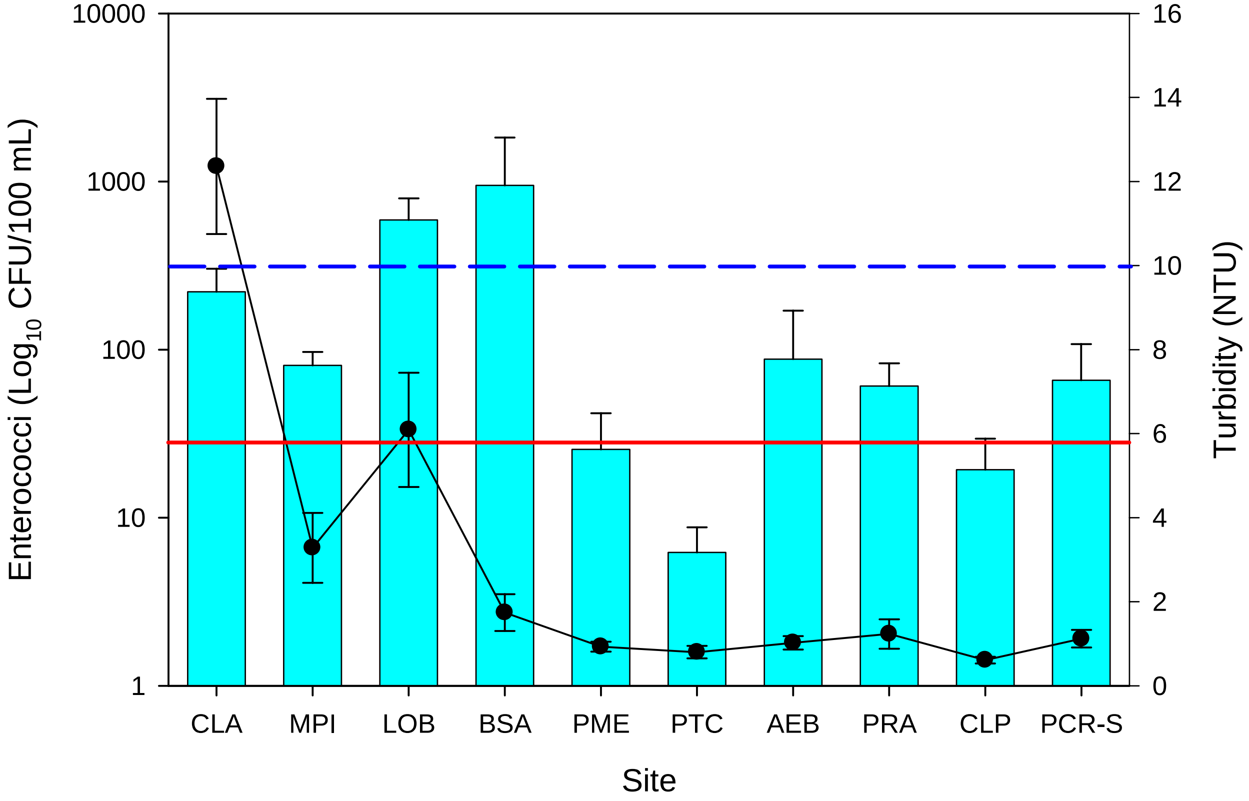

FIGURE 12. FC counts (turquoise bars) as a function of water turbidity by site (mean±one standard error). Red solid line represents FC standard (200 cfu/100 mL). Blue dashed line represents water turbidity standard (10 NTU).

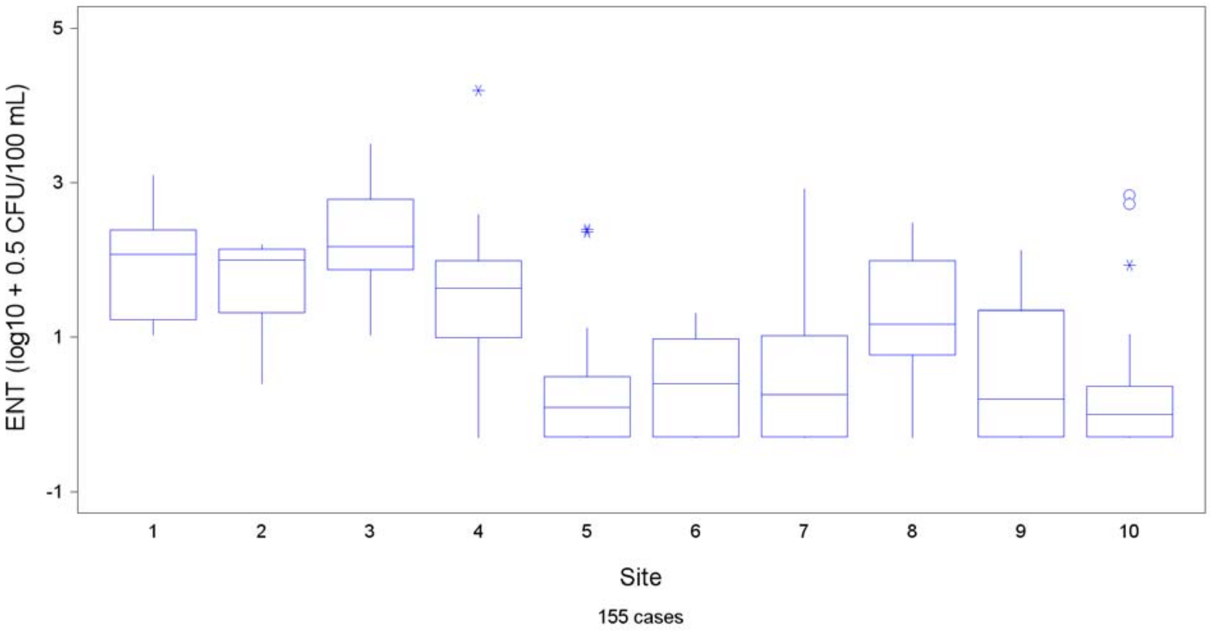

FIGURE 13. Box and whisker plot of enterococci data per study site. Sites arranged from higher to lower pollution as follows: (1) Ensenada Honda at Clark (CLA); (2) Ensenada Honda at Municipal Pier (MPI); (3) Lobina Channel (LOB); (4) Bahía Sardinas (BSA); (5) Punta Melones (PME); (6) Punta Tamarindo Chico (PTC); (7) Arrecife El Banderote (AEB); (8) Punta Rompeanzuelos (PRA); (9) Cayo Luis Peña-North (CLP); and (10) Playa Carlos Rosario (PCR).

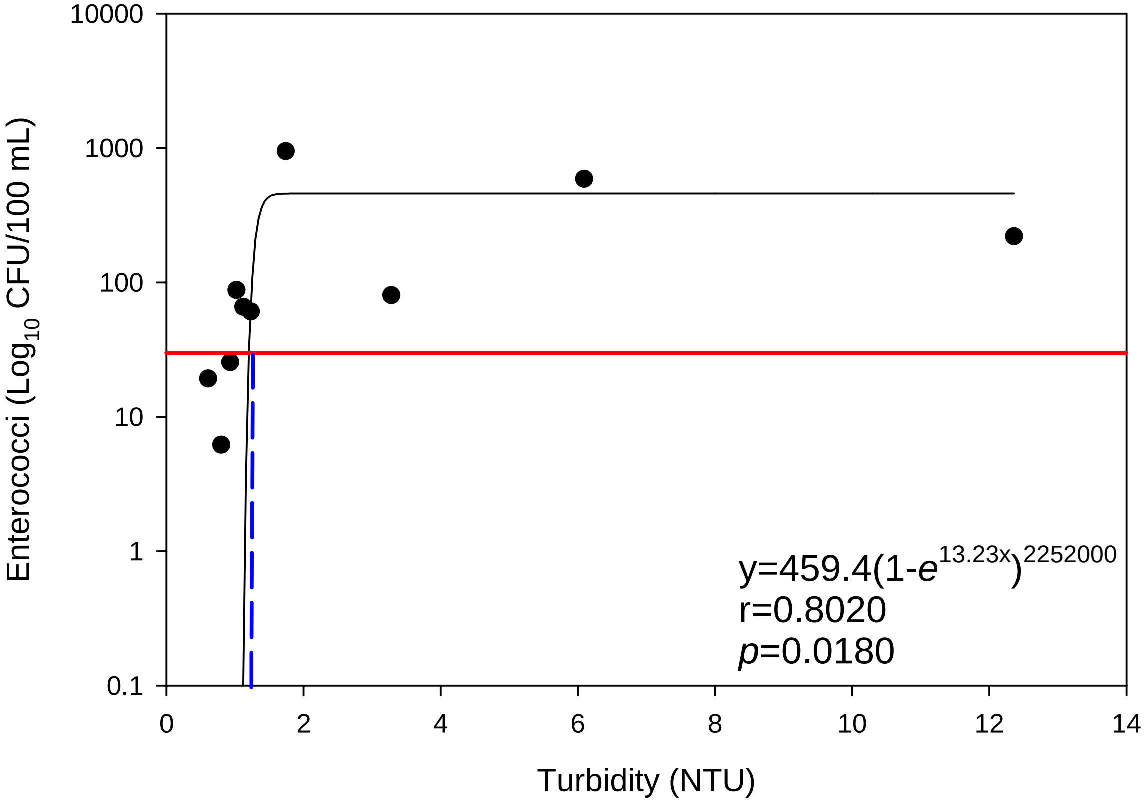

FIGURE 14. Chapman non-linear regression for enterococci counts and water turbidity by site. Red solid line represents FC standard (35 cfu/100 mL). Blue dashed line represents approximate turbidity level at which FC counts may exceed current standards. Water turbidity values approximately above 1.5 NTU suggest enterococci counts above current legal standards for coastal waters. Significantly higher turbidity values did not result in further increases in enterococci counts probably as a result of growth interference by other opportunistic microbes in culture media.

Water quality gradient.

Cluster analysis and multi-dimensional scaling (MDS) analysis showed that water turbidity can significantly indicate a water quality gradient, with a very low stress of 0.01 (Figure 15). There were three clusters at the 80% similarity cutoff level that evidenced a strong turbidity gradient: one highly turbid cluster (CLA), two moderately turbid sites (LOB, MPI), and a third low turbidity cluster composed of the remaining seven sites. A similar approach was used with FC, but spatial resolution of the gradient was moderate, which was reflected by a lowed stress of 0.12 (Figure 16). There were six clusters at the 40% similarity cutoff level that evidenced a moderate water quality gradient, with known polluted sites clustering together (CLA, LOB, MPI, BSA), and several isolated clusters of the remaining sites. But, cluster and MDS analyses using ENT showed also a very strong gradient of non-point source sewage pollution, with a very low stress resolution of 0.04 (Figure 17). There were four clusters that evidenced a strong water quality gradient, with known polluted sites clustering together (CLA, LOB, MPI, PRA), and BSA separately. CLP, PCR, AEB, and PME clustered as a moderately polluted group. PTC clustered separately as a low-pollution site.

A multivariate correlation analysis showed that there was a strongly significant positive correlation between ENT and water turbidity (Rho=0.613, *p*=0.0008), suggesting the relationship between sewage and runoff pulses. But no correlation was found between FC and water turbidity (Rho=0.135, *p*=0.2500). FC growth showed significant interference by background bacteria under high turbidity when using standard methods. Thus, high data variability could probably explain the observed lack of spatial resolution and correlation.

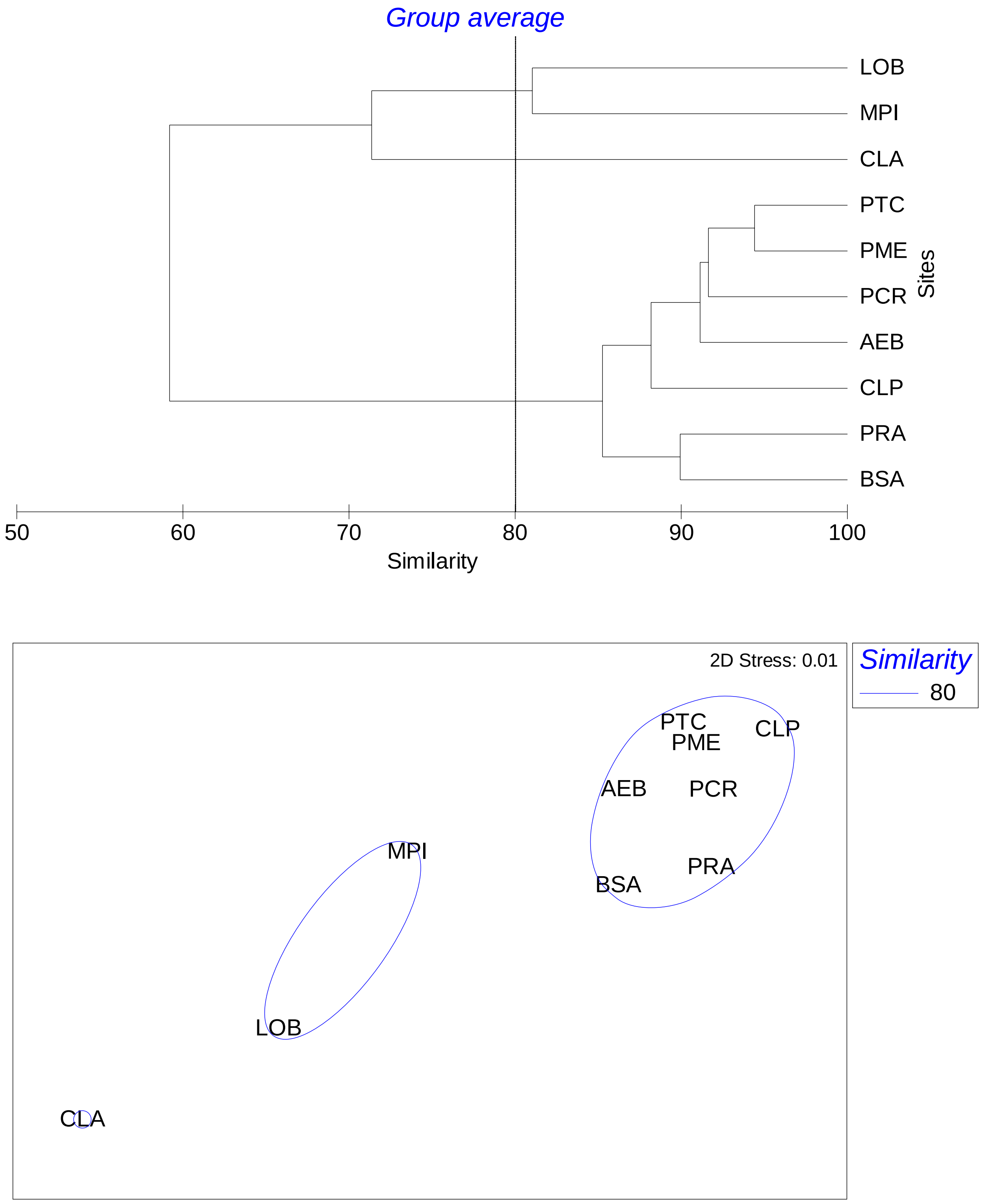

FIGURE 15. Cluster analysis and multi-dimensional scaling (MDS) plot based on water turbidity. Vertical bar in cluster chart and blue contour line in MDS plot represent the 80% similarity cutoff level. There were three clusters that evidenced a strong turbidity gradient: one highly turbid site (CLA), two moderately turbid sites (LOB, MPI), and a third low turbidity cluster.

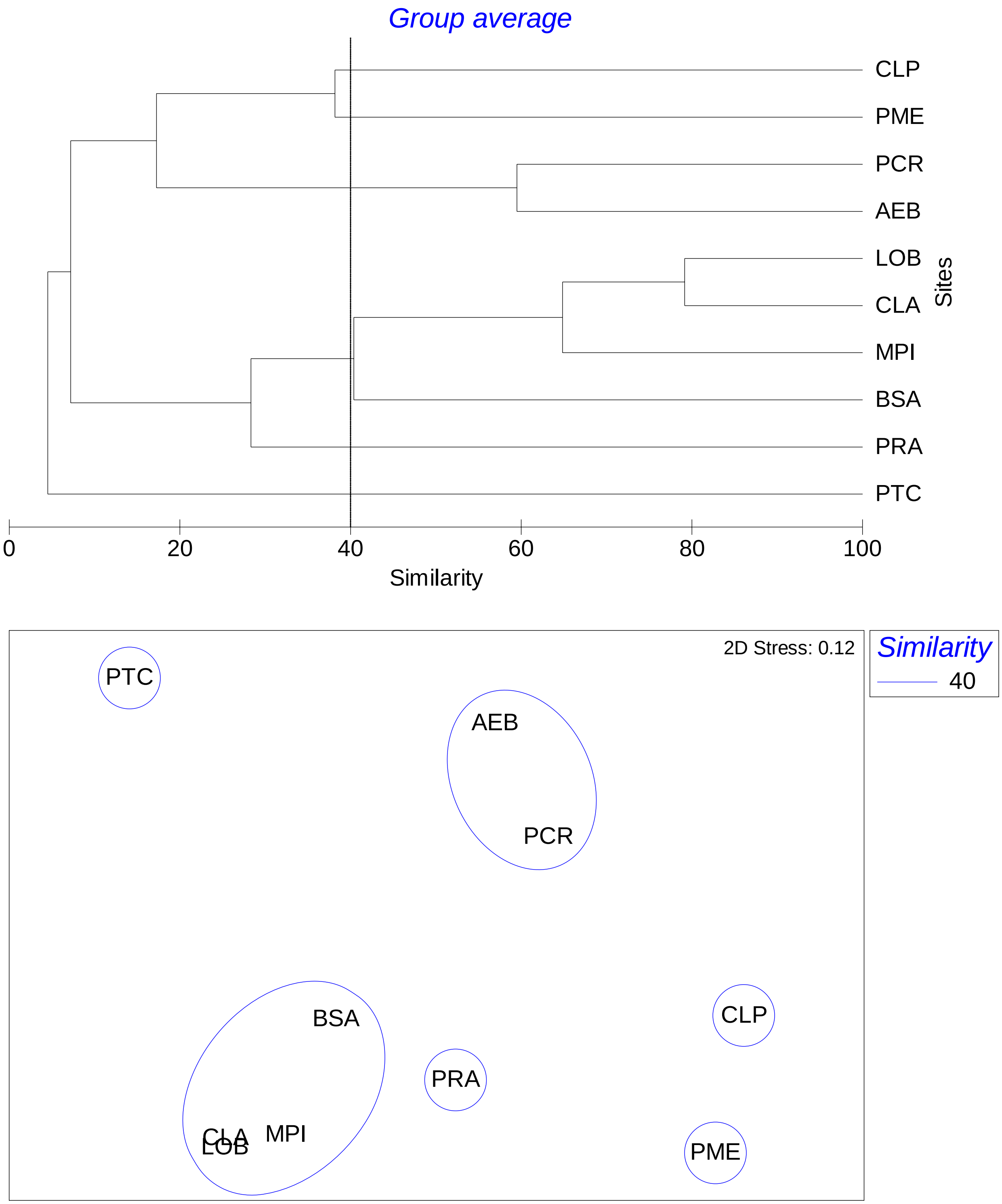

FIGURE 16. Cluster analysis and multi-dimensional scaling (MDS) plot based on FC counts. Vertical bar in cluster chart and blue contour line in MDS plot represent the 40% similarity cutoff level. There were six clusters that evidenced a moderate water quality gradient, with known polluted sites clustering together (CLA, LOB, MPI, BSA), and several isolated cluster of the remaining sites.

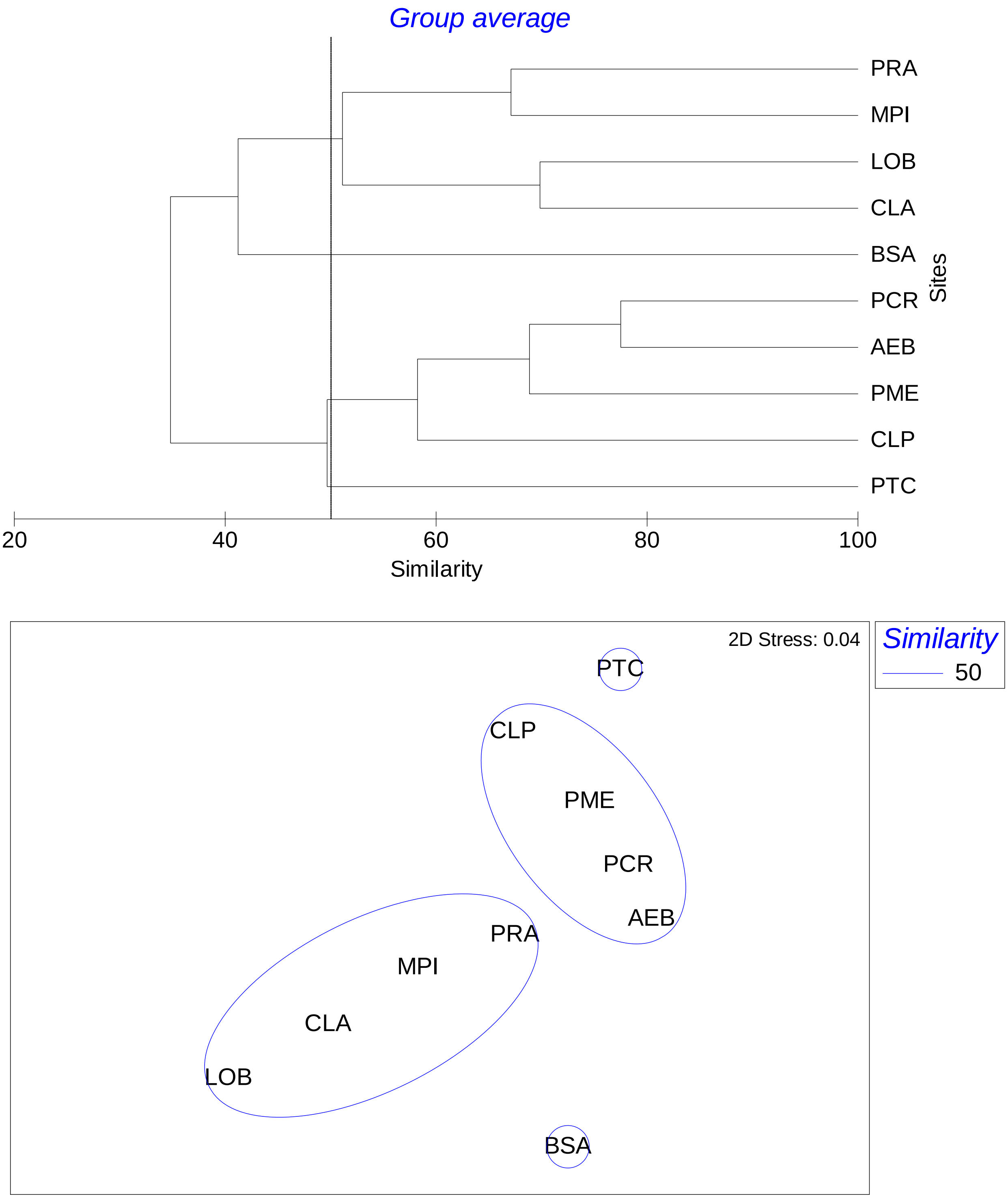

FIGURE 17. Cluster analysis and multi-dimensional scaling (MDS) plot based on enterococci counts. Vertical bar in cluster chart and blue contour line in MDS plot represent the 50% similarity cutoff level. There were four clusters that evidenced a strong water quality gradient, with known polluted sites clustering together (CLA, LOB, MPI, PRA), and BSA separately.

Benthic community characterization

Coral species richness, colony abundance and % cover

Most coral reef benthic community parameters showed significant differences among sites that suggest a significant water quality gradient impact. Total coral (scleractinian, hydrocoral, octocoral) species richness was significantly higher (Table 8) at PTC (9.2 species/transect), followed by PRA (7.5 species/transect) (Figure 18). Total coral species richness was significantly lower at BSA (3.7 species/transect). This reef was the closest to polluted urban areas. Hard coral (scleractinian, hydrocoral) species richness was significantly higher at PTC and AEB (6.5 species/transect) (Figure 18). Hard coral species richness was significantly lower at BSA (3.7 species/transect). Total coral colony abundance was higher at PTC (25 colonies/transect), followed by PRA (23 colonies/transect) (Figure 19). Total coral colony abundance resulted lower at CLP-N (10 colonies/transect), and at BSA (14 colonies/transect). These differences were only marginally significant. Hard coral colony abundance was significantly higher at PTC (17 colonies/transect), and significantly lower at CLP-N (7 colonies/transect).

Percent living coral cover was higher at PME (40%), closely followed by PRA (39%) (Figure 20). It resulted lower at CLP-N (17%), and at BSA (22%). These differences were only marginally significant. However, scleractinian coral % cover was significantly higher at PTC, and AEB, with significantly lower values at AEB, CLP-N, and PME (Figure 21). Hydrocoral % cover was significantly higher at AEB and CLP-N, with significantly lower values at PCR-S and BSA. Octocoral % cover was significantly higher at PRA, with significantly lower values at BSA.

TABLE 8. Summary results of one-way ANOVA analysis of coral reef benthic parameters among sites (d.f.= 6,32).

Parameter	**F statistic**	***P* value**
Species richness (total)	3.95	**0.0046**
Species richness (hard)	2.93	**0.0217**
Colony abundance (total)	2.23	0.0656
Colony abundance (hard)	2.70	**0.0309**
% Coral	2.32	0.0569
% Scleractinian	4.83	**0.0013**
% Hydrocoral	3.90	**0.0049**
% Octocoral	7.07	**0.0001**
% *Acropora palmata*	7.59	**<0.0001**
% Partial mortality	4.85	**0.0013**
% Recent mortality	8.64	**<0.0001**
H’n (total)	5.57	**0.0005**
J’n (total)	5.09	**0.0009**
H’n (hard)	3.42	**0.0101**
J’n (hard)	3.04	**0.0180**
% Sponges	3.12	**0.0123**
% Zoanthids	27.6	**<0.0001**
% Macroalgae	5.73	**0.0004**
% Turf	4.76	**0.0014**
% CCA	36.2	**<0.0001**
% Cyanobacteria	5.58	**0.0005**
% Sand, pavement, rubble	5.57	**<0.0001**

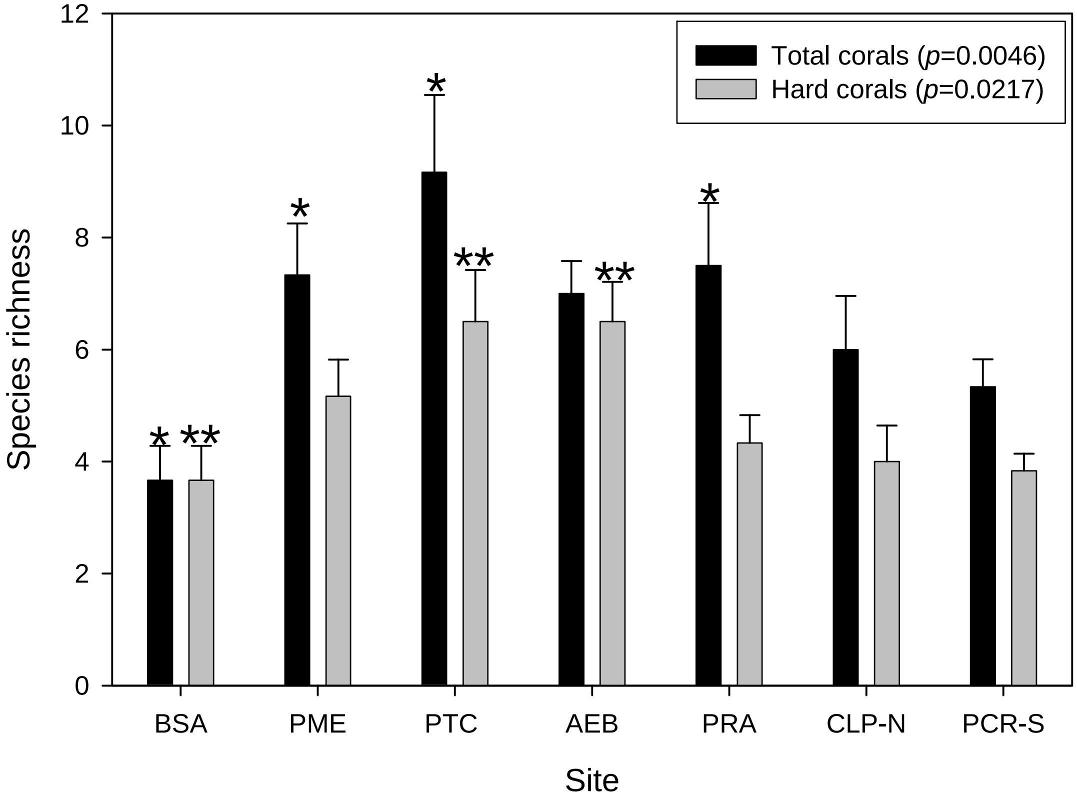

FIGURE 18. Species richness (total and hard corals).

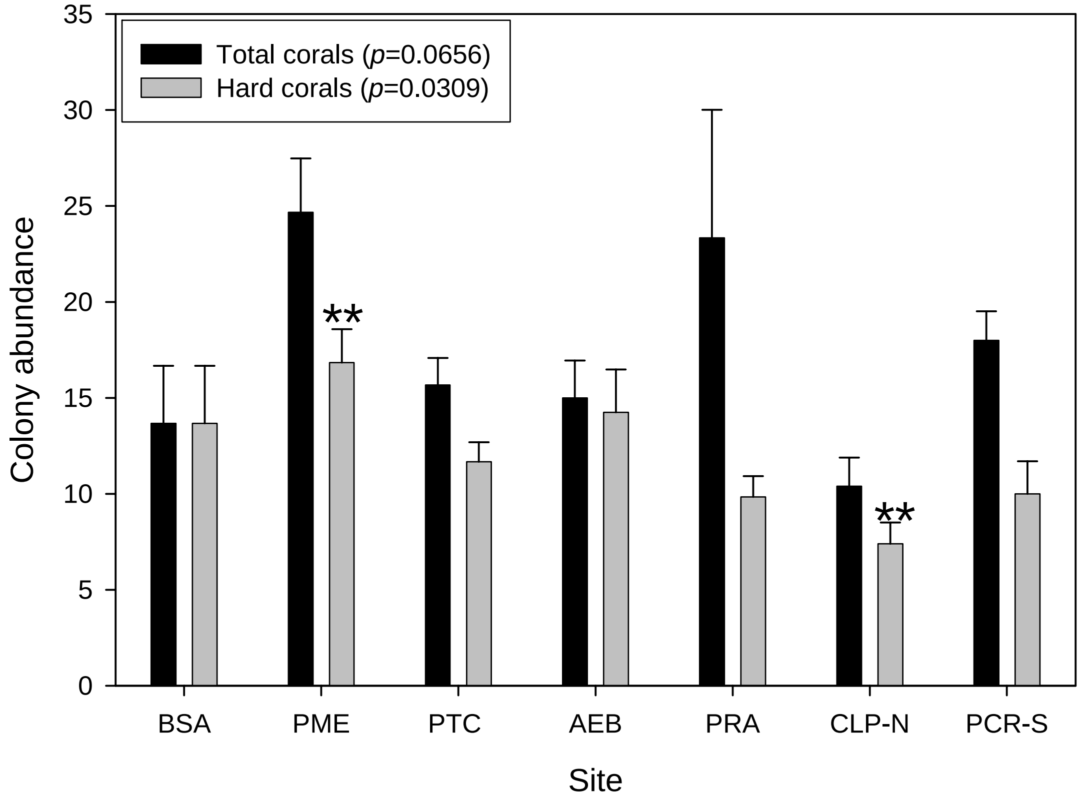

FIGURE 19. Colony abundance (total and hard corals).

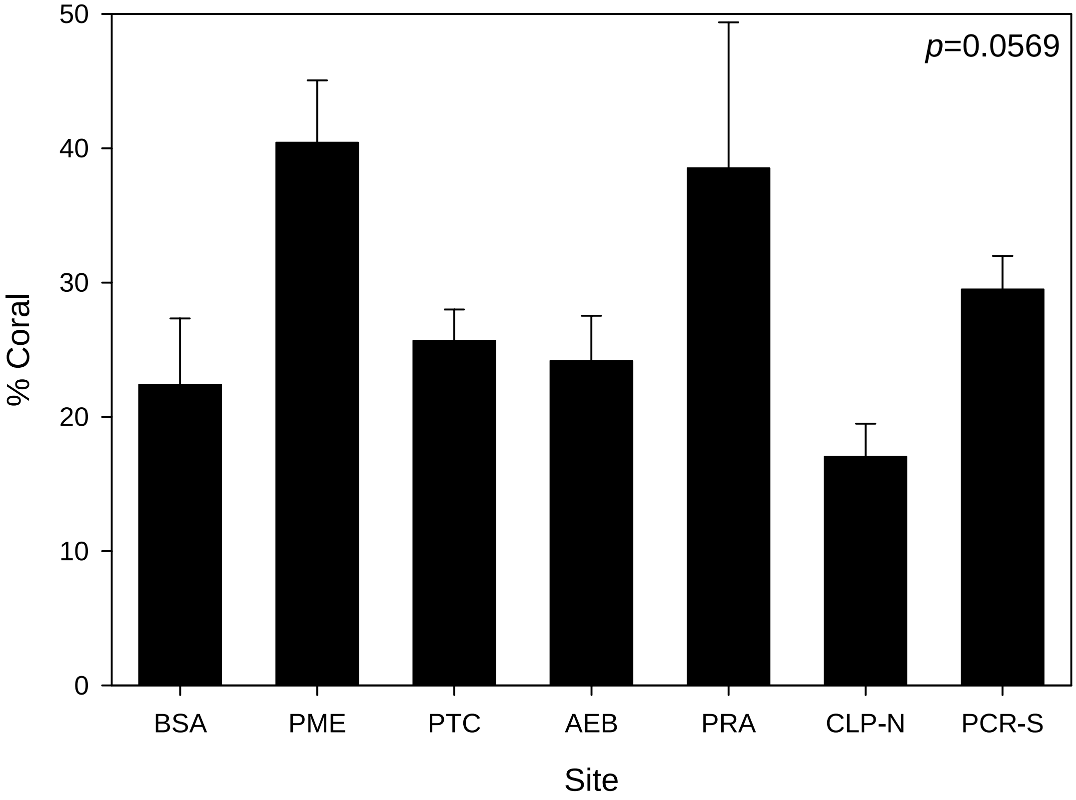

FIGURE 20. Percent living coral cover.

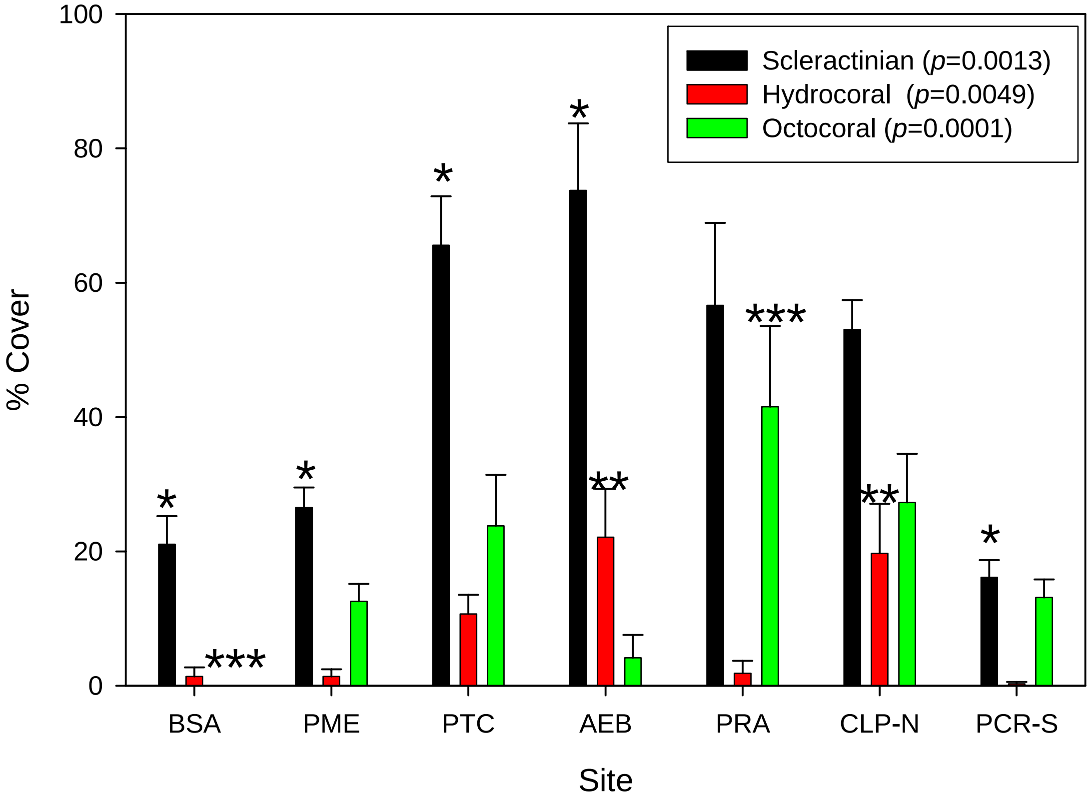

FIGURE 21. Percent relative coral cover.

Coral colony mortality

Percent frequency of old mortality of coral colonies was significantly higher at BSA (49%), followed by PCR-S (32%). Lowest values were documented at AEB (5%), followed by PTC, and CLP-N, with 7%, respectively (Figure 22). This pattern suggests two long-term trends of reef degradation. The first one points out at the long-term decline of BSA, which is the reef located closest to Dewey downtown area. The second pattern implies significant long-term decline associated to significant sediment bedload at PCR-S. Percent frequency of recent mortality of coral colonies was also significantly higher at BSA (41%), followed by PCR-S (38%). No recent mortality was observed at CLP-N, and only 1% recent mortality was detected at PTC. This suggests that mortality trends are still continuing. Further, the fact that 90% of the living hard corals at BSA, and 70% of the living hard corals at PCR-S are showing at least some degree of mortality suggests that stressful conditions are significant and that both, impacts by non-point source pollution, as well as sediment bedload, can have similar adverse long-term effects on shallow-water coral assemblages. Coral mortality is nearly absent at coral reefs subjected to strong water circulation.

Coral H'n and *J'n*

Total coral species diversity index (H'n) was significantly higher at PTC (2.0230), and significantly lower at BSA (0.8937) and PCR ((1.3202) (Figure 23). Hard coral H'n was significantly higher at AEB (1.7101) and at PTC (1.6969), and significantly lower at BSA (0.8937). Total coral species evenness (J'n) was significantly higher at PTC (0.9377), AEB (0.9294), CLP-N (0.9126), and PRA

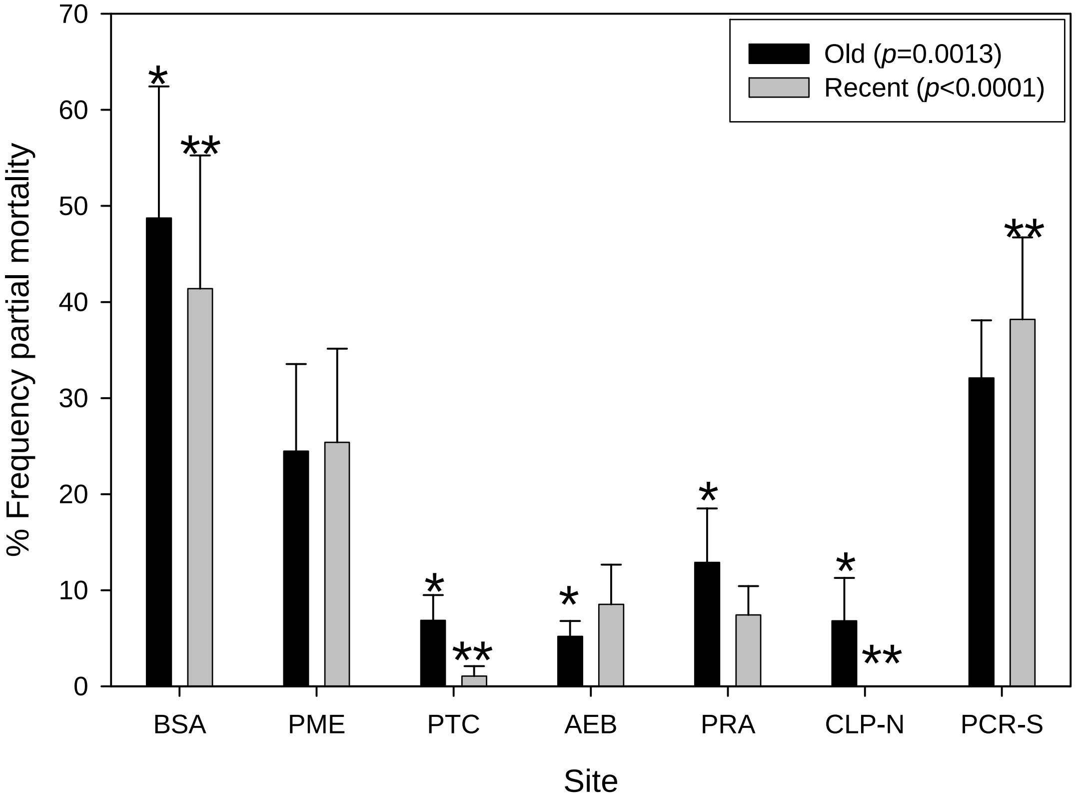

FIGURE 22. Percent relative frequency of hard coral old partial colony mortality and recent colony mortality.

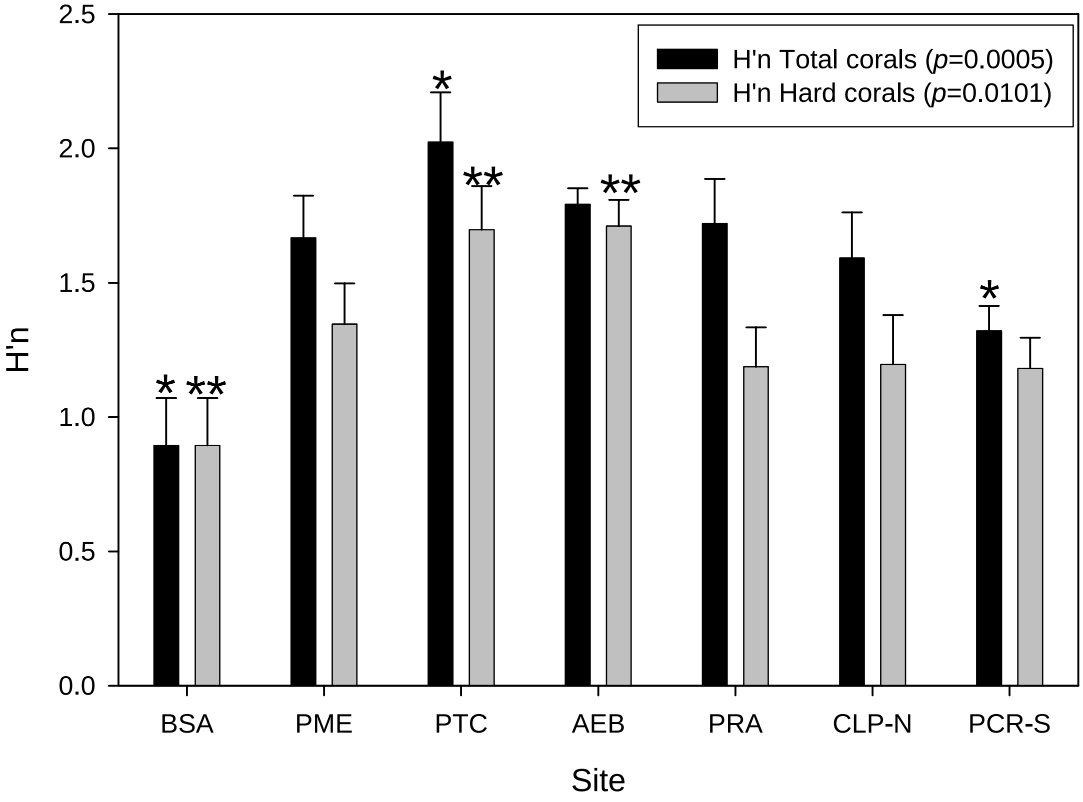

FIGURE 23. Species diversity index of total and hard corals.

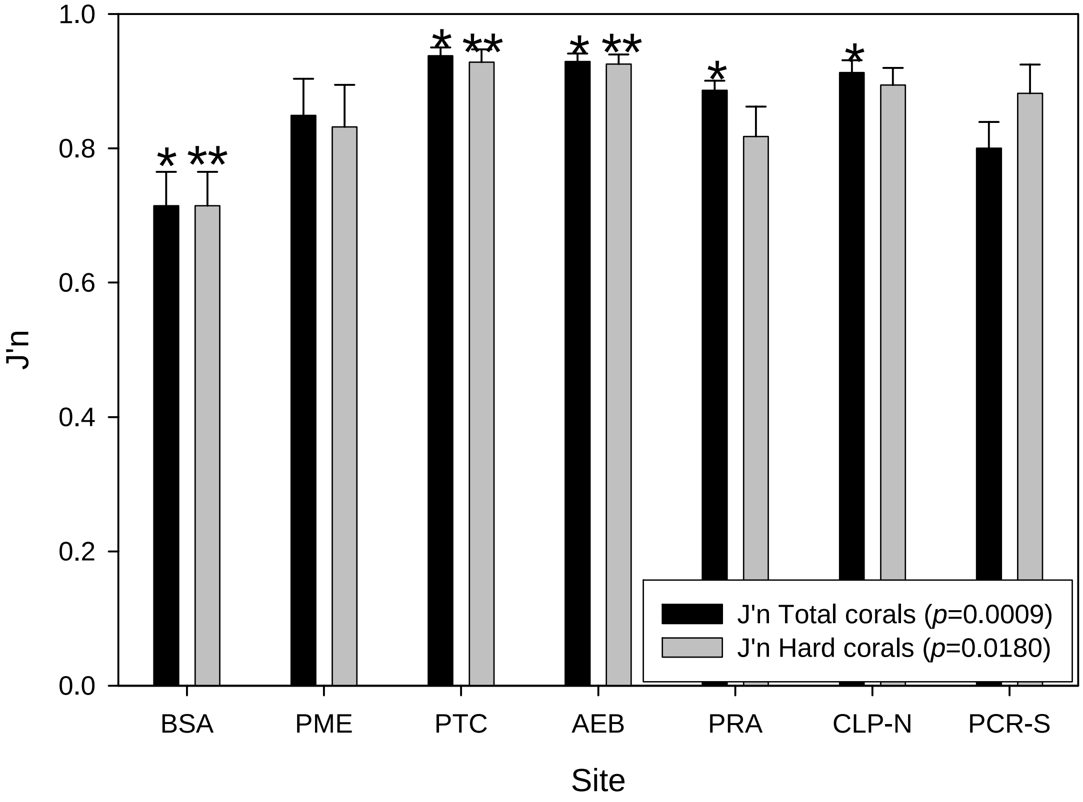

FIGURE 24. Species evenness of total and hard corals.

(0.8863) (Figure 24). Total coral J'n was significantly lower at BSA (0.8000). Hard coral J'n was significantly higher at PTC (0.9281) and at AEB (0.9252), and significantly lower at BSA (0.7145). Both indexes showed significant influences by the quality gradient that has resulted from non-point source pollution.

Other benthic components

There was no significant difference in % zoanthid cover (Figure 25), although they showed higher % cover at higher energy locations, particularly PTC. AEB showed a significantly higher % sponge cover (Figure 25). Macroalgal distribution showed unequivocal influences of the non-point source pollution gradient, with significantly higher % cover at the non-point source polluted BSA (16%), followed by downstream reefs at PTC (14%), and PRA (11%), with values as low as 2% at PCR-S (Figure 26). Percent algal turf cover was significantly higher at CLP (49%) and BSA (43%), with the lowest value at PME (<1%) (Figure 27). *Halimeda* spp cover was significantly higher at BSA (Figure 28). CCA was significantly higher at AEB (Figure 29). This site posses the highest density of long-spine sea urchins (*Diadema antillarum*), thus the largest grazing intensity on other algal groups. Cyanobacterial cover was significantly higher at PME and PTC (Figure 30). Sandy bottoms were significantly more abundant at PCR-S, bedrock at PRA, and rubble at BSA (Figure 31).

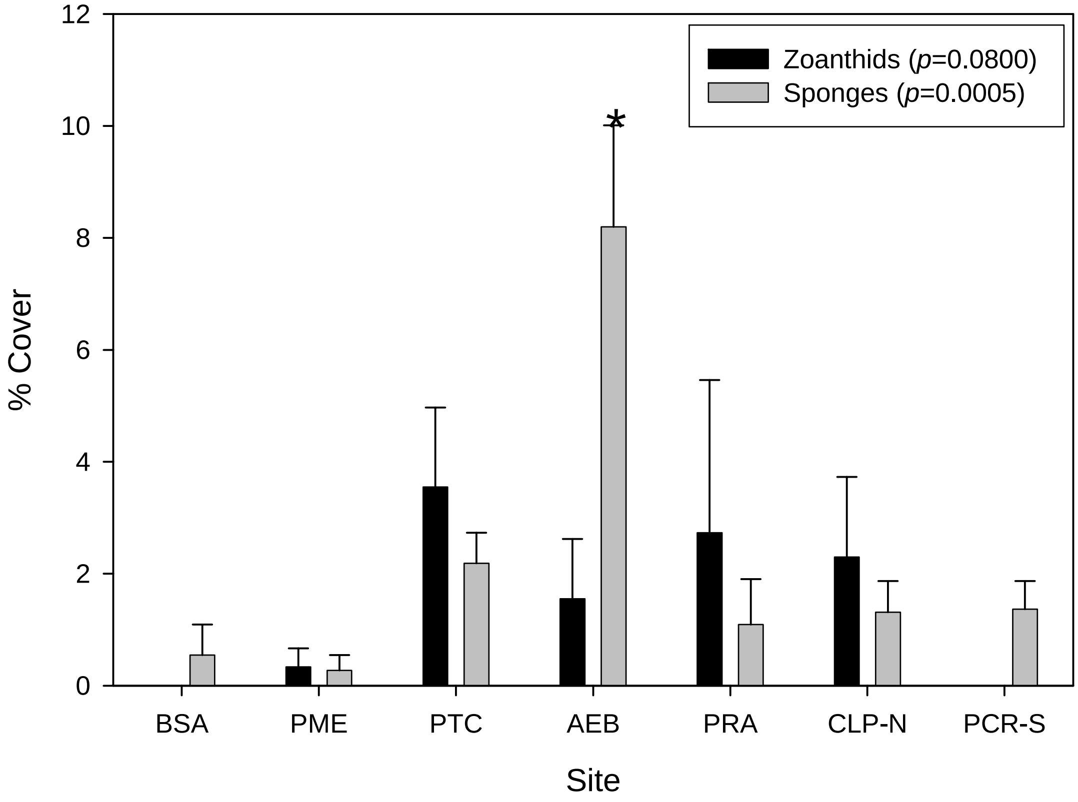

FIGURE 25. Percent zoanthid and sponge cover.

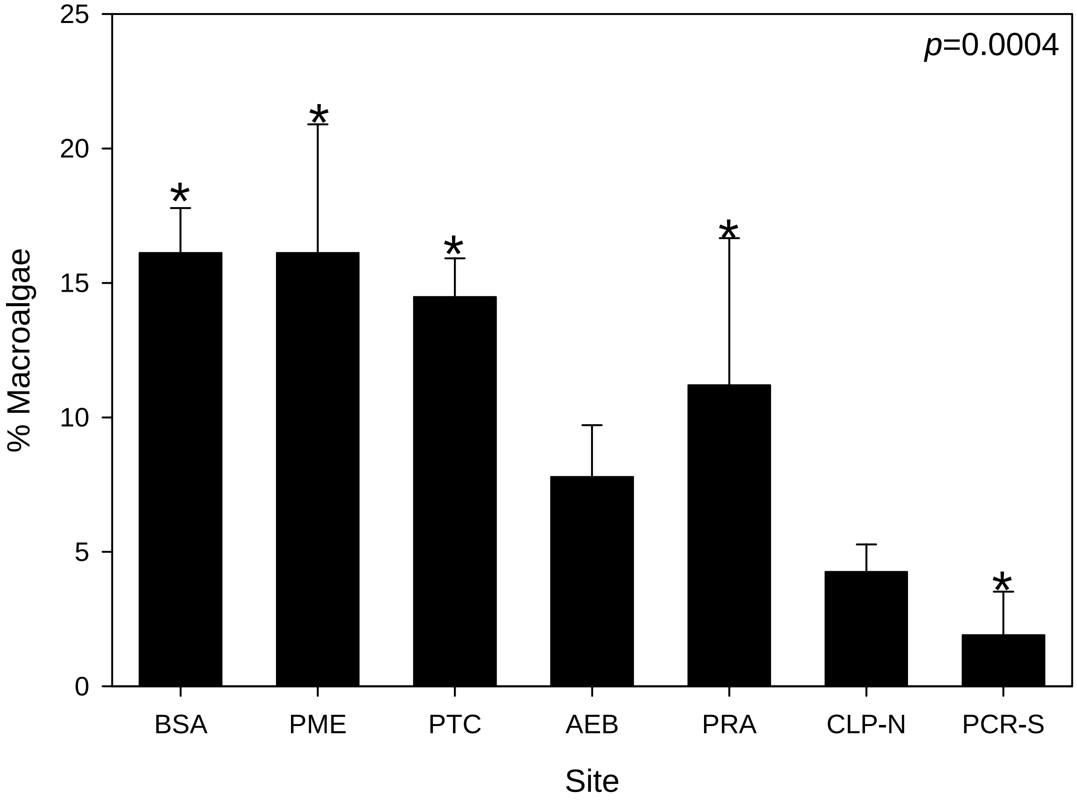

FIGURE 26. Percent macroalgal cover.

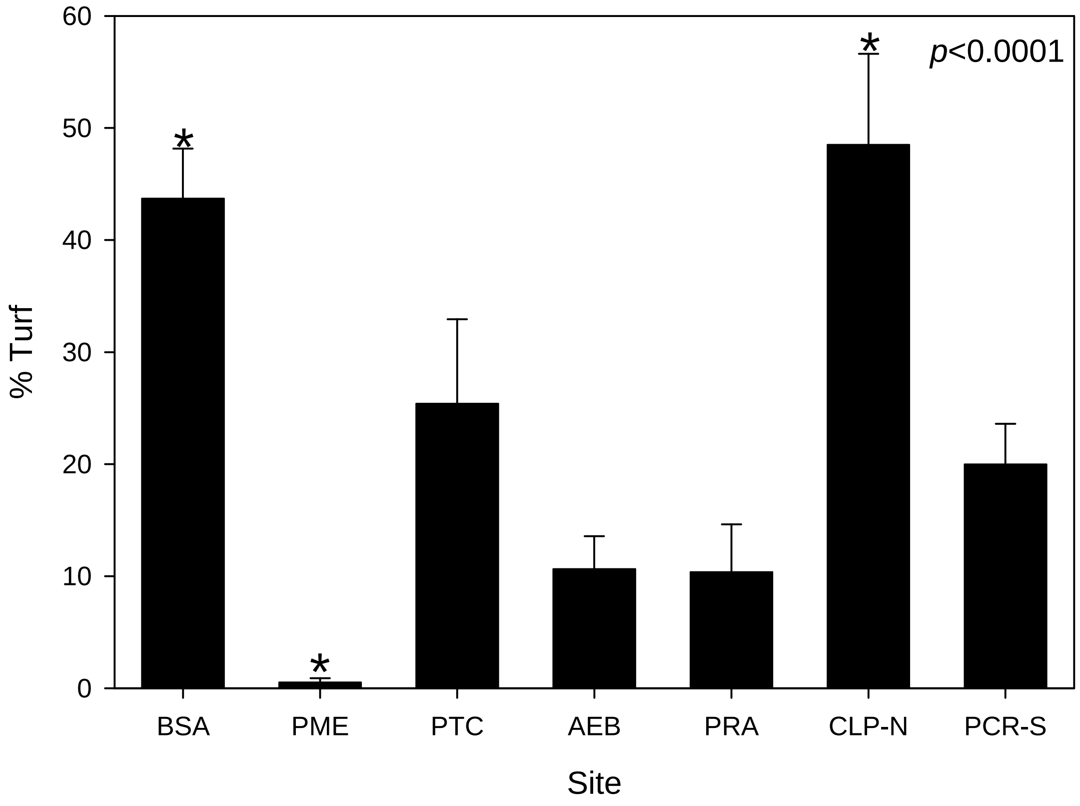

FIGURE 27. Percent algal turf cover.

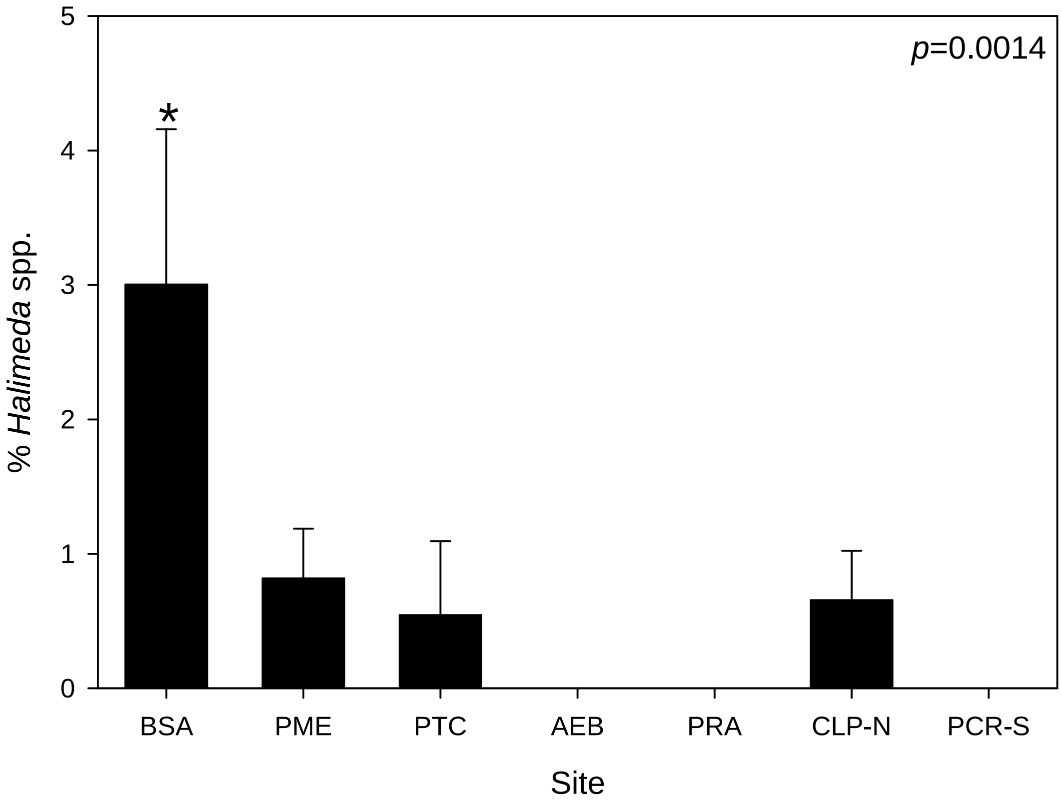

FIGURE 28. Percent *Halimeda* spp. cover.

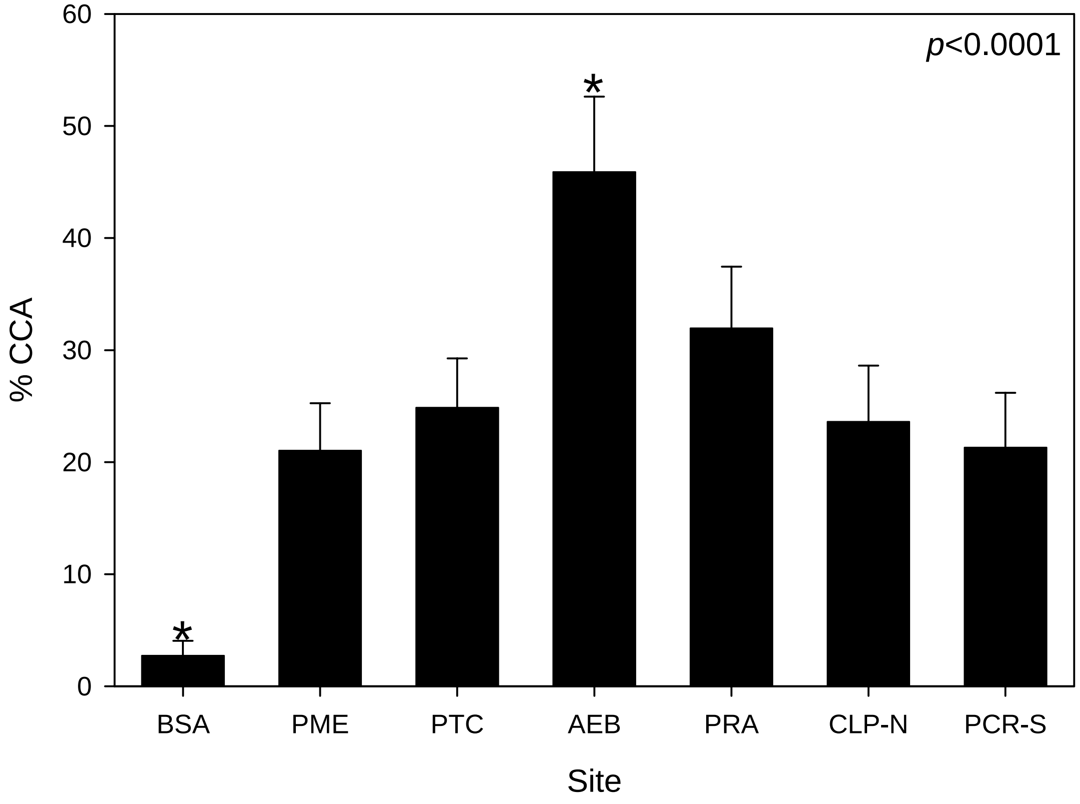

FIGURE 29. Percent crustose coralline algal (CCA) cover.

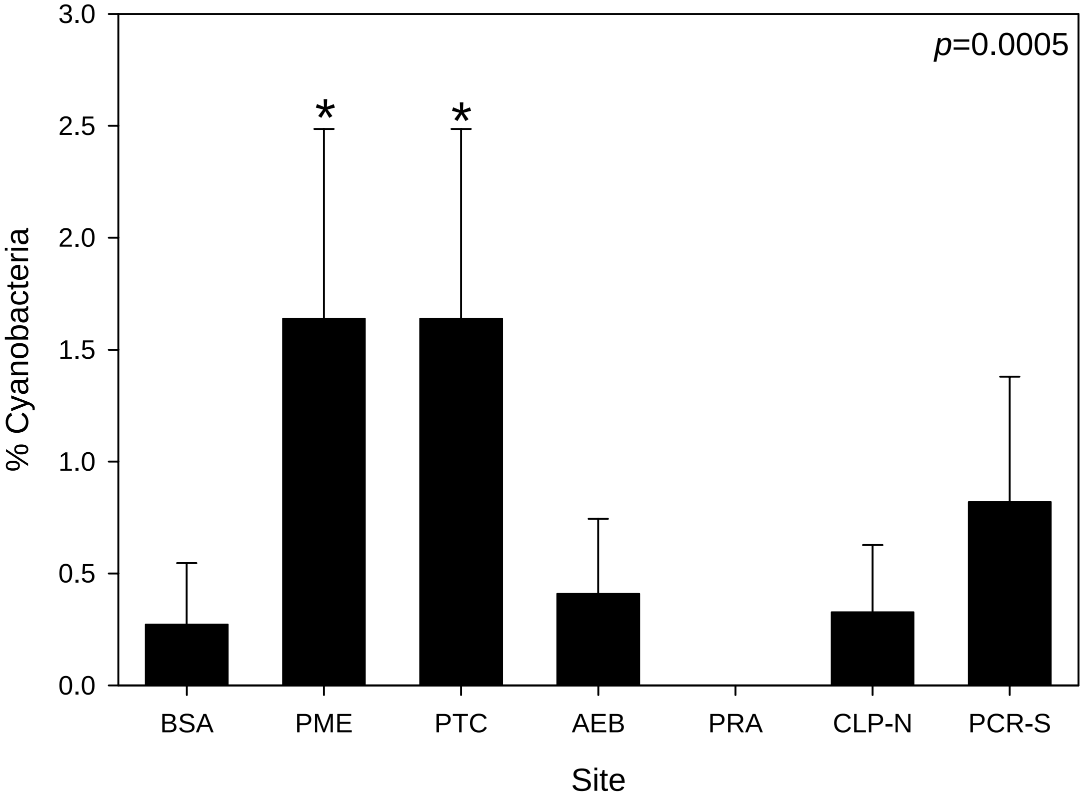

FIGURE 30. Percent cyanobacterial cover.

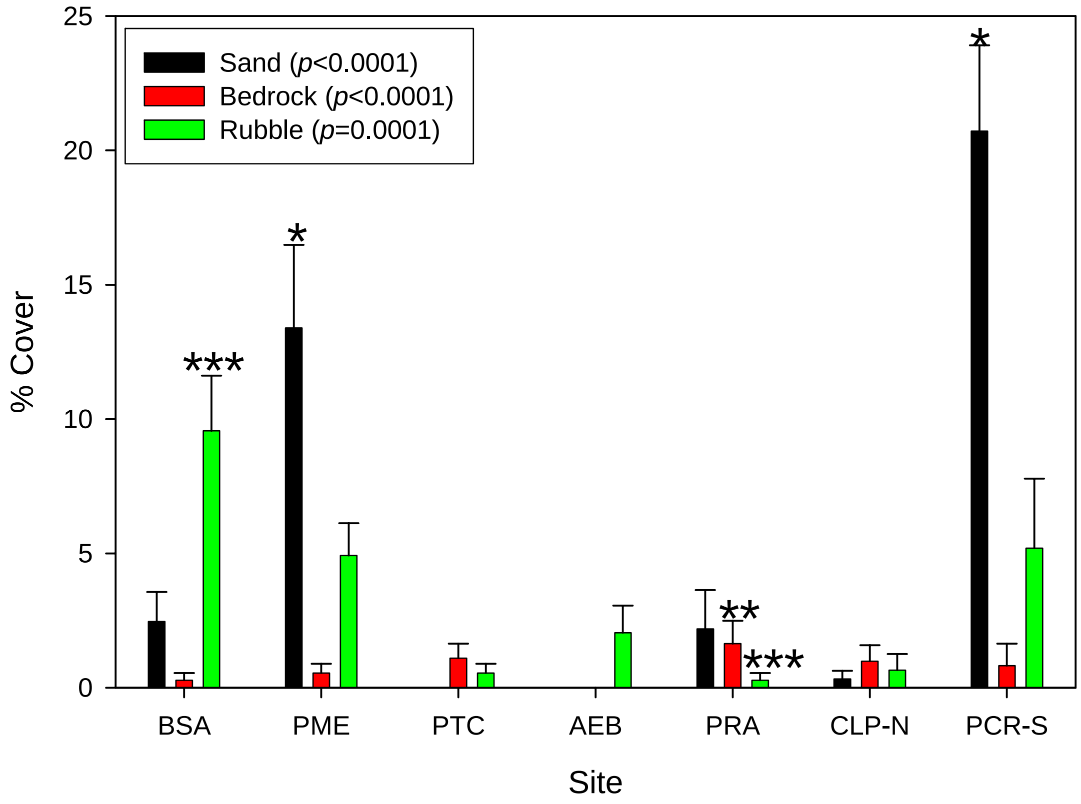

FIGURE 31. Percent cover of other benthic components (sand, bare bedrock substrate, rubble).

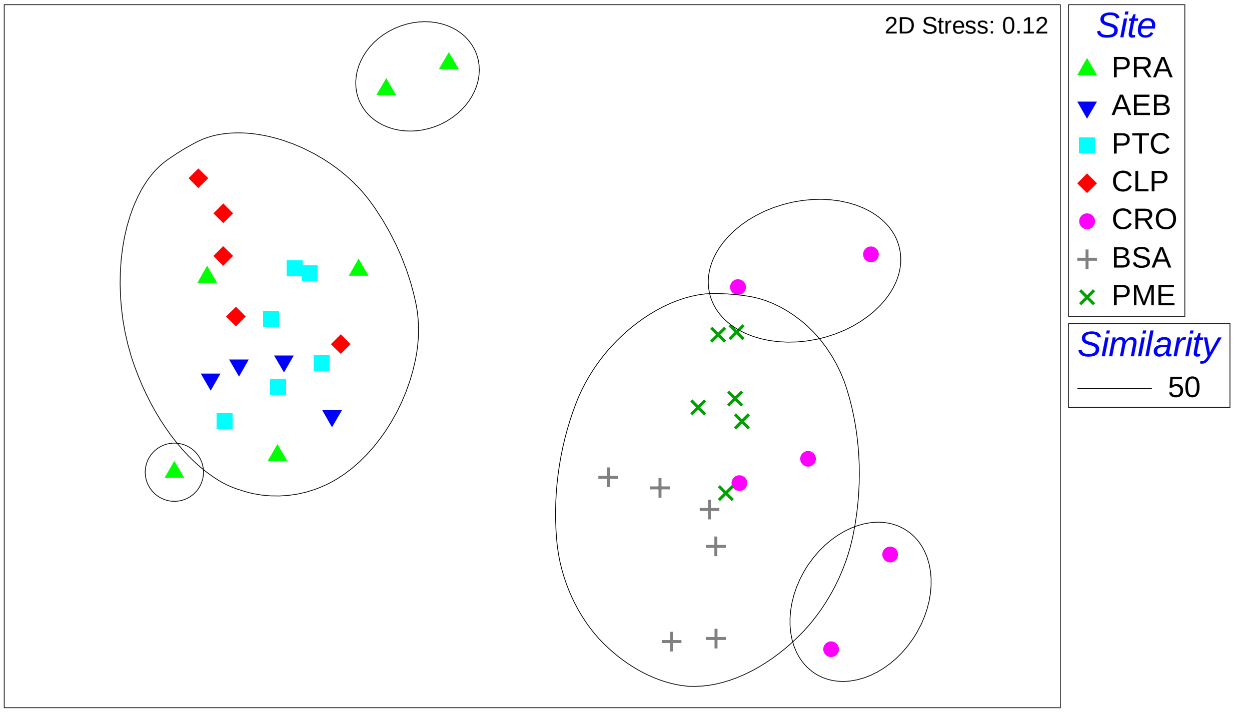

FIGURE 32. Multi-dimensional scaling analysis of shallow-water coral reef benthic communities among sites. Data shows six clustering patterns split into two major patterns at the 50% community similarity cutoff level. Locations impacted by either recurrent sediment-laden runoff pulses (BSA, PME) or recurrent sediment bedload pulses (PCR-S or CRO at this figure) clustered within the same general pattern. Other communities exposed to stronger circulation clustered separately.

TABLE 9. Results of a one-way ANOSIM test of benthic community structure. Based on 5000 permutations. NS= Not significant (*p*>0.0500).

Factors	**Global R**	**Significance**
Global test		
Site	0.730	0.0002
Pairwise test – Site		
PRA vs. AEB	0.155	0.1290 NS
PRA vs. PTC	0.254	0.0130
PRA vs. CLP-N	0.131	0.1560 NS
PRA vs. PCR-S	0.974	0.0002
PRA vs. BSA	0.946	0.0002
PRA vs. PME	0.933	0.0002
AEB vs. PTC	0.278	0.0430
AEB vs. CLP-N	0.600	0.0160
AEB vs. PCR-S	0.992	0.0005
AEB vs. BSA	1.00	0.0005
AEB vs. PME	1.00	0.0005
PTC vs. CLP-N	0.165	0.1190 NS
PTC vs. PCR-S	1.00	0.0002
PTC vs. BSA	1.00	0.0002
PTC vs. PME	1.00	0.0002
CLP-N vs. PCR-S	0.992	0.0002
CLP-N vs. BSA	1.00	0.0002
CLP-N vs. PME	1.00	0.0002
PCR-S vs. BSA	0.443	0.0002
PCR-S vs. PME	0.504	0.0004
BSA vs. PME	0.693	0.0002

Multivaraiate analysis

Multi-dimensional scaling analysis of shallow-water coral reef benthic communities among sites showed six clustering patterns split into two major patterns at the 50% community similarity cutoff level (Figure 32). Locations impacted by either recurrent sediment-laden runoff pulses (BSA, PME) or recurrent sediment bedload pulses (PCR-S) clustered within the same general pattern. Other communities exposed to stronger circulation and lower sediment deposition impacts clustered separately. In spite of the fact that there was no significant difference in many of the individual benthic community parameters as tested using univariate statistics, a one-way multivariate ANOSIM test (Table 9) showed highly significant differences in overall benthic community structure among sites (p=0.0002). Benthic community structure at the runoff impacted sites BSA and PME sites was significantly different from other sites. Similarly, benthic community at sediment bedload-impacted PCR-S was significantly different than other sites.

The benthic community at the sediment-laden runoff-impacted BSA was dominated by *Porites porites*, rubble and macroalgae (67%) (Table 10). Dominance at the sediment bedload-impacted PCR-S was exerted by sand, rubble, and *P.porites* (71%). Dominance at sites with strong water circulation (PTC, PRA, AEB) was exerted by CCA, algal turf, macroalgae, sponges, and disturbance adapted *Porites astreoides* (52-65%). PME was the study site with higher spatial relief and showed the highest biodiversity, with dominance by *Gorgonia ventalina*, rubble and *P. porites* (40%). Dominance tends to be higher by few taxa on stressed sites, but more evenly distributed (lower dominance) at areas with lower sediment deposition.

Table 10. SIMPER routine cumulative percent of the three major components of each site along the Luis Peña Channel

Site	Benthic components	Cummulative %
BSA	*Porites porites*, rubble, macroalgae	66.66
PME	*Gorgonia ventalina*, rubble, *P. porites*	40.31
PTC	CCA, algal turf, macroalgae	52.89
AEB	CCA, algal turf, sponges	54.30
PRA	CCA, macroalgae, *Porites astreoides*	64.94
CLP-N	Algal turf, CCA, *P.astreoides*	68.70
PCR-S	Sand, rubble, *P.porites*	71.15

Table 11. SIMPER routine major component differences between sites.

Sites	BSA	PME	PTC	AEB	PRA	CLP-N
BSA						
PME	*Gorgonia ventalina*					
PTC	*Porites porites*	CCA				
AEB	*Porites porites*	CCA	Algal turf			
PRA	*Porites porites*	CCA	Algal turf	Sponges		
CLP-N	*Porites porites*	Algal turf	Algal turf	Algal turf	Algal turf	
PCR-S	Sand	*Gorgonia ventalina*	Sand	CCA	*Porites porites*	Algal turf

A SIMPER test revealed that algal turf explained 33% of the benthic community differences sites, *P. porites* explained 24%, CCA explained (19%), *G. ventalina* and sandy bottoms (10%, respectively, and sponges about 5% of the variation (Table 11). BSA benthic community was significantly different from the rest largely due to dominance of surviving patches of *P. porites*, while benthic communities at PME were significantly difference due to dominance of open substrates covered with high CCA cover in most other sites that sustain large populations of *D. antillarum*. Algal turfs explained most differences between PTC and other sites. Sponges, algal turf and CCA explained differences between PRA and other sites. Algal turf and *P. porites* explained differences at PRA, and algal turfs at CLP-N. Sand became also an important factor at PCR-S.

Vulnerability analysis to sediment-laden runoff and non-point source sewage pollution

We calculated the expected risk vulnerability to sediment-laden runoff and non-point source sewage pollution at different areas that may directly or indirectly impact water quality within LPCNR using data obtained in this project and historical observations (E. Hernández-Delgado, pers. obs. and unpub. data). For the purpose of calculations, we assumed slack tides. Ebbing tides may cause stronger peak pulse impacts on waters adjacent to Ensenada Honda Bay (via bay mouth or Lobina Channel towars Bahía Sardinas). The expected risk of peak water turbidity pulses following heavy rainfall is significantly higher within Ensenada Honda due to a nearly total lack of erosion controls and its proximity to urban areas (Figure 33). Waters in the southeastern boundaries of LPCNR (i.e., PME to PRA) and often in portions of its northeaster

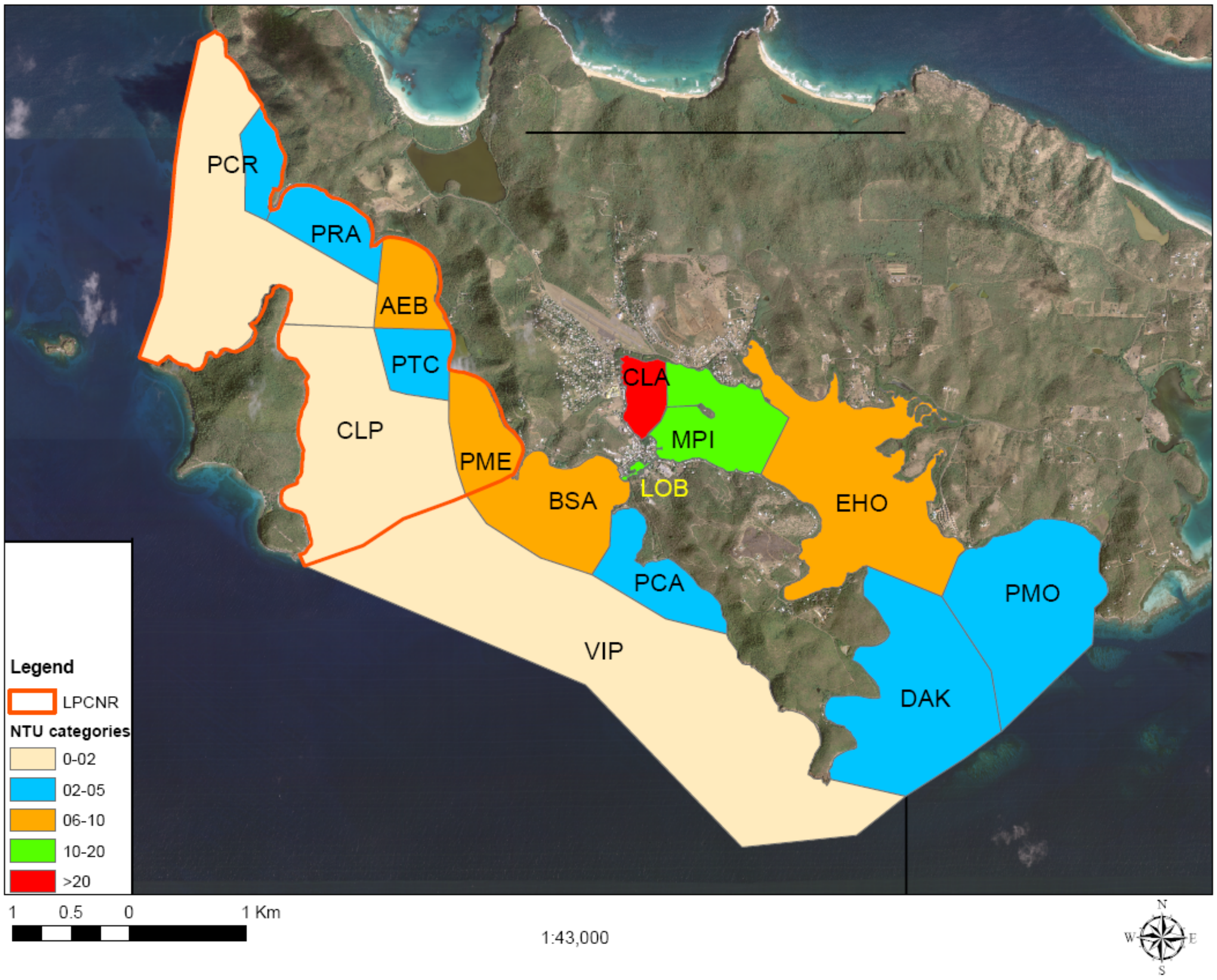

FIGURE 33. Expected peak water turbidity categories following heavy rainfall events and runoff pulses. Units are in NTU.

quadrant (PRA to PCR) may receive frequent sediment- and nutrient-laden runoff pulses following heavy rainfall. As a result, reefs may be recurrently impacted by runoff pulses causing frequent cyanobacterial and macroalgal blooms, as well as occasional coral mortality outbreaks, particularly in threatened Acroporid corals. Sedimented runoff pulse vulnerability risk is higher in areas adjacent to Culebra's center and southwest watersheds (Figure 34). These have either been subjective to extensive poorly planned construction or are being currently parceled for extensive construction. Thus risks of significant turbidity/sediment impacts are expected to increase in the near future.

Risk by FC pollution was more difficult to interpret due to its high variability (Figure 35). High variability is largely the result of particle interference in standard culturing procedures if water samples are highly turbid. But Ensenada Honda and LOB have the higher FC pollution risk, followed by BSA. Depending on future development plans high risk may expand to other areas as well. BSA, LOB and Inner Ensenada Honda and LOB have the higher enterococci pollution risk. Areas in the eastern quadrants of LPCNR have the higher FC pollution risk. Also Ensenada Honda mouth and areas in the eastern quadrants of LPCNR have a moderately high enterococci pollution risk. Depending on future development plans high risk may expand to other areas as well.

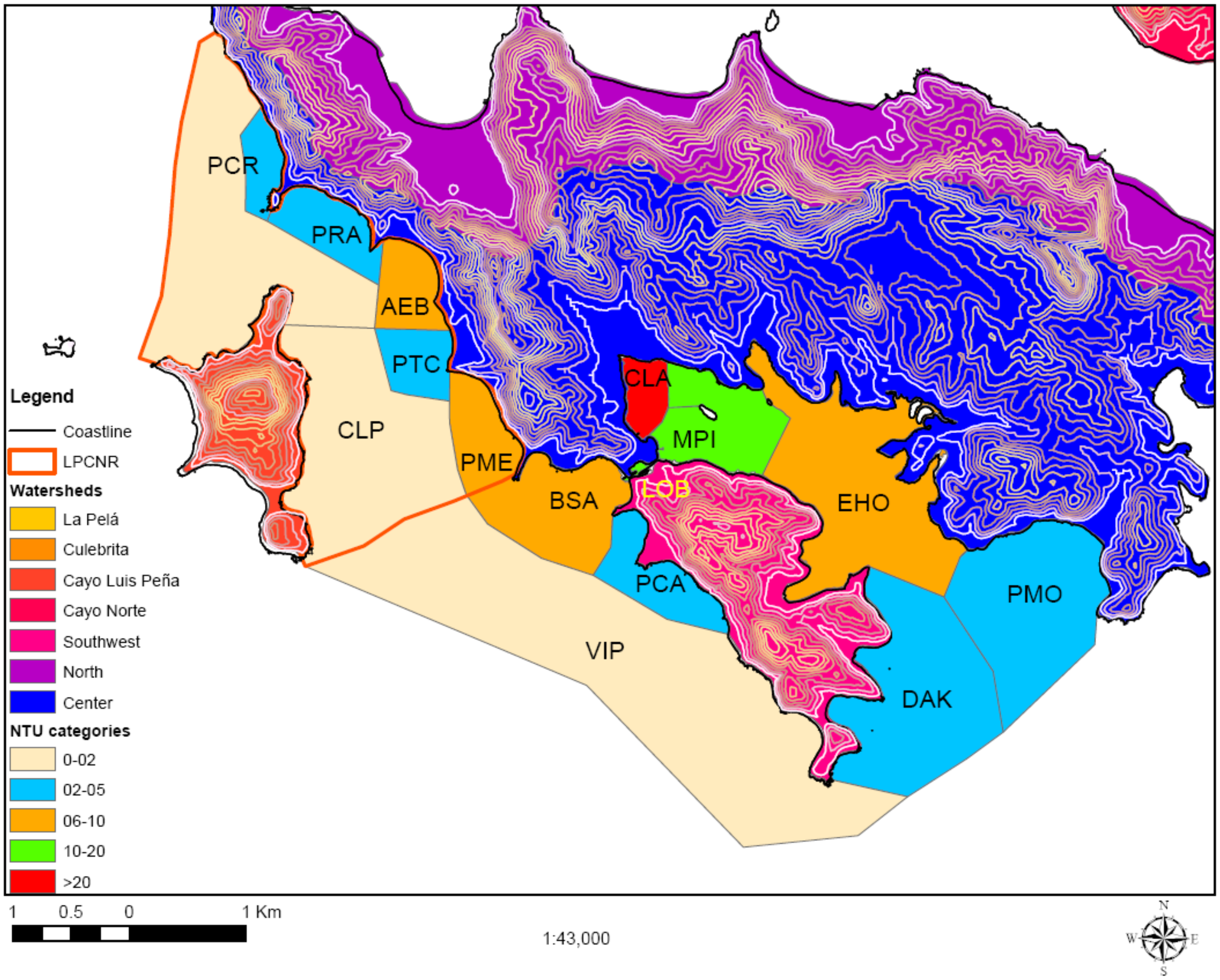

FIGURE 34. Expected peak water turbidity categories following heavy rainfall events and runoff pulses as a function of the digital elevation model and drainage areas of Culebra Island.

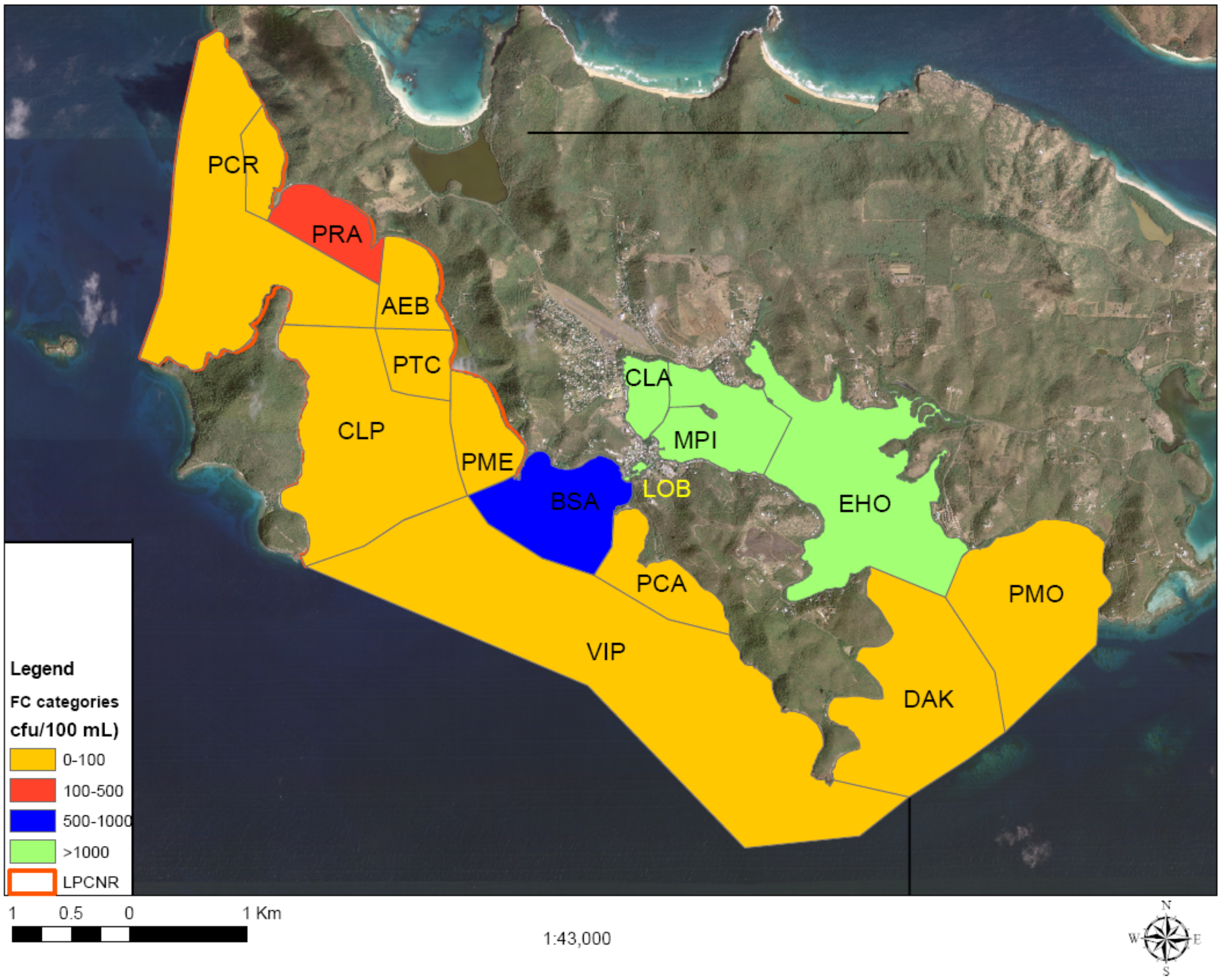

FIGURE 35. Expected peak FC categories following heavy rainfall events and runoff pulses

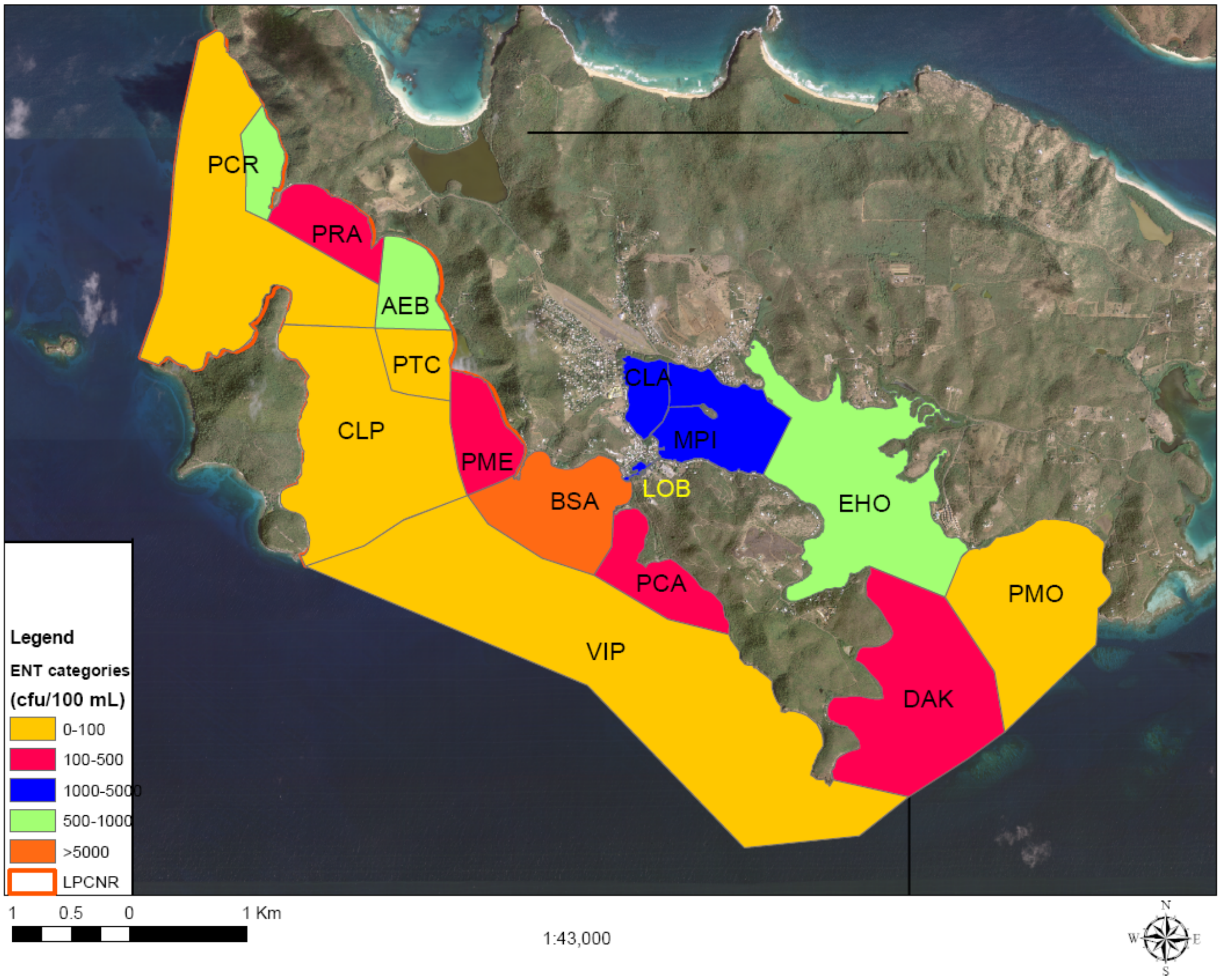

FIGURE 36. Expected peak enterococci categories following heavy rainfall events and runoff pulses

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