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Abstract
Determining the efficacy of efforts to conserve natural systems requires that environmental baseline data exist; without such baseline data, it is impossible to determine if management actions are working. This study presents water quality baseline data (nutrients) for the coastal waters of the island of Vieques, Puerto Rico. As the island's economy shifts more towards tourism, these data can be used to verify that conservation efforts to preserve the ecology of the coastal waters are succeeding. Surface waters were sampled at 40 sites, selected using a stratified random sampling design, on 7 occasions between July 2007 and March 2008. Nutrient concentrations were similar to what has been observed in other systems in Puerto Rico, except for in the near coastal lagoons which had significantly higher observed concentrations. Variations in nutrients between lagoons are driven by connectivity to the ocean and lagoon depth. Because of these relationships, and because there are no obvious major sources of point or non-point sources of pollution on the island that would affect only the lagoons, it is hypothesized that these high nutrient levels are the natural status of the system, rather than evidence of eutrophication.

Keywords: nutrients, corals, eutrophication, Caribbean

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Introduction

Nutrients often control primary productivity in marine systems. Most commonly, photosynthetic productivity in these systems is controlled by nitrogen (N), but at certain times of year some systems can be co-limited by both nitrogen and phosphorus (P). Although not typically a pollutant, silica distribution can also have impacts on diatom communities because diatoms need silica for frustule growth. In coastal systems, nutrient enrichment can result in algal blooms, changes in algal community composition (including harmful algal blooms) and increases in hypoxia/anoxia [1]. Furthermore, in tropical systems, excess nutrient loads can have both indirect and direct deleterious impacts on coral reefs. Nutrients can indirectly affect corals by causing increases in macroalgae which can outcompete and overgrow corals. Corals can be directly impacted by elevated levels of nitrogen and phosphorus by lowering fertilization success [2], and reducing both photosynthesis and calcification rates [3].

"New" (i.e. exogenous) nutrients can reach coastal systems from a variety of sources and pathways. Nitrogen and phosphorus can originate from animal/human waste, chemical fertilizers (lawns, golf courses, agriculture), and industrial sources [4]. Unlike phosphorus, nitrogen can be added to the system via atmospheric deposition and biological nitrogen fixation[5]. Silica is the second most abundant element in the earth's crust and is generally not considered to be a pollutant. Silica reaches the marine environment through erosion.

The island of Vieques lies approximately 11 km southeast of the main island of Puerto Rico in the U.S. Caribbean (Figure 1). Vieques is 34 kilometers long, 5 km wide and is home to a population of approximately 9,300 people [6]. Between 1940 and 2003, the U.S. Navy used portions of Vieques for military training, including the storage and firing of live munitions. The far western side of the island served as a storage area for munitions and other materials, while the eastern side was used for military training exercises that included naval gunfire and aerial bombardments. With the departure of the Navy in 2003, most of these areas have been transformed into the Vieques National Wildlife Refuge, operated by the U.S. Fish and Wildlife Service. Most of the eastern half of the island is still closed to the public due to concerns about unexploded ordnance. The two population centers on Vieques, Isabel Segunda and Esperanza, are located in the central portion of the island, on the north and south shores, respectively.

A comprehensive island wide nutrient budget is beyond the scope of this study, but there are several generalizations that can be made. There is no significant agricultural activity and no industrial sources of N or P on the island. Populations of free roaming horses and wild dogs may be adding to the nutrient budget, and human waste, from the WWTP, septic systems and possibly untreated waste, is almost certainly an important source.

The wastewater treatment plant (WWTP) in Vieques is located on the north shore of the island, west of Isabel Segunda and east of the airport (Figure 1). The plant, which has a capacity of 0.5 million gallons per day, uses a lagoon treatment system, consisting of four evaporation/percolation cells, to perform secondary sewage treatment [7] with no discharge to surface waters [8]. This type of treatment system relies on a combination of evaporation/volatilization to the atmosphere and a percolation of treated liquid into the groundwater. Volatilization of ammonia from the lagoon system may result in atmospheric deposition of ammonia-N to the landscape (resulting in runoff to marine systems) and/or directly to coastal waters. Similarly, nitrogen that leaches into the groundwater may eventually end up in the coastal environment. Although this is the only WWTP on the island, it only serves a population of 4,000 (less than half the population of the island) with the rest of the population not being served by a WWTP. Unsewered households may have septic tanks, or in rare cases

may not have treatment systems in place. This portion of the population not connected to the WWTP may be especially important to the nutrient budgets of the island.

The lagoon ecosystems of Vieques are of great ecological significance, ranging from bird, fish and crab habitats to the unique dinoflagellate populations of the bioluminescent bay [9]. Following the departure of the Navy in 2003, the economy of Vieques has slowly shifted towards tourism. With this shift, significant development is occurring and is expected to continue. This development may lead to increased nutrient loads from increased human waste (as both permanent and tourist populations increase) and increased fertilizer inputs (from heavily landscaped areas such as golf courses, hotel grounds and lawns). Baseline data are critical to be able to detect these potential changes, and make coastal management decisions to protect the natural resources of Vieques.

The goals of this study are to:

1. Establish a baseline of nutrient condition against which to measure changes in the future;
2. Determine if there are any hotspots of nutrient enrichment in the coastal waters;
3. Characterize the spatio-temporal variability in nutrient concentrations;
4. Compare nutrient conditions in Vieques to other systems in Puerto Rico.

Methods

Sampling Design

Because water column nutrients in tropical systems may vary greatly between the wet and dry season, the temporal sampling design was structured to collect samples throughout a hydrologic year. Field missions were conducted in July, August, September, October, and November of 2007 (wet season), and February and March of 2008 (dry season). Using a stratified random sampling design, forty sites were selected (Fig. 1). These samples were stratified by location: inshore (<1.5 km from shore), offshore (>1.5 km from shore) and lagoons. The historical land use by the US Navy resulted in the exclusion of human populations from the eastern and western ends of the island. Today, the entire eastern end of the island is part of the Vieques Natural Wildlife Refuge, most of which is off limits to visitors. This allowed the sampling design for this study to effectively sample areas likely to be impacted by humans (western island) versus areas that are less likely to be impacted by humans (eastern island).

Sites were evenly distributed between the eastern (uninhabited) and western (inhabited) halves of the island. During each field mission, an attempt was made to re-sample each of these forty sites; however, due to weather and boat related problems, not all sites could be sampled at every time point (Appednix 1). During the study period, a total of 193 samples were collected for nutrient analysis.

Sample collection methods

Nutrient samples were collected in high density polyethylene (HDPE) bottles from 0.1 m below the surface. In extremely shallow lagoons (<0.5 m), samples were taken at half the distance to the bottom; in this situation, care was taken to exclude sediment from the samples. Bottles were never previously used and were rinsed three times with site water prior to sampling. Nitrile or latex gloves were worn by field personnel to avoid contamination of the samples during handling. On each sampling mission, replicate samples were collected at four (randomly selected) of the 40 sites to ensure precision in sampling methodology. Unfiltered samples were

stored on ice, in the dark while in the field and frozen at -20 degrees C upon returning to the lab and not thawed until immediately prior to analysis.
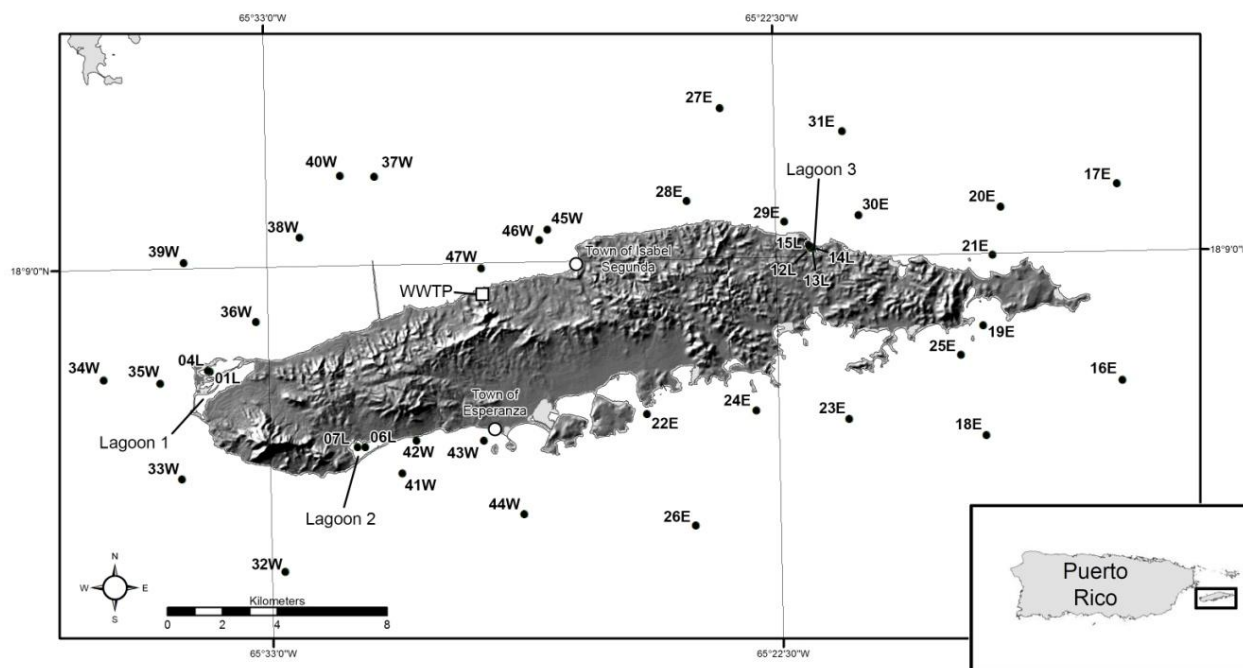

Fig. 1. Location of sampling sites, sampled lagoons, towns and wastewater treatment plant (WWTP).

Analytical methods used for the analysis of nutrients in water

Laboratory analyses were conducted at the NOAA National Status and Trends Program contract lab (TDI-Brooks International; College Station, TX). Water samples were analyzed for a standard suite of nutrient analytes: nitrate (NO_3^-), nitrite (NO_2^-), orthophosphate ($HPO_4^=$), ammonium (NH_4^+), urea ($(NH_2)_2CO$), total nitrogen and total phosphorus (Appendix 2).

Nitrate and nitrite analyses were based on the methodology of Armstrong et al [10]. Orthophosphate was measured using the methodology of Bernhardt and Wilhelms [11]. with the modification of hydrazine as reductant. Silicate determination was accomplished using the methods of Armstrong et al. [10] using stannous chloride. Ammonium analysis was based on the method of Harwood and Kuhn [12] using dichloro- isocyanurate as the oxidizer. Urea was measured using diacetyl-monoximine and themicarbozide. The total concentrations of nitrogen and phosphorus were determined after an initial decomposition step. This method involves persulfate oxidation while heating the sample in an autoclave ($115^\circ C$, 20 minutes) [13]. After oxidation of the samples, nutrient determination was conducted on the Technicon II analyzer for nitrate and orthophosphate.

Because data were not normally distributed (Shapiro-Wilk W test), non-parametric statistics (Wilcoxon test, $\alpha = 0.05$) were used to evaluate differences between strata and between lagoon types.

Results

Because the lagoons and the offshore environment are very different systems (based on both qualitative observations and water quality/nutrient data), summary statistics are presented by lagoon and inshore/offshore separately in Appendix 3 and 4.

Precipitation data during the study period were acquired from the RAWS USA Climate Archive [14] for the station in Vieques (18° 07' 18", 65° 24' 58"). Precipitation can generate nutrient laden runoff which can be an important driver of nutrient concentrations in some systems.

Because there were only two sampling dates during the dry season, and boat/weather problems prevented complete sampling during these dates, it is not statistically valid to compare wet versus dry seasons. Furthermore, there are significant gaps in the precipitation data record at this station, making precipitation analysis problematic. Across seasons, nutrient data were not well correlated with precipitation patterns.

No significant differences in nutrient concentrations between the eastern and western strata were observed, with the lone exception being for urea; urea concentrations were significantly higher in the western zone (Fig. 2 and 3). This pattern is somewhat unexpected because population centers in the western portion of the island were hypothesized to be a significant source of nutrients. This expected geographic pattern only holds true for urea, but not for other nutrient species. It is unclear whether this observed pattern in urea concentrations represents a human or animal waste signal. Additional data would be required to determine the source of this urea and why this pattern is not observed in other nutrient species. It is possible that in near coastal waters, human-derived sources of nutrients are not particularly important to the nutrient budget. This possibility is supported when comparing differences between inshore and offshore waters and the absolute magnitudes of nutrient concentrations when compared to other systems in Puerto Rico (see detailed discussion below). A possible alternative hypothesis would be that military activities led to increased nutrients. However, any military activities which affected the nutrient budget of the island (e.g. increase human waste due to personnel stationed there) were likely small (relatively small number of personnel stationed there) and transient effects that have not persisted over time (the Navy left the island in 2003). Other studies have investigated additional potential water quality impacts of military activities on Vieques [6].

Statistically significant differences between strata for TP (offshore>inshore) were observed, but no differences were seen for orthophosphate (Fig. 4). This is counterintuitive based on expectations of land based sources of phosphorus being important. This pattern can potentially be explained by higher uptake of phosphorus by near shore primary producers. Across all nutrient species, mean lagoon concentrations were significantly higher than marine (inshore or offshore sites) concentrations (Fig. 4 and 5). Field observations showed that the lagoons are shallow, poorly flushed and visibly high in humic materials. Fringing mangroves, submerged aquatic vegetation and benthic microbial mats all contribute to high organic material in the lagoons. It is hypothesized that high nutrient concentrations of these lagoons represent their natural state, and differences in nutrient status between lagoons can be explained by hydrologic differences rather than nutrient sources via runoff.
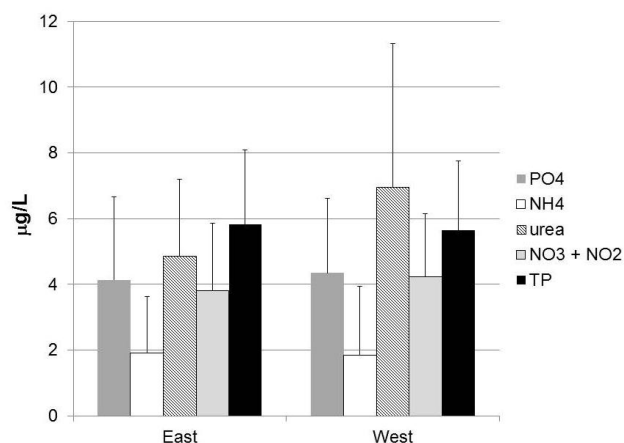
Fig. 2. Mean concentrations of ortho phosphorus (PO$_4^{3-}$-P), ammonium (NH$_4^+$-N), urea-N, nitrate plus nitrite (NO$_3^-$-N + NO$_2^-$-N) and total phosphorus (TP), by strata (east vs. west). Error bars are one standard deviation. Urea was statistically higher (α = 0.05) in the west (no significant differences among other analyses).
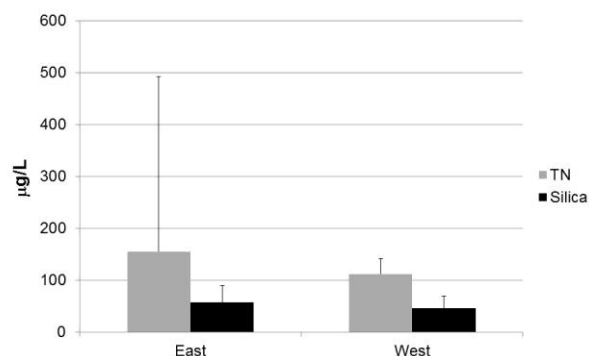

Fig. 3. Mean concentration of total nitrogen (TN) and silica by strata (east vs. west). Error bars are one standard deviation. No statistically significant differences (α = 0.05).
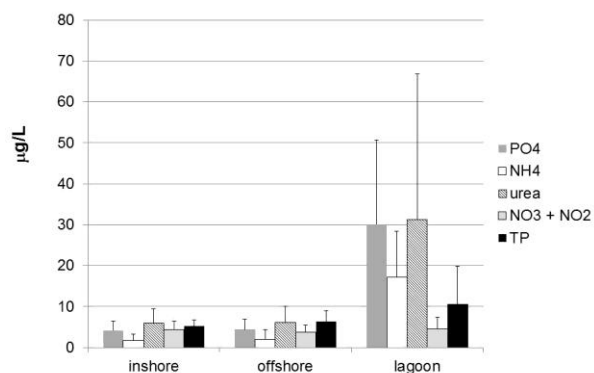

Fig. 4. Mean concentrations of ortho phosphorus (PO$_4^{3-}$-P), ammonium (NH$_4^+$-N), urea-N, nitrate plus nitrite (NO$_3^-$-N + NO$_2^-$-N) and total phosphorus (TP), by strata (offshore vs. inshore vs. lagoon). Error bars are one standard deviation. TP was significantly higher (α = 0.05) offshore compared to inshore. All nutrient species were significantly higher (α=0.05) in the lagoons compared to inshore and offshore.
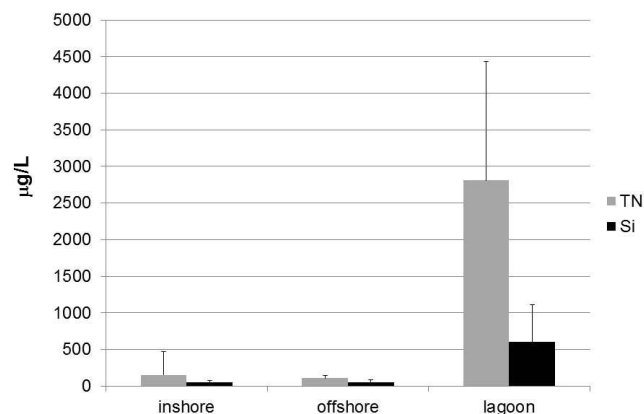

Fig. 5. Mean concentration of total nitrogen (TN) and silica by strata (offshore vs. inshore vs. lagoon). Error bars are one standard deviation. No statistically significant differences (α = 0 .05). All nutrient species were significantly higher (α = 0.05) in the lagoons compared to inshore and offshore.

In order to more thoroughly explore this, the lagoons were classified based on their level of connectivity to the ocean (based on satellite imagery and field observations) and their depth (based on field observations). Two of the three lagoons sampled were categorized as "shallow" (lagoons 2 and 3), with depth of less than 0.25 m. The only deeper lagoon is Laguna Arenas (lagoon 1 on Figure 1), located in the northwest portion of the island in part of the National Wildlife Refuge. It has depths of 1-2 meters. Shallow lagoons had significantly higher ammonium, total phosphorus and silica concentrations than the deeper lagoon. (Fig. 6 and 7).
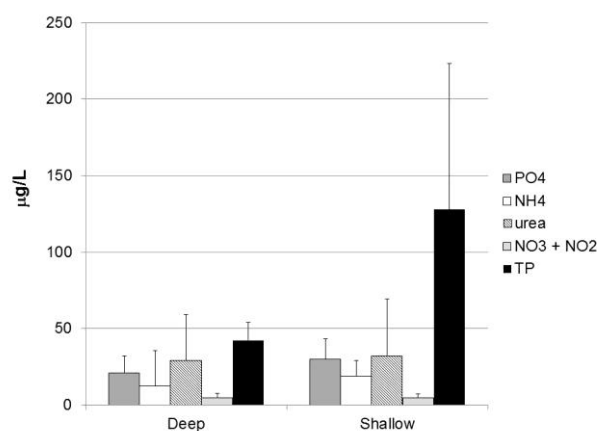

Fig. 6. Mean lagoon concentrations of ortho phosphorus (PO_4^{3-}-P), ammonium (NH_4^+-N), urea-N, nitrate plus nitrite (NO_3^--N + NO_2^--N) and total phosphorus (TP), by strata (deep vs. shallow). Error bars are one standard deviation. TP and ammonium were significantly higher (α = 0.05) in shallow lagoons.
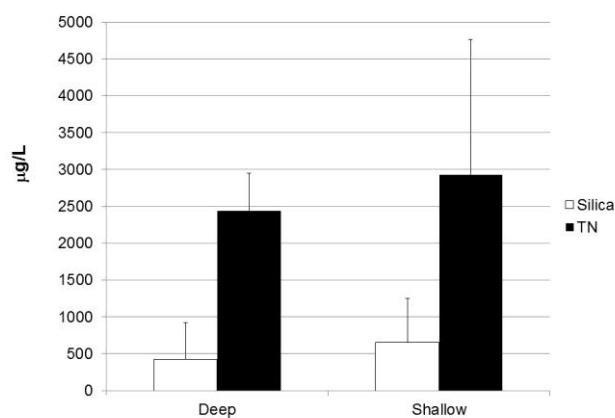
Fig. 7. Mean lagoon concentration of total nitrogen (TN) and silica by strata (deep vs. shallow). Error bars are one standard deviation. Silica was significantly higher (α = 0.05) in the shallow lagoons.
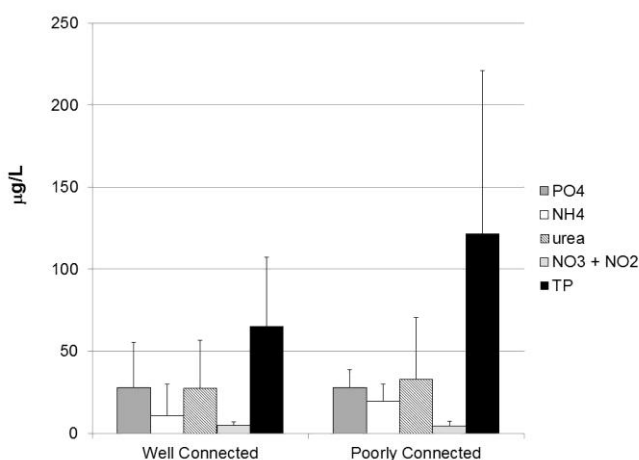
Fig. 8. Mean lagoon concentrations of ortho phosphorus (PO_4^{3-}-P), ammonium (NH_4^+-N), urea-N, nitrate plus nitrite (NO_3^--N + NO_2^--N) and total phosphorus (TP), by strata (ocean connectivity; well connected vs. poorly connected). Error bars are one standard deviation. Ammonium was significantly higher (α = 0.05) in poorly connected lagoons.
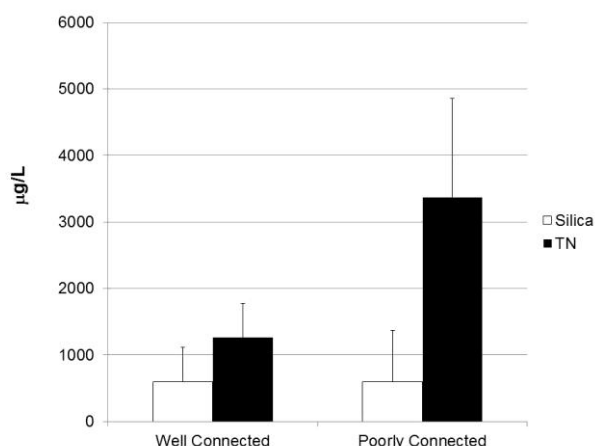
Fig. 9. Mean concentration of total nitrogen (TN) and silica by strata (ocean connectivity; well connected vs. poorly connected). Error bars are one standard deviation. Total nitrogen (TN) was significantly higher (α = 0.05) in poorly connected lagoons.

Two of the three lagoons sampled were categorized as "poorly connected" to the ocean (lagoons 1 and 3). The only lagoon with extensive tidal connectivity to the ocean is Laguna Playa Grande (Lagoon 2 on Figure 1). The other two lagoons have no visible connection to the ocean, and no observed tidal influence. Ammonium and total nitrogen concentrations are significantly higher in lagoons with poor connectivity to the ocean (Fig. 8 and 9).

This hydrologic analysis builds on earlier work [6] and explains some of the observed variability in nutrient concentrations between lagoon types. Furthermore, this type of hydrologic analysis can be applied to other lagoon systems in the Caribbean.

Discussion

Temporal patterns in precipitation (e.g. storm events) are not well correlated with nutrient concentrations in lagoon, or inshore and offshore waters. This observed disconnect between rainfall, and therefore runoff, could be explain with several possible hypotheses. First, it is possible that inputs of nutrients from the watershed are not the primary driving factor controlling ambient nutrient concentrations in marine waters. It is also possible that biological activity, such as uptake during primary productivity or denitrification, overwhelms any runoff signal. Finally, because nutrient concentrations can change on short time scales (minutes to hours), it is possible that there are short term fluctuations in water column concentrations that were not captured in this dataset. Similarly, there could be long term trends in nutrient concentrations that were not captured during this one year study. These research questions could be answered with further study and monitoring.

The magnitude of nutrient concentrations in the coastal surface waters of Vieques was similar to what has been observed in other recent studies in Puerto Rico. In total, these data do not suggest that there is currently a problem with anthropogenic nutrient over enrichment in Vieques.

No regulatory nutrient criteria exist for U.S. coastal waters. However, previous studies have postulated that, for coral reef ecosystems, 14 µg-N/L dissolved inorganic nitrogen (DIN) and 31 µg-P/L soluble reactive phosphorus (SRP) are threshold values above which macroalgal growth can threaten coral reefs [15]. In Vieques, the marine (i.e. non-lagoon) sites never exceeded 14 µg/L of DIN (nitrate + nitrite + ammonium). Although a proposed threshold for total nitrogen (TN) does not exist, TN concentrations in the coastal waters of Vieques did exceed the level for DIN. This is not necessarily indicative of an ecological problem, because DIN is much more readily available for plant or phytoplankton uptake than TN. The suggested threshold value for phosphorus (31 µg-P/L) is exceeded only in the lagoons, suggesting that P is not a problem in nearshore or offshore waters.

Previous studies that have measured nutrient concentration in other locations in Puerto Rico can be valuable for comparison. Nutrient data reported from southwest Puerto Rico [16] were lower than reported here for Vieques (comparing TN and TP); however, it should be noted that the southwest Puerto Rico data is from one sampling period in August, so it is possible that this is not representative due to temporal nutrient variability. Conversely, a long term monthly dataset for Jobos Bay, Puerto Rico [17], including orthophosphate, ammonium, nitrate and nitrite, has observed concentrations that were very similar to lagoon data for Vieques. Jobos Bay has been documented to be impacted by both point and non-point source nutrient pollution

[18]. However, Jobos Bay is both hydrographically and ecologically dissimilar from the lagoons on Vieques, in that Jobos Bay is much larger, much deeper (maximum depth=10m) [19], better flushed and has less organic matter than the lagoons of Vieques. As noted above, it is hypothesized here that the high nutrient levels in the Vieques lagoons are the natural state and do not represent anthropogenic enhancement.

Implications for Conservation

There are many strategies for environmental conservation, ranging from ameliorating current degradation (e.g. habitat restoration and reducing pollution) to preventing future damage (e.g. land use planning and pollution prevention). In order to determine the effectiveness of conservation efforts, environmental data must be collected against which to measure future change. Ideally, future change could be compared against historical data from a long term data record, but in many coastal systems, especially in tropical areas, there is very little historical water quality data. In these cases, it is critical to collect baseline information describing current conditions so that future changes in water quality (e.g. nutrient over-enrichment) can be measured.

The data presented here will serve as critical baseline information that will allow coastal managers to take proper steps to insure that development pressures on the island do not increase the nutrient flux to coastal waters, thereby increasing stressors to coral reef ecosystems.

Acknowledgments

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Appendix 1: Dates of field sampling at each site ("x" means site was sampled). Missing sample dates were due to weather or boat problems.

Sites	7/07	8/07	9/07	10/07	11/07	2/08	3/08
01L	x	x	x	x	x		x
04L	x	x	x	x	x	x	x
06L	x	x	x	x	x	x	x
07L	x	x	x	x	x	x	x
11L				x	x	x	
12L	x	x	x	x	x	x	
13L	x	x	x	x	x	x	
14L	x	x	x	x	x	x	
15L	x	x	x		x	x	
16E	x	x	x		x		
17E	x	x	x		x		
18E	x	x	x		x		
19E	x	x	x		x		
20E	x	x	x		x		
21E	x	x	x		x		
22E	x	x	x		x		
23E	x	x	x		x		
24E	x	x	x		x		
25E	x	x	x		x		
26E	x	x	x		x		
27E	x	x	x				
28E	x	x	x		x	x	
29E	x	x	x		x		
30E	x	x	x		x		
31E	x	x	x				
32W		x	x		x		
33W	x	x	x		x	x	
34W	x	x	x		x	x	
35W	x	x	x		x	x	
36W	x	x	x		x	x	
37W	x	x	x		x	x	
38W	x	x	x		x	x	
39W	x	x	x		x	x	
40W	x	x	x		x	x	
41W	x	x	x		x		
42W	x	x	x		x		
43W	x	x	x		x		
44W	x	x	x		x		
45W	x	x	x		x	x	
46W	x	x	x		x	x	
47W	x	x	x		x	x	

Appendix 2: Details on analytical methods for nutrients

Analyte	Method Detection Limit (µM)	Method Detection Limit (mg/L)	Standard Range (µM)	Standard Range (mg/L)
NO_3^-	0.177	0.010	3.85 - 30.14	0.23 - 1.86
NO_2^-	0.010	0.0004	0.09 - 0.72	0.006 - 0.033
$HPO_4^=$	0.030	0.002	0.35 - 2.18	0.021 - 0.21
$HSIO_3^-$	0.155	0.014	4.05 - 30.08	0.25 - 2.80
NH_4^+	0.070	0.001	0.42 - 3.44	0.026 - 0.062
Urea	0.205	0.012	0.59 - 4.42	0.036 - 0.265

Appendix 3: Lagoon nutrient summary statistics (July 2007 to March 2008). Concentrations in μg/L.

Analyte	Mean	Stdev	Min.	Max
HPO_4	30.1	20.6	2.1	112
TP	106.8	91	2.4	556.7
NH_4^+	17.2	11.2	1.1	48.8
NO_3^-	2.7	2.6	0.04	13.5
NO_2^-	1.7	1	0.03	5
Urea	31.2	35.5	4.4	172.4
TN	2810.4	1624.9	218.4	7331.4

Appendix 4: Coastal (inshore and offshore) nutrient summary statistics (July 2007 to March 2008). Concentrations in 𝜇g/L.

Analyte	Mean	Stdev	Min.	Max
HPO_4	4.3	2.8	1.2	11.5
TP	5.7	2.2	0.5	13.8
NH_4^+	1.9	1.9	0.06	11.2
NO_3^-	3.6	1.8	0.04	1.1
NO_2^-	0.5	0.3	0.04	1.1
Urea	6	3.7	1.8	25.2
TN	131.3	229.7	83.6	2766.4