

Bioluminescence Levels and Dinoflagellates Abundances at the Bioluminescent Bay Puerto Mosquito, Vieques, PR

Prepared by

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Introduction

Puerto Mosquito (PM) in Vieques is one of the three most important bioluminescent bays in Puerto Rico, which are characterized by high cell densities of the bioluminescent dinoflagellate *Pyrodinium bahamense* var. *bahamense*. The wind-driven water circulation patterns (Margalef, 1961; Seliger et al., 1971), nutrient conditions (Burkholder and Burkholder, 1958; Soler-Figueroa and Otero, 2014), shallow depths and water resident times (Margalef, 1957; Smayda, 1970) along with the fringing mangrove forest, have been proposed to be important features which helps in the maintenance of the bioluminescent organisms and other phytoplankton species inside these bays.

During the past months it has been reported that the observed bioluminescence at PM has diminished. As a result, a moratorium for visiting this bay was established by the *Department of Natural and Environmental Resources of Puerto Rico* (DNER) and visits were restricted only to Fridays, Saturdays, and Sundays from 6:00 pm to 12:00 am.

Representatives from various institutions (*DNER, Puerto Rico Environmental Quality Board - PREQB, The Vieques Conservation and Historical Trust - VCHT, Fish and Wildlife Service - FWS and Ecoeléctrica, LP*) met on May 23, 2014 with DNER Secretary, Carmen Guerrero, at the agency headquarters. During the meeting a request was made to the UPR scientists to conduct bioluminescence measurements at PM and to collected samples to determine the

dinoflagellate abundances (i.e. *Pyrodinium bahamense* and *Ceratium furca*) in order to assess the recovery of the bay to conditions pre-moratorium (normal dinoflagellate abundances and bioluminescence).

Bioluminescence measurements were conducted at Puerto Mosquito on June 11, 2014 and surface water samples were collected and preserved to evaluate the dinoflagellate abundances. Results from this study confirmed the low bioluminescence levels reported. Furthermore, *Pyrodinium bahamense* cell densities were low, while high abundances of *C. furca*, a non-bioluminescent dinoflagellate, were observed. The low precipitation regimes (and probably low nutrient concentrations) were suggested to be one of the causal factors for the decrease. A report was prepared and shared with *DNER*, *VCHT*, and other *UPR* scientists. In addition, a presentation with the results, as well as other relevant information on bioluminescence and BioBays, was given to the Vieques community and tour operators.

The following report presents the findings from a follow-up study conducted on July 11, 2014, a month after the first sampling schedule. Hence, the methods used are the same as the previous work.

Goals and Objectives

This study supports the efforts conducted at Puerto Mosquito by *DNER*. The specific objectives of this work were 1) to determine the actual bioluminescence levels at PM and compared them with the measurements observed in the previous study, and 2) to assess the extant dinoflagellate cell densities (i.e. *Pyrodinium bahamense* and *Ceratium furca*) at PM and compare them with the abundances observed in the previous study. This work provides useful information for the management of PM, as well as other bioluminescent systems.

Methods

Study Area

PM is located in the southwest coast of Vieques Island at 16 km of the east coast of Puerto Rico (18° 6' N; 65° 26' W). The sampling stations were selected in order to cover a wider area of the bay and to possibly evaluate different bioluminescence levels and dinoflagellate abundances (Figure 1). The exact position for each of the stations was assessed with a handheld GPS during the sampling day.



Figure 1. Sampling stations at Puerto Mosquito, Vieques, P.R. (Source: Google Earth)

Bioluminescence Measurements

Bioluminescence levels at PM were recorded on July 11, 2014 at six stations (S1-S6) with an *Underwater Bioluminescence Assessment Tool* (UBAT). Five of the stations were localized inside the bay and one station at the entrance. The bioluminescence measurements were recorded in all stations during three consecutive minutes. Averages were calculated for each of the stations and the bioluminescence levels are reported as photons $\text{sec}^{-1} \text{L}^{-1}$.

Pyrodinium bahamense and Ceratium furca cell densities

Field Work - Triplicates water samples were collected at each of the stations with 9 L carboys while the bioluminescence measurements were recorded. Samples were filtered through a 25 µm mesh, concentrated to 50 ml in polystyrene jars and preserved with buffered formalin (~1% final concentration) for later enumeration of cells.

Laboratory Work - The abundances of both dinoflagellate species were determined with a FlowCAM®, an imaging-based particle analyzer, using a 4x objective and a 200 µm Tygon flow cell. Filters previously created for BF were used to classify the organisms by species. At least 100 cells in each sample were counted in order to have a confidence limit of 95 % (Lund et al., 1958). Determination of cell densities were based on average cell counts calculated from triplicate samples and results are expressed as cells L⁻¹.

Environmental Parameters

Temperature (°C), salinity, dissolved oxygen (DO - mg L⁻¹), pH, and turbidity (NTU) were measured at each of the stations with an YSI probe (Model 6920, 650 Data Logger) while the bioluminescence measurements were recorded.

Meteorological Conditions

Precipitation data were obtain from a meteorological station located at approximately 3.8 km northeast of the bay (18°07'18"N 65°24'58"W). Data were accessed in the following address: www.raws.dri.edu

Statistical Analysis

Differences in the spatial variability of bioluminescence levels were evaluated with a One-Way Analysis of Variance (One-Way ANOVA). Since data were not normally distributed, the spatial variability of *P. bahamense* and *C. furca* cell densities were evaluated using a non-parametric Kruskal-Wallis Analyses of Variance (KW-ANOVA) and to compare the overall abundances of both dinoflagellates a Mann-Whitney U test was performed. A Spearman rank correlation was used to evaluate relationships between *P. bahamense* cell densities and bioluminescence levels. All statistical analyses were conducted using Sigma Plot 12.0.

Results

Environmental Parameters

Table 1 shows the average temperature, salinity, dissolved oxygen, pH, and turbidity recorded at Puerto Mosquito on July 11, 2014. The average temperature and salinity was 29.5 °C and 37.7, respectively. A high turbidity of 7.33 NTU was observed at S6 (the outer station), probably due to the presence of sand observed in the collected samples.

Table 1. Environmental parameters measured at Puerto Mosquito on July 11, 2014.

Station	Temperature (°C)	Salinity	Dissolved Oxygen (mg L ⁻¹)	pH	Turbidity (NTU)
1	29.33	38.28	6.06	8.11	2.73
2	29.60	37.71	6.73	8.15	2.63
3	29.75	37.94	6.78	8.14	3.17
4	29.67	38.08	6.74	8.12	2.57
5	29.78	37.69	6.88	8.14	3.17
6	29.12	36.42	6.11	8.11	7.33

Meteorological Conditions

Cumulative rain ten days prior the sampling day was 8.89 mm, with 7.11 mm recorded on the sampling day (Figure 2).

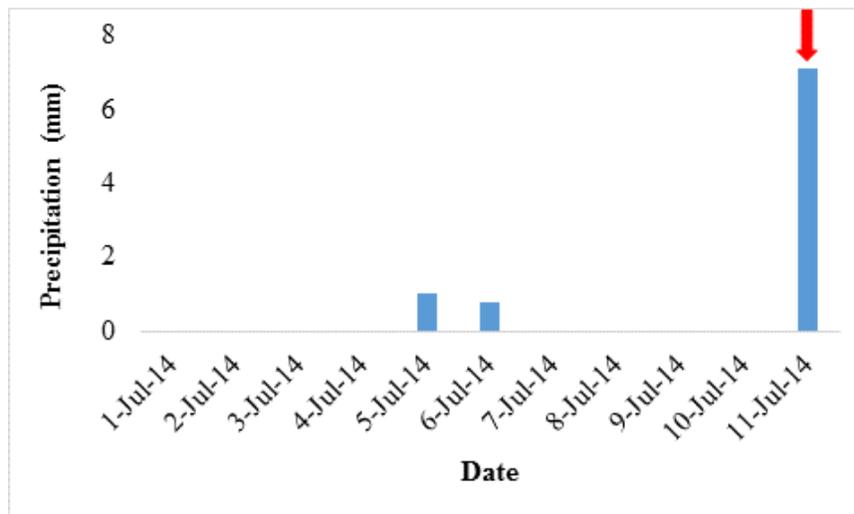


Figure 2. Cumulative precipitation ten days prior the sampling day (red arrow represents the sampling day).

Bioluminescence Levels

High bioluminescence levels were observed on July 11, 2014 with an average of $1.49 \times 10^{12} \pm 5.49 \times 10^{10}$ photons $\text{sec}^{-1} \text{L}^{-1}$, standard error (shown in the graph as 1.5E+12; Figure 3).

Differences among stations were statistically significant ($p < 0.001$). Higher levels were observed at S3 and S5 with $3.72 \times 10^{12} \pm 6.84 \times 10^{10}$ and $2.41 \times 10^{12} \pm 1.50 \times 10^{11}$ photons $\text{sec}^{-1} \text{L}^{-1}$, respectively. The lowest bioluminescence levels were observed at S6 with $6.40 \times 10^{10} \pm 1.92 \times 10^9$ photons $\text{sec}^{-1} \text{L}^{-1}$.

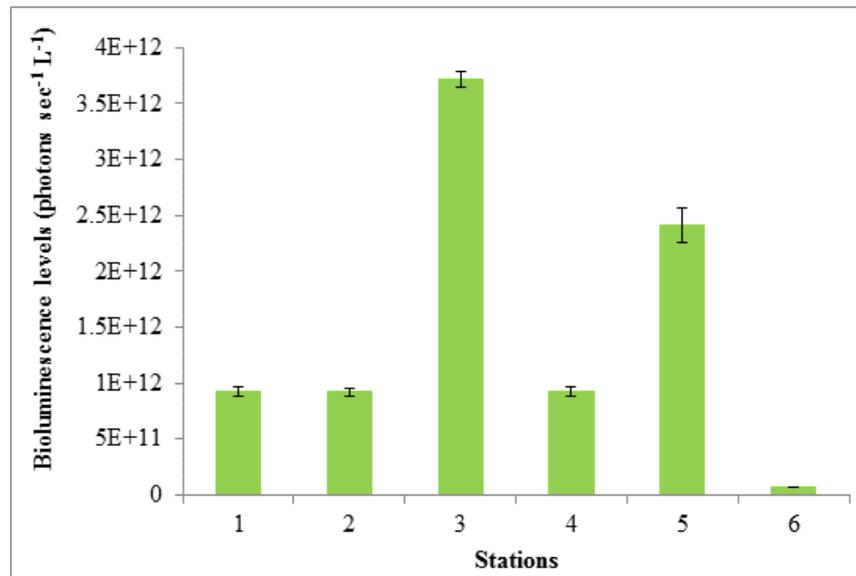


Figure 3. Bioluminescence levels recorded at Puerto Mosquito on July 11, 2014 (Bars represents standard errors).

Pyrodinium bahamense and Ceratium furca cell densities

Pyrodinium bahamense cell densities were higher than those of *C. furca* ($p < 0.001$), with averages of $46,700 \pm 3,780$ and $3,025 \pm 300$ cells L^{-1} , respectively (Figure 4). A spatial variability ($p < 0.01$) in the abundance of *P. bahamense* was observed, with a bloom condition (i.e. $\sim 100,000$ cells L^{-1}) at S3 and S5 (Figure 5). For *C. furca* ($p < 0.001$), the highest cell densities were observed at S3 and S4 with $7,744 \pm 1,038$ and $6,273 \pm 145$ cells L^{-1} , respectively (Figure 6).

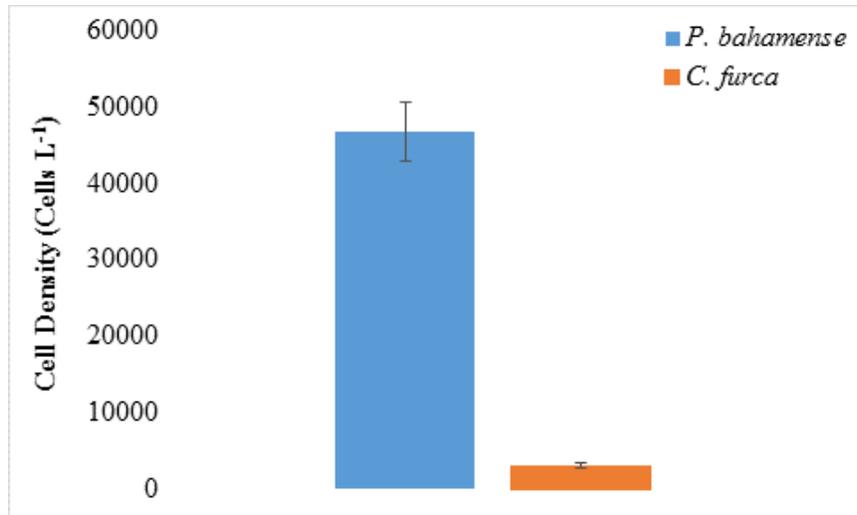


Figure 4. Overall cell densities of *P. bahamense* and *C. furca* at Puerto Mosquito on July 11, 2014 (Bars represents standard errors).

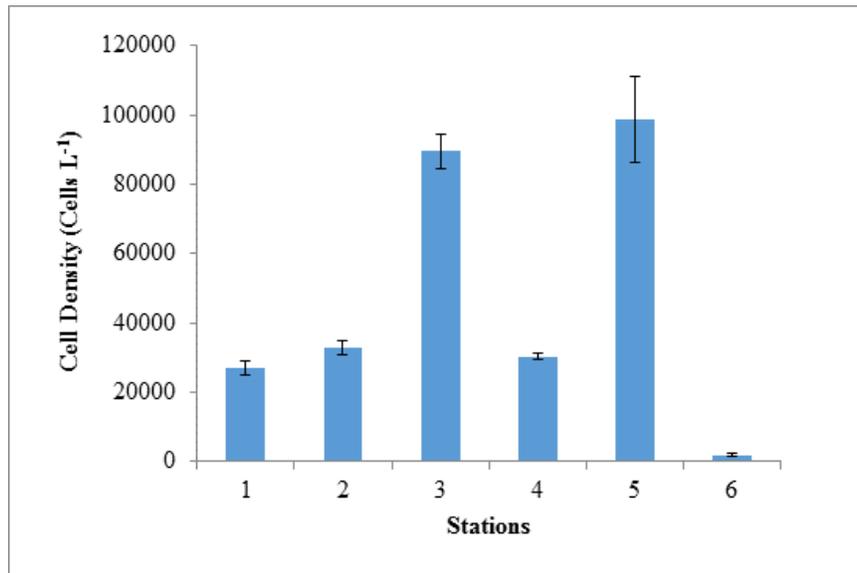


Figure 5. *Pyrodinium bahamense* cell densities observed at Puerto Mosquito on July 11, 2014 (Bars represents standard errors).

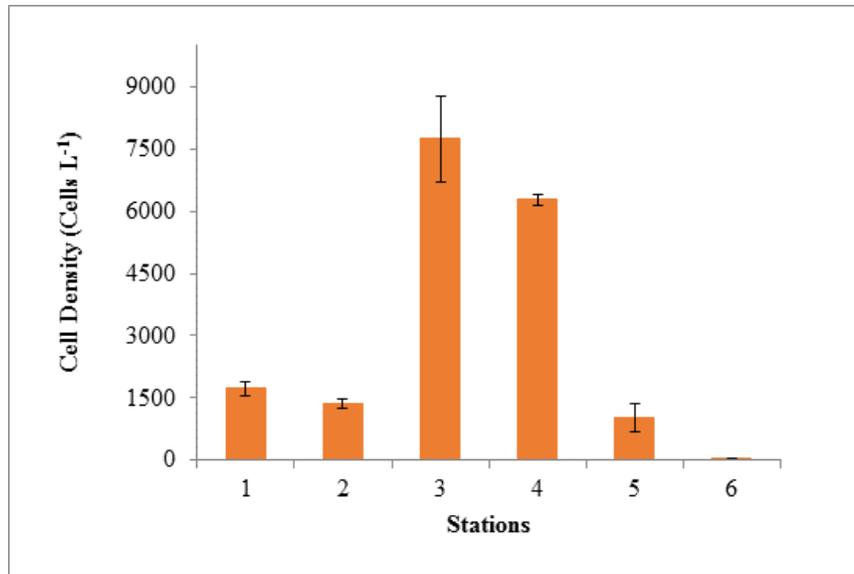


Figure 6. *Ceratium furca* cell densities observed at Puerto Mosquito on July 11, 2014 (Bars represents standard errors).

Correlations between P. bahamense cell densities and bioluminescence levels

A significant positive correlation was found between *P. bahamense* cell densities and the bioluminescence levels (Spearman Rank Correlation – $r = 0.86$, $N = 18$, $p < 0.001$; Figure 7).

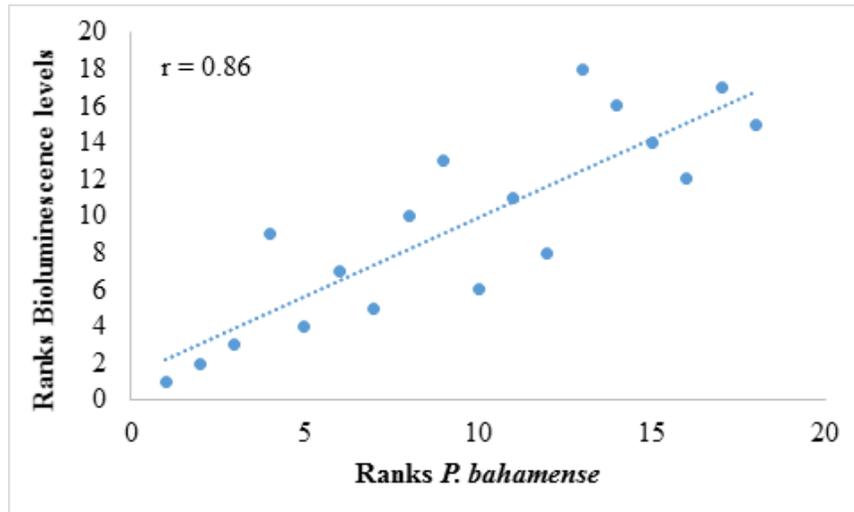


Figure 7. Spearman rank correlation between *P. bahamense* cell densities and bioluminescence levels ($p < 0.001$, $N = 18$).

Discussion

During the Puerto Mosquito sampling conducted on July 11, 2014 the bay was notably bright, and even the Full Moon condition do not opaque the visual perception of the light emitted. The bioluminescence measurements were in accordance with these observations, showing high bioluminescence levels and a concomitant bloom condition of *P. bahamense* (~ 100,000 cells L⁻¹), especially at S3 and S5. The strong positive correlation between *P. bahamense* cell densities and bioluminescence levels confirms that this organism was responsible for most of the bioluminescence observed. However, the occurrence of other bioluminescent dinoflagellates such as *Protoperidinium* spp. (data not shown), at those same stations, suggests their possible but minor contribution to the total bioluminescence budget.

The environmental variables measured during this and the previous study were similar, with only slightly higher salinities in the present study. Furthermore, no substantial differences were observed in the cumulative rain registered 10 days before the sampling days. While the bioluminescence levels and *P. bahamense* abundances observed in this study represents 25 and 60 times, those observed on our previous study; *C. furca* cell densities remain essentially the same. However, due to the short period (i.e. 1 sampling/month) of the studies conducted, the insufficiency in the environmental data acquired, and the scarcity of previous and continuous research, the factors associated with the increase in *P. bahamense* populations during this study remain unclear. For example, increases in the abundance of *P. bahamense* could be due to an increased in nutrient availability due to suspension of sediments by winds or due to the suspension of *P. bahamense* cysts. None of these data is available.

These studies confirm the inherent complexity of natural fluctuations of dinoflagellate abundances at this and other similar coastal bays, which may be due to the influence of environmental changes modulated by the interaction of climate forcing, nutrient cycling and trophic interactions. Phytoplankton organisms are subject to complex interactions among a wide range of environmental variables including nutrients (i.e. type, concentration and ratios), light intensity/water transparency, wind direction/intensity, water circulation patterns, and intensity/interval of pluviosity, among others. These factors and interactions, in conjunction with the short generation times (i.e. hours to days) of phytoplankton, has a profound influence on the

presence, abundances and community structure of aquatic systems including bioluminescent bays. Therefore, approaches that increase the resolution of phytoplankton sampling, assessment of taxonomic composition and monitoring of water quality are expected to yield vital information to develop a robust link between potential environmental change, whereas natural or anthropogenic, and the response of the planktonic community in these unique and economically important coastal systems.

Acknowledgments

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