

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.

Persons 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 in order to assess the recovery of the bay to conditions pre-moratorium (normal dinoflagellate abundances and bioluminescence).

Goals and Objectives

This study supports the efforts conducted at Puerto Mosquito by DNER and the PREQB. The specific objectives of this work were 1) to determine the actual bioluminescence levels at PM and compared them with baseline measurements observed in a previous study and to the levels observed at Bahía Fosforescente (BF) in La Parguera, 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 from previous studies and from the ones observed at BF. This work will provide information useful to for the management of PM, specifically in what is related to the present day DNER moratorium.

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 June 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 June 11, 2014. The highest temperatures and salinities were observed at S3 and the lowest at S6.

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

Station	Temperature (°C)	Salinity	Dissolved Oxygen (mg L⁻¹)	pH	Turbidity (NTU)
1	29.74	37.77	6.22	8.08	4.47
2	29.97	37.55	7.15	8.15	2.93
3	30.36	37.84	6.94	8.14	4.83
4	29.95	37.50	6.84	8.12	3.17
5	29.80	37.41	7.31	8.17	2.73
6	29.28	36.45	7.09	8.20	3.23

Meteorological Conditions

Cumulative precipitation, from January to June, for the years 2011, 2012, 2013, and 2014 was 477, 354, 376, and 277 mm, respectively (Figure 2). The cumulative rain ten days prior the sampling day was 11.18 mm (Figure 3), which represents about 4% of the cumulative rain observed for June 2014.

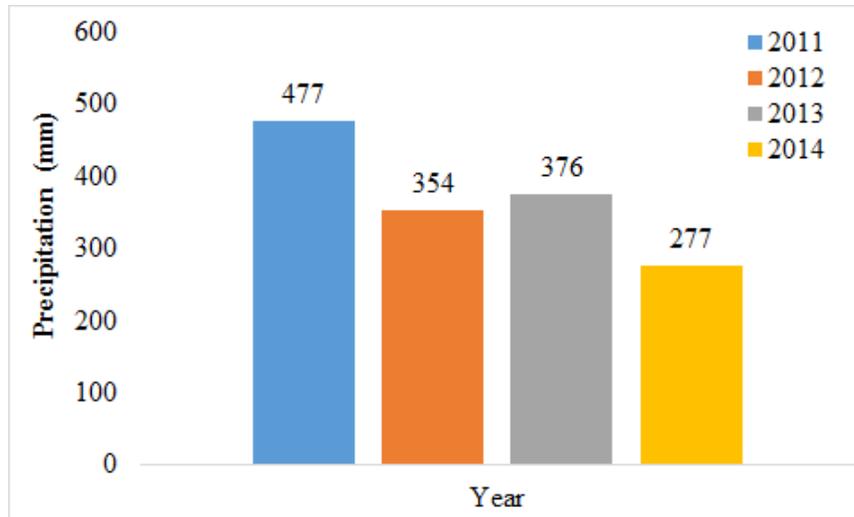


Figure 2. Cumulative precipitation from January to June for the years 2011, 2012, 2013, and 2014.

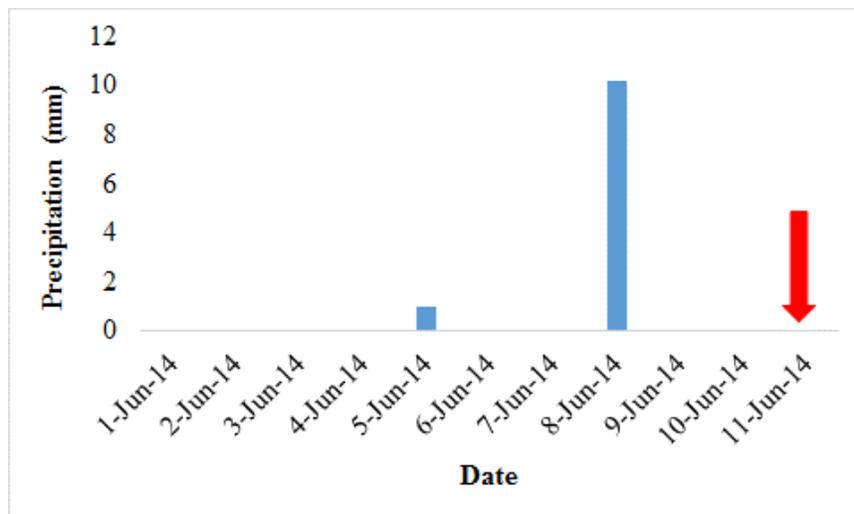


Figure 3. Cumulative precipitation ten days prior the sampling day (red arrow represents the sampling day).

Bioluminescence Levels

Figure 4 shows the bioluminescence levels recorded at PM on June 11, 2014. Significant differences in bioluminescence ($p < 0.001$) were found among the six sampling stations. Higher bioluminescence levels were recorded at S2 and S3 with 1.06×10^{11} (shown in the graph as $1.06\text{E}+11$) $\pm 1.32 \times 10^9$ photons $\text{sec}^{-1} \text{L}^{-1}$, standard error and $1.36 \times 10^{11} \pm 3.22 \times 10^9$ photons sec^{-1}

L⁻¹, respectively. The lowest bioluminescence levels were observed at S6 with $1.16 \times 10^9 \pm 5.13 \times 10^7$ photons sec⁻¹ L⁻¹.

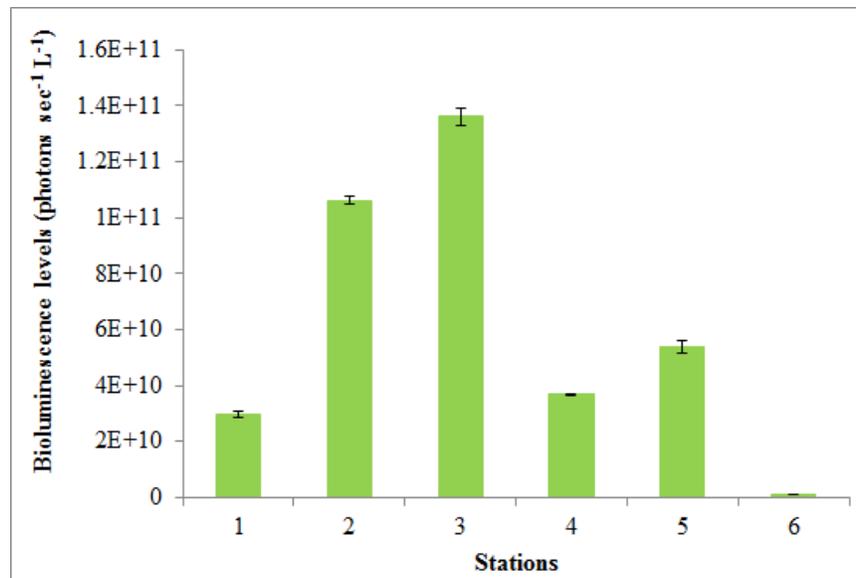


Figure 4. Bioluminescence levels recorded at Puerto Mosquito on June 11, 2014 (Bars represents standard errors).

***Pyrodinium bahamense* and *Ceratium furca* cell densities**

Significant differences ($p < 0.05$) were found in the cell densities of both dinoflagellates among the six sampling stations. *Pyrodinium bahamense* cell densities were higher at S2 and S3 with an average of $1,493 \pm 48$ and $1,782 \pm 416$ cells L⁻¹, respectively, while the lowest cell densities were observed at S6 with 27 ± 21 cells L⁻¹ (Figure 5). For *C. furca*, the highest cell densities were observed at S2 and S5 with $7,361 \pm 444$ and $4,270 \pm 191$ cells L⁻¹, respectively (Figure 6). Overall, the cell densities of *C. furca* were higher than those of *P. bahamense* ($p < 0.05$; Figure 7).

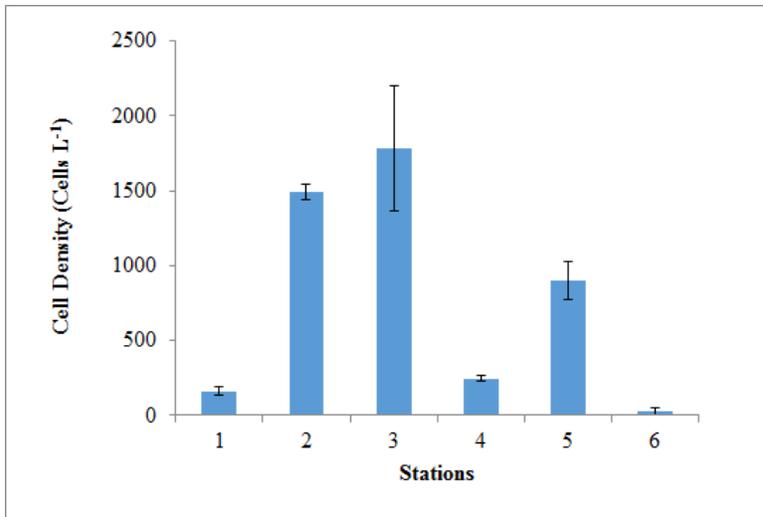


Figure 5. *Pyrodinium bahamense* cell densities observed at Puerto Mosquito on June 11, 2014 (Bars represents standard errors).

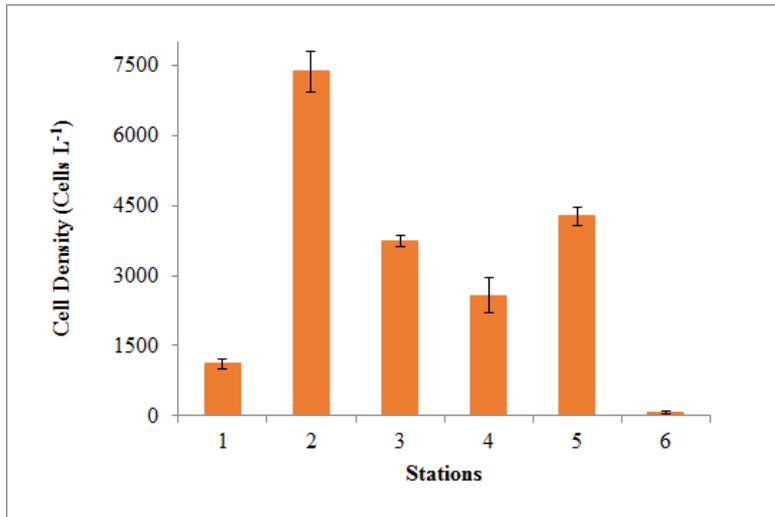


Figure 6. *Ceratium furca* cell densities observed at Puerto Mosquito on June 11, 2014 (Bars represents standard errors).

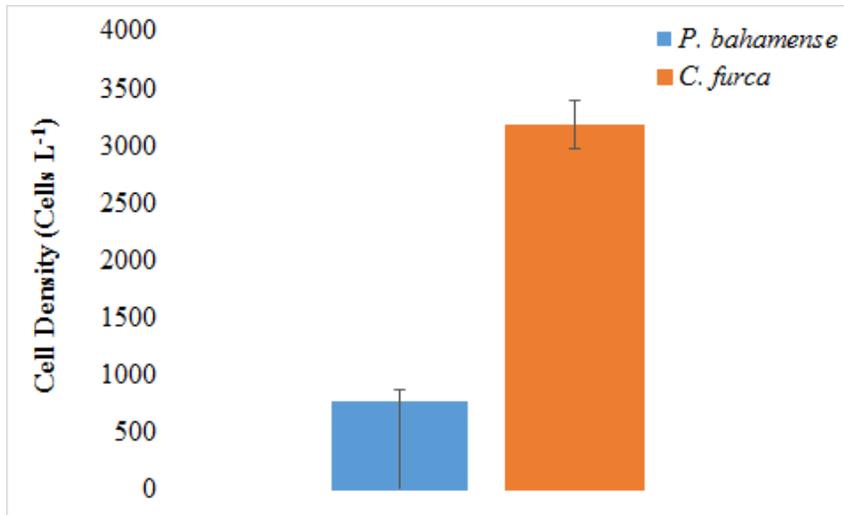


Figure 7. Overall cell densities of *P. bahamense* and *C. furca* at Puerto Mosquito on June 11, 2014 (Bars represents standard errors).

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 – $r = 0.96$, $N = 18$, $p < 0.01$; Figure 8).

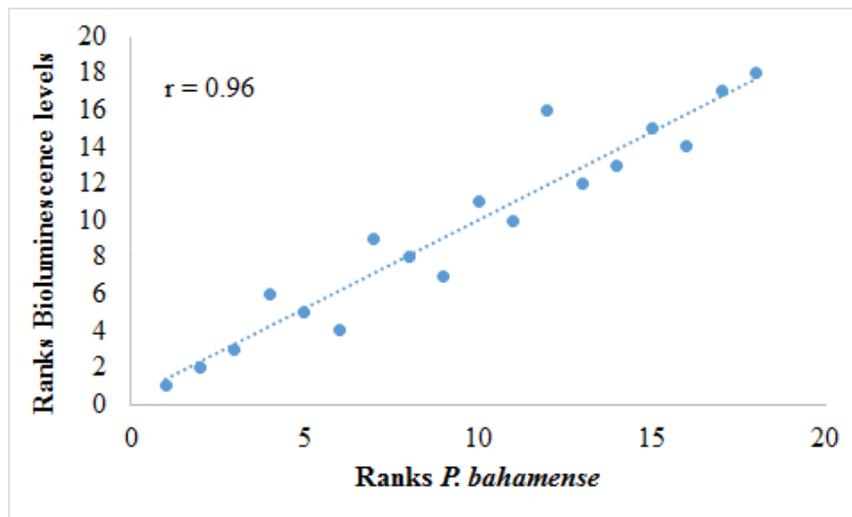


Figure 8. Spearman rank correlation between *P. bahamense* cell densities and bioluminescence levels ($p < 0.001$, $N = 18$).

Discussion

The temperatures and salinities observed during this study were similar to the ones observed in previous studies (Walker, 1997; Soler-Figueroa, 2006) and in others bioluminescent systems (i.e. Bahía Fosforescente - Soler-Figueroa, 2006; Cedeño-Maldonado, 2008; Soler-Figueroa and Otero, 2014). The temperatures observed during this study characterized these bays during the summer period, while the high salinities reflect the lower precipitation observed during 2014 until the sampling day. The cumulative rain from January to June for 2014 only represents 58, 78, and 74 % of the precipitation observed for the years 2011, 2012, and 2013, respectively.

The observed ranges in DO, pH and turbidity fell well between the Water Quality Standards for coastal class SB waters (category used by Latz 2011 studies)

(http://www2.pr.gov/agencias/jca/LeyesyReglamentos/Documents/Reglamentos/Water_Quality_Standards_Reg_2010.pdf – DO: > 5 mg L⁻¹; pH: 7.3 – 8.5; Turbidity: < 10 NTU) and are similar to values observed at Bahía Fosforescente (Soler-Figueroa and Otero, data to be published).

In general, the bioluminescence levels detected during this study were low, with an average of $6.06 \times 10^{10} \pm 1.35 \times 10^9$ photons sec⁻¹ L⁻¹. These low levels were expected, since *P. bahamense* cell densities were also low, with an average of 768 ± 108 cells L⁻¹. Low bioluminescence levels, similar to the ones observed during this study, were also previously reported for Puerto Mosquito by Latz 2011 (unpublished data provided by Mark Martin) and were related to the meteorological conditions observed.

A similar scenario have been also observed at Bahía Fosforescente during the dry season 2013, with bioluminescence levels of $2.54 \times 10^{10} \pm 3.04 \times 10^9$ photons sec⁻¹ L⁻¹ and *P. bahamense* cell densities of 14 ± 14 cells L⁻¹ (Soler-Figueroa and Otero, pers. com.). These low cell densities, observed during the dry season, are part of the natural fluctuations experienced by the system and have been attributed to low nutrient concentrations (Soler-Figueroa and Otero, 2014). At the same time, a shift towards higher abundances of *C. furca*, a non-bioluminescent mixotrophic dinoflagellate, occurs, probably due to its ability to prey on small organisms (i.e. ciliates and other microzooplankton) under low nutrient concentrations (Smalley et al., 2003; Baek et al., 2008). This suggests that the low bioluminescence levels observed at Puerto Mosquito, as well as

the *P. bahamense* cell densities, were probably the result of low nutrient concentrations expected during the low precipitation regimes observed. In addition, higher *C. furca* cell densities were observed at Puerto Mosquito, confirming its competitive advantage over *P. bahamense*, to thrive under low nutrient concentrations. The link of increased availability of nutrients due to suspension of sediments by winds is at present uncertain and thus should be evaluated in future work.

The strong significant positive correlation observed during this study between *P. bahamense* cell densities and the bioluminescence levels confirms that this organism was responsible for most of the bioluminescence observed and that the contribution of other bioluminescent organisms, such as *Protoperdinium* sp., was less prominent. A significant positive correlation between *P. bahamense* and *C. furca* (data not shown) suggests a system-wide accumulation mechanism that tends to concentrate the organisms in specific areas of the bay (i.e. S2, S3 and S5). In order to better understand the spatial variability of the organisms, the water circulation pattern of the system needs to be assessed by means of tracers, drogues or acoustic approaches.

In summary, it is suggested that the low bioluminescence levels and *P. bahamense* cell densities observed at Puerto Mosquito are part of the natural fluctuations of these systems and are the result of the actual weather conditions, i.e. low precipitation regimes and therefore, low nutrient concentrations. *Pyrodinium bahamense* is a photosynthetic organism that requires nutrients, and probably other humic/organic material, to grow and develop. Therefore, to better understand the presence/absence of this and other phytoplankton organisms at bioluminescent bays, concurrent and continuous measurements of inorganic/organic nutrients, colored dissolved organic matter (CDOM), and suspended particulate matter (SPM), among other water quality measurements, are vital. These studies should focus on the relationships of organisms with environmental conditions at a wide range of timescales encompassing days through months through years. In addition, to understand the dinoflagellate dynamics of the bioluminescent systems and their fate due to climate change, a more comprehensive short-term/long-term studies need to be assessed. Also, effective management and conservation practices need to be implemented in order to safeguard these rare and unique ecosystems.

Acknowledgments

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