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RESUMEN
La Parguera is the site of a Marine Reserve and counts among its unique habitats a phosphorescent bay, important reef systems and a diverse and healthy mangrove system including significant cays. Areas of its waters see some of the heaviest use in the US. A study, underway since 1998, has analyzed inshore and near-shore waters for a number of biological, chemical and physical parameters and related these to onshore development, a sewage treatment plant discharge and known stormwater discharges. Among the thirty parameters monitored were bacteriological indicators (total and fecal coliforms, Escherichia coli, Enterococcus) total suspended solids, chlorophyll, light attenuation (light reaching the bottom compared to surface insolation) and nutrients (NH₃, NO₃, NO₂ and PO₄). In addition, bacteriological monitoring data were analyzed according to US EPA standards for recreational waters and violations also related to onshore development. A number of sampling stations were monitored, selected for the onshore development, stormwater runoff and the littoral and coastal systems located there. At minimum discretion the categories used were presence or absence of mangroves and presence or absence of onshore development.


INTRODUCCIÓN
Thirty percent of the National Wildlife Refuges in the Caribbean and nearly all of the remaining subtropical dry forest are located in southwest Puerto Rico. Until recently, most of the commercial fishery activity in Puerto Rico was centered on southwest Puerto Rico because of the high productivity in this area. The area is enclosed to the north by the Palmarojo Hills (or Sierra Bermeja, locally) short hills that produce a rain shadow for this area; annual rainfall to the north of the hills is 120 cm (48") and in La Parguera, 60-70 cm (24-28"). Rainfall occurs mostly in two rainy seasons, March-May and September-December. There are no permanent streams in the area. To the south or seaward, there is an extensive system of reefs and mangrove cays.

La Parguera was a small village with an economy based on fishing and small-scale, local tourism. Tourists have been attracted to the sport fishing and aesthetics of the area. Development was limited and mangrove stands and seagrass meadows were extensive. In 1990, approximately 1,200 people lived in La Parguera (US Census Bureau 1990). The population developed infrastructure that allowed the co-existence of the fishing community and moderate recreational use by other area residents. Part of this infrastructure was an advanced wastewater treatment plant (0.15 MGD), utilizing activated sludge in a package plant and discharging effluent onto sand beds for polishing. From the sand beds effluent flowed into salt flats behind the mangrove fringe. In the last four years the discharge was changed, with headers feeding laterals in the gravel beds with spray nozzles elevated about three feet. The caps on the headers blowing off have vitiated any improvement
due to this method of discharge and the result is direct discharge into the salt flats behind the mangrove fringe.

In 1994, the Junta de Planificación de Puerto Rico (Puerto Rico Planning Board) created a Tourist Zone in and around La Parguera. In effect, this created an entirely new town, allowing development in a large portion of the dry forest and salt flat areas. Since this rezoning, development has destroyed much of the remaining tropical dry forest habitat. By November, 1998 an informal survey counted at least 650 additional housing units in La Parguera, approximately doubling the housing units and tripling the population in the area. Other impacts of this rezoning might include direct habitat damage due to construction; increased pressure on infrastructure; increased effects of sedimentation due to poor control of erosion during and after construction; resuspension effects due to heavier boat traffic; increased runoff of nutrients and incidentals of urban land-use, such as chemical and petroleum products; and groundwater contamination.

Popular belief in Puerto Rico has as a given that marine water quality in La Parguera is poor and that this is the result of houses built into the mangrove fringe and of the wastewater treatment plant. Paradoxically, there has never been a single reported violation of bacteriological standards. This is likely due, in part, to the fact that only a single station is sampled bimonthly between Punta Papayo and Punta Parguera, a distance of about 2 km and an area of about 600,000 m². A multi-year study was undertaken with three specific goals:

1. to verify chemical, physical and bacteriological water quality in the area;
2. to provide baseline data for management of the area and;
3. to elicit, insofar as possible, the source of system degradation if and where encountered.

We report here bacteriological water quality data and chemical/physical water quality data. In addition, we report on a number of other measures of system health to compare with more traditional indicators of water quality.

MATERIALS AND METHODS

Station Selection and Categorization

The area has an east-west long-shore current, approximately 2 knots outside the embayment and 200-400 meters per hour in the areas sampled. Station 1 is south of an undeveloped, naturally vegetated hill and has a mangrove fringe. Station 2 is south of a steep, unvegetated, undeveloped hill and has no fringe mangroves. Station 3 is adjacent to low density development with high erosion potential with an interrupted mangrove fringe. Station 4 has extensive development onshore, with an extensively cut mangrove fringe and experiences high water traffic. Station 5 has extensive onshore development and very high erosive potential; the mangrove fringe is extensively cut. Station 11 is in a channel that has very high boat traffic and land development is high on the hill just north of this station; erosion potential is very high. Station 14 has extremely sparse onshore vegetation and extensive development. Station 6 has no development and mangroves and is just down-current of the wastewater treatment plant. Station 22 is located outside direct influence from onshore development and has extensive mangrove fringe. Stations 5 and 6 are immediately offshore of significant inflow from onshore development and may have additional input from intermittent overflows from the sanitary collection system adjacent to the bay. Finally, categorized by development, stations 1, 2 and 21 are undeveloped, stations 3 and 4 are affected only by older development, stations 5-14 are affected mostly by development that has occurred over the last 4 years and station 22 is a downstream control outside areas of direct effects of particulate carry-in. See Figures 1 and 2.

Field Methods

Samples for bacteriological analyses are collected in sterile, clean polypropylene bottles and stored with ice packs in the dark until transfer to a refrigerator in the laboratory. All samples are analyzed within 30 hours of collection. Samples for chemical analyses are collected in acid-washed polypropylene and polyethylene bottles of from 120 mL to 40L capacity. These are stored, protected from light, until analysis begins the same day in the laboratory. Dissolved oxygen, salinity, conductivity and temperature are measured with an Orion Model 85 calibrated for each use. Light is measured with Licor dataloggers and photometric sensors.
Field and laboratory pH are measured with meters from various manufacturers calibrated for each day of use.

**Figure 1. Sampling stations off La Parguera.**

**Figure 2. Photograph of a portion of La Parguera. Photo corresponds approximately to rectangle in Figure 1.**
Laboratory Methods

Bacteriological analyses for total coliform are performed according to Standard Methods, 19th Ed., Method 9222B. Fecal coliforms are positive subcultures of a representative portion of typical colonies that grow in EC broth under appropriate culture conditions. Enterococci have been analyzed using both multiple-tube (9230B) and membrane filter methods (9230C). Chlorophyll a is analyzed by fluorometry, utilizing an adaptation of Method 10200 H. Filtrable and non-filtrable solids are analyzed utilizing adaptations of Methods 2540B, D and E. Nutrients are analyzed utilizing: Method 4500-NH₃ F for ammonia, 4500-NO₃ E for nitrate-nitrite (corrected for nitrite), 4500-NO₂ B for nitrite and 4500-P E for phosphate. All results are reported as NOₓ-N and PO₄-P.

All glassware used is washed with 10% hydrochloric acid, rinsed repeatedly with tap and then with RO/DI, and dried completely before use. Whatman GF/F glass fiber filters (0.70 µm) are prepared by heating them at 500°C for 4 hours in a muffle furnace; they are then rinsed and dried for 24 hours. All material to be weighed was first placed in a dessicator for 6 hours then weighed to the nearest 0.00001 grams.

Chlorophyll a
All glassware used in this procedure must be acid free. This procedure is from Strickland and Parsons (1972). One liter of water is filtered through an ashed Whatman filter while working in the dark. The filters are immediately wrapped in aluminum foil and placed in the freezer. Samples are shipped to ANS frozen in an upright position. At ANS, samples are extracted from glass fiber filters in subdued light using 10 ml of 90% acetone. Samples are refrigerated overnight, approximately 18 hours, and centrifuged at 3000 rpm for 20 minutes. A clean Pasteur pipette is used to transfer approximately 9 ml of the solution to the fluorometer cuvette which is then analyzed using a Turner Designs TD-700 Fluorometer. Two drops of a 5% HCL solution are added and mixed. After two minutes, the sample is analyzed.

Turbidity, Total Suspended Solids and Organic Content
One liter of water from the sample carboy is filtered through each of three preweighed filters. The filters are placed in a petri dish, frozen and held until weighed. At weighing, the filters are dried to a constant weight and weighed, ashed at 450°C for 4 hours and reweighed. The particulate material is calculated by subtracting the preweight of the filter from the dried weight. The organic fraction is determined by subtracting the ashed weight from the dried weight. The material remaining on the filter constituted the inorganic fraction. Turbidity was also measured using a nephelometer (Standard Methods 2130B).

Nutrients
Nitrate, nitrite, ammonia, and phosphate analysis according to Strickland and Parsons (1972) at Interamerican University within twenty-four hours of their collection. A subsample is frozen and shipped to ANS for analysis and comparison.

Light Measurements
Light (photosynthetically active radiation, PAR) measurements were made at the surface and at one-foot intervals until bottom is reached. Light measurement units are µmol second⁻¹ m⁻².¹ The percent light reaching the bottom was determined as follows:

\[
\text{Percent Reaching Bottom} = \frac{\text{Bottom Measurement (LI-193SA)}}{\text{Top Measurement (LI-190SA)}} \times 100
\]

The extinction coefficient is the slope of the line \(\log_{10}(P/P)\) vs. depth in dms.

RESULTS

Bacteriological
For waters that are intensively used for bathing, Puerto Rico applies the US EPA bacteriological standard for Enterococcus. The standards for Enterococcus are no sample > 35 CFU/100 mL and no sample count > 75% of a one-sided confidence level based on sufficient samples or a confidence level of 0.7 otherwise. In fact, the

¹ 1 µmol/second-m² is 6.02*10¹⁷ photons.
regulation may be read as no sample count > 75% of the confidence level or no sample count > the running GM of the site plus 75% of the confidence level. We report both these variants. So, bacteriological standards are: Sample count > 35 (standard 1); sample count > sampling station GM + 75% one-sided confidence level (standard 2); and sample count > 75% sampling station one-sided confidence level (standard 3).

**By Station**

In our sampling one-way ANOVA for Enterococcus (geometric mean) vs. station and for Enterococcus vs. our onshore system categories were significantly different, p<0.001, df=8,144 and p<0.001, df=3,149, respectively. In all, stations monitored violated Enterococcus standards 186 times, with frequencies ranging from 0 to 20 times per station until February, 2000. These are summarized at Table 1. These violations are 21 for standard 1, 63 for standard 2 and 102 for standard 3. By station violations of standard 1 are shown at Figure 3. Violations by station for standard 1 are significantly different by $\chi^2$ (18.947, df=8, exact sig. 0.017) and by Cramer’s V (0.347, p= 0.015). By station violations of standard 2 are shown at Figure 4. Violations by station for standard 2 are not significantly different by $\chi^2$ (1.468, df=8, exact sig.= 0.994) and by Cramer’s V (0.0987, exact sig.= 0.994). By station violations of standard 3 are shown at Figure 5. Violations by station for standard 3 are not significantly different by $\chi^2$ (14.898, df=8, exact sig.= 0.058) and by Cramer’s V (0.316, exact sig.= 0.058).
By Development Category

These classifications are no development, old development, new development and downcurrent. For standard 1, violations are not significantly different by $\chi^2$ (3.584, df=3, exact sig.= 0.289) and by Cramer’s V (0.151, exact sig.= 0.289). For standard 2, violations are not significantly different by $\chi^2$ (0.219, df=3, exact sig.= 0.993) and by Cramer’s V (0.038, exact sig.= 0.993). For standard 3, violations are significantly different by $\chi^2$ (8.611, df=3, exact sig.= 0.034) and by Cramer’s V (0.240, exact sig.= 0.034). This is shown in Figure 6, and number of violations in Table 1.

<table>
<thead>
<tr>
<th>Station</th>
<th>Geometric Mean</th>
<th>Std Dev</th>
<th>n</th>
<th>Characteristic</th>
<th>Violations (std. 1, 2, 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.46</td>
<td>0.450</td>
<td>30</td>
<td>No development, mangroves</td>
<td>1, 9, 14</td>
</tr>
<tr>
<td>2</td>
<td>0.73</td>
<td>0.919</td>
<td>27</td>
<td>No development, no mangroves</td>
<td>5, 10, 12</td>
</tr>
<tr>
<td>3</td>
<td>0.62</td>
<td>0.501</td>
<td>27</td>
<td>Development, no mangroves</td>
<td>2, 10, 14</td>
</tr>
<tr>
<td>4</td>
<td>0.81</td>
<td>0.576</td>
<td>26</td>
<td>Development, mangroves</td>
<td>1, 8, 16</td>
</tr>
<tr>
<td>5</td>
<td>1.14</td>
<td>0.682</td>
<td>27</td>
<td>Development, mangroves</td>
<td>8, 11, 20</td>
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<tr>
<td>14</td>
<td>0.94</td>
<td>0.664</td>
<td>10</td>
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<td>2, 3, 4</td>
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<td>11</td>
<td>0.56</td>
<td>0.406</td>
<td>9</td>
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<td>0, 2, 2</td>
</tr>
<tr>
<td>6</td>
<td>0.77</td>
<td>0.526</td>
<td>27</td>
<td>No development, mangroves</td>
<td>1, 8, 18</td>
</tr>
<tr>
<td>22</td>
<td>0.46</td>
<td>0.608</td>
<td>10</td>
<td>No development, mangroves</td>
<td>1, 2, 2</td>
</tr>
</tbody>
</table>
Chemical and Physical

Of the parameters tested, chlorophyll a was significantly different by both station and development/on-shore system category, $F=3.802$, df=8,100, $p=0.001$ and $F=2.755$, df=3,105, $p=0.046$, respectively. Results by station are shown in Figure 7.

None of the nutrients (ammonia, nitrite, nitrate, nitrite-nitrate (i.e., cadmium-reduction results without separate nitrite determinations) and phosphate) were significantly different by either category. These are shown in Table 2. None of the nutrients (ammonia, nitrite, nitrate, nitrite-nitrate (i.e., cadmium-reduction results without separate nitrite determinations) and phosphate) were significantly different by either category. Data are shown in Table 3.

Total suspended solids were significantly different by station ($F=296.2$, df 8,92, $p<0.001$) but not by category. Results by station are shown at Figure 8. Turbidity was significantly different by station and by development category, $F=6.755$, df=8,93, $p<0.001$ and $F=10.863$, df=3,98 and $p<0.001$, respectively. The percent of incident light reaching the bottom was also significantly different by development category, though not by station, $F=3.944$, df=3,14, $p=0.031$, and is shown at Figure 9. The maximum depth is also shown at Figure 9 for purposes of comparison. The coefficient of extinction is also used to compare the transmissivity of marine waters for light. These are shown at Figure 10, and are significantly different by (by regression) development category, though not by station, $R^2=0.286$, $F=6.423$, df=1,13 and $p=0.022$.

Figure 7. Chlorophyll a by station.

![Figure 7. Chlorophyll a by station.](image)

Table 2. Chlorophyll a, Nitrogen and Phosphorus by station

<table>
<thead>
<tr>
<th>Station</th>
<th>Chlorophyll, mg/L</th>
<th>Nitrate-nitrite as N, mg/L</th>
<th>Phosphate as P, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std.Dev.</td>
<td>Mean</td>
</tr>
<tr>
<td>1</td>
<td>0.219</td>
<td>0.113</td>
<td>0.312</td>
</tr>
<tr>
<td>2</td>
<td>0.157</td>
<td>0.082</td>
<td>0.356</td>
</tr>
<tr>
<td>3</td>
<td>0.218</td>
<td>0.108</td>
<td>0.340</td>
</tr>
<tr>
<td>4</td>
<td>0.169</td>
<td>0.079</td>
<td>0.347</td>
</tr>
<tr>
<td>5</td>
<td>0.208</td>
<td>0.103</td>
<td>0.342</td>
</tr>
<tr>
<td>14</td>
<td>0.177</td>
<td>0.095</td>
<td>0.424</td>
</tr>
<tr>
<td>11</td>
<td>0.086</td>
<td>0.007</td>
<td>0.394</td>
</tr>
<tr>
<td>6</td>
<td>0.342</td>
<td>0.199</td>
<td>0.366</td>
</tr>
<tr>
<td>22</td>
<td>0.115</td>
<td>0.012</td>
<td>0.388</td>
</tr>
</tbody>
</table>
Table 3. Chlorophyll a, nitrogen and phosphorus by development category

<table>
<thead>
<tr>
<th>Development category</th>
<th>Chlorophyll, mg/L</th>
<th>Nitrite-nitrate as N, mg/L</th>
<th>Phosphate as P, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Std.Dev.</td>
<td>Mean</td>
</tr>
<tr>
<td>None</td>
<td>0.190</td>
<td>0.105</td>
<td>0.334</td>
</tr>
<tr>
<td>Old</td>
<td>0.193</td>
<td>0.094</td>
<td>0.342</td>
</tr>
<tr>
<td>New</td>
<td>0.260</td>
<td>0.169</td>
<td>0.362</td>
</tr>
<tr>
<td>Down-current</td>
<td>0.115</td>
<td>0.016</td>
<td>0.388</td>
</tr>
</tbody>
</table>

Figure 8. TSS by station.

Figure 9. Percent of insolation reaching bottom.
CONCLUSIONS

It is apparent that the parameters measured suggest that there are effects from onshore development, with stations down-current and those influenced by new development generally with higher concentrations of chemical and physical constituents and with higher Enterococcus counts. It is apparent that the sewage treatment plant does not contribute to all or even most of the differences noted, with the exception of chlorophyll $a$. The sewage plant discharge is located up-current of Station 6, and the only likely affected measurement reported here is chlorophyll. Chlorophyll has been suggested as a sensitive indicator of nutrient loadings (Dixon, 2000; Neely, 2000), but our data to date do not allow correlation. Chlorophyll-a measurements (2.5 µg/L) in the vicinity of the waste treatment facility were three times the level of other areas with similar topography and development. However, if we add a category for the sewage treatment plant, for light extinction coefficient for example, we can see that the difference from none and old development to new development is still significantly different, $t=-3.823$, $df=12$, and $p=0.002$. These data are shown at Figure 11. We can see, though, that the highest coefficients are still at the sewage treatment plant and the far downstream site. For the latter, we again note the possible or likely effect of the long-shore current that would tend to concentrate solutes and suspended material at or near that station.

During the third year sediment traps will be placed at Stations 1, 4, 5, 11, 14, and 6 based on the high total suspended solids at these stations. In addition, rain events greater than 0.25 inches will be targeted for instantaneous sampling. Further, the contribution of each of the watersheds identified will be estimated and verified by sampling during rain events and x-ray diffraction analyses of sediments will be employed to ascertain origin and constituents of those sediments.
Our preliminary analysis of water quality suggests areas adjacent to new development are significantly impacted compared to undisturbed habitats nearby. This has, in turn, impacted seagrass beds in the area, though data to that effect are not presented here.

ACKNOWLEDGEMENT

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BIBLIOGRAPH REFERENCES