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THE CONCENTRATION AND DISTRIBUTION OF BIOLUMINESCENT DINOFLAGELLATES IN VIEQUES, PUERTO RICO

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INTRODUCTION

The dinoflagellate *Pyrodinium bahamense* var. *bahamense* is chiefly responsible for the observed bioluminescence in Puerto Mosquito, Vieques, Puerto Rico. Concentrations of *P. bahamense* in Puerto Mosquito are reported to range from 20,000 to 150,000 organisms per liter (Seliger, 2001). Two bays, Bahia Tapon and Puerto Ferro, are located less than 10 km east of Puerto Mosquito. These two bays have not received the same study attention as Puerto Mosquito. In 2006-2007, seawater samples from all three bays were treated with a preservative, settled, and counted to determine concentrations per liter of water column dinoflagellates for each bay. The highest concentration found within Bahia Tapon is 150 dinoflagellates per liter. The highest concentration found in Puerto Ferro is 400 dinoflagellates per liter. Bahia Tapon and Puerto Ferro are protected by the Vieques National Wildlife Refuge. A sample from Puerto Mosquito yielded the highest concentration level of all three bays at 27,300 organisms per liter. Dinoflagellates were found in concentrations generally at least one order of magnitude, and usually two to three orders of magnitude, greater in Puerto Mosquito than the other two bays. Puerto Mosquito lies outside the VNWR boundaries. Given the known high concentrations of *P. bahamense* found within Puerto Mosquito, further studies must be undertaken to preserve this unique environment from future anthropogenic effects.

DINOFLAGELLATES

General

Dinoflagellates are small, mainly unicellular protists split fairly evenly between autotrophs and heterotrophs (Dodge, 1984). They are mostly microscopic but the largest dinoflagellate, Noctiluca, has a diameter of 2 mm. They are characterized by possessing two flagella; one transverse and one longitudinal. The transverse flagellum is usually contained in a groove, called the cingulum, located on the equator of the dinoflagellate (Spector, 1984). The transverse flagellum provides the dinoflagellate with forward motion and spin (Olney, 2004). The cingulum may divide the dinoflagellate into an upper, or epitheca, and lower, or hypotheca, portion. The longitudinal flagellum extends from the posterior of the cell. The longitudinal flagella provides some propulsive force but functions mainly as a steering mechanism (Olney, 2004). The motility of dinoflagellates allows them to migrate downwards in the water column during the night to access higher nutrient levels and then rise up during the day to photosynthesize (Olney, 2004).

Another characteristic of dinoflagellates is the theca, or rigid outer wall covering. Early classification was based upon the apparent absence or presence of theca, dividing dinoflagellates into armored and unarmored species, but detailed electron microscope studies found this distinction to be unclear and inconsistent (Dodge, 1984). Modern classification is still based on the theca, but focuses on the pattern, or tabulation, of the plates.

P. bahamense Identification

Individual *P. bahamense* organisms range in size from 30-60 microns (Wood, 1968, personal observations). SEM images reveal the plate tabulation and obvious apical horn (Fig. 1). A hand-drawn figure of *P. bahamense* from Plate, 1906 shows a spherical body with clear apical horn, one to two large spines, and epitheca and hypotheca of nearly equal size (Wood, 1968). Such high resolution features were less clear using light microscopy, but the spherical shape, plate junctions, and flagella are diagnostic of this species (Fig. 2).

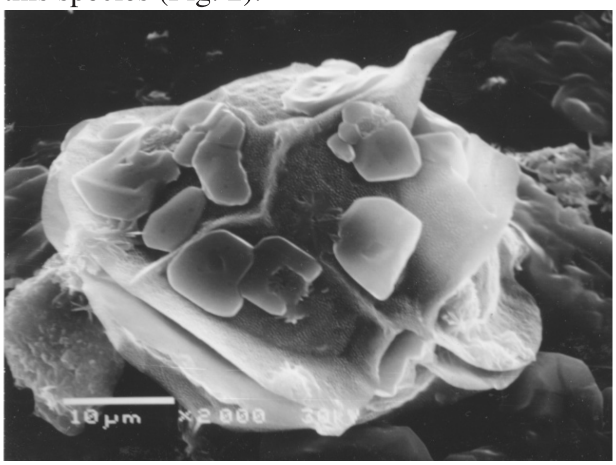

Figure 1. SEM image of *Pyrodinium bahamense*.

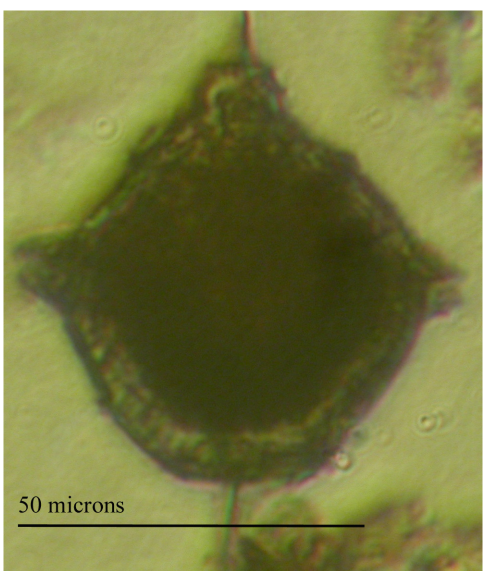

Figure 2. Inverted light microscope image of *Pyrodinium bahamense*.

BIOLUMINESCENT MECHANISM

Bioluminescent organisms have a vast phyletic distribution, with 17 phyla and at least 700 genera containing luminescent species (Thomson, 2001). In general, light is produced by the excitation of an electron to a higher energy level and the emission of a photon when the electron falls down to a lower energy level. In fluorescence and phosphorescence, an electron is excited by light energy. In bioluminescence, an electron is excited by energy produced through a chemical reaction within a living organism. This reaction involves the oxidation of a luciferin substrate, catalyzed by luciferase, producing an inactive oxyluciferin and light, or luciferin + O2 → oxyluciferin + light. There are five known luciferin structures, among all the phyla and genera containing luminescent species (Thomson, 2001). The luciferin found in dinoflagellates is thought to be derived from chlorophyll. Upon agitation, the above reaction proceeds within *P. bahamense* and produces a flash of light. Control factors, such as protein-binding components on the product side of the reaction, cause dinoflagellates to flash rather than glow (Loeblich, 1984). The exact reason dinoflagellates display bioluminescence is not certain, but defense and food attraction are two hypotheses.

METHODS

Data Acquisition

Seawater samples were collected at various depths within the three bays using a Van Dorn bottle apparatus. At most sites, 2,000 mL of seawater was collected to use for a variety of tests. Approximately 33 mL of seawater sample per site was used to preserve water column dinoflagellates. 1 mL of Lugols iodine preservative was pipetted into a 35 mL Nalgene bottle. This bottle was filled with approximately 33 mL of seawater sample within 3 hours of collection. In total, 59 samples were preserved; 27 samples from Puerto Mosquito, 21 from Puerto Ferro, and 11 from Bahia Tapon. An Utermohl settling device was used to settle the dinoflagellates. Each Utermohl chamber holds 25 mL but only 20 mL of sample was used per chamber. Therefore, 5 mL of DI water was added to each 25 mL settling chamber to fill the chamber and allow for proper settling. Each sample settled for 11 hours. The entire slide was counted using a 0.9mm square ocular on a Zeiss IM 35 inverted microscope at 100x magnification.

Data Analysis

The number of dinoflagellates counted per 20 mL of sample was converted to the number of dinoflagellates per liter and plotted in ArcGIS 9.1 using graduated bubble symbology and 12 classes in the Jenks natural breaks classification with a merged data table for all three bays. 29 sample sites total were plotted, with the average concentration used for sites with samples from multiple depths.

RESULTS

The concentration of dinoflagellates in Puerto Mosquito is significantly greater than that of Puerto Ferro or Bahia Tapon. Figures 3, 4, and 5, show plots of the dinoflagellate concentration per liter at 31 sampling locations in the three bays. Locations annotated with SSBP indicate sites where the bathyphotometer was deployed. The rest of the sites correspond to core locations where seawater was also collected. The highest concentration of dinoflagellates found in Puerto Mosquito is 27,300 organisms per liter. The lowest concentration in the Puerto Mosquito proper, that is not in the nearby mangrove swamp, is 100 in the bay mouth and 1,350 deeper within the bay. The highest and lowest concentrations for Puerto Ferro are 425 and 0, respectively. In Bahia Tapon, the highest concentration counted is 150 organisms per liter. Out of the 6 sampling sites in Bahia Tapon, 4 of the sample sites contained 0 dinoflagellates.

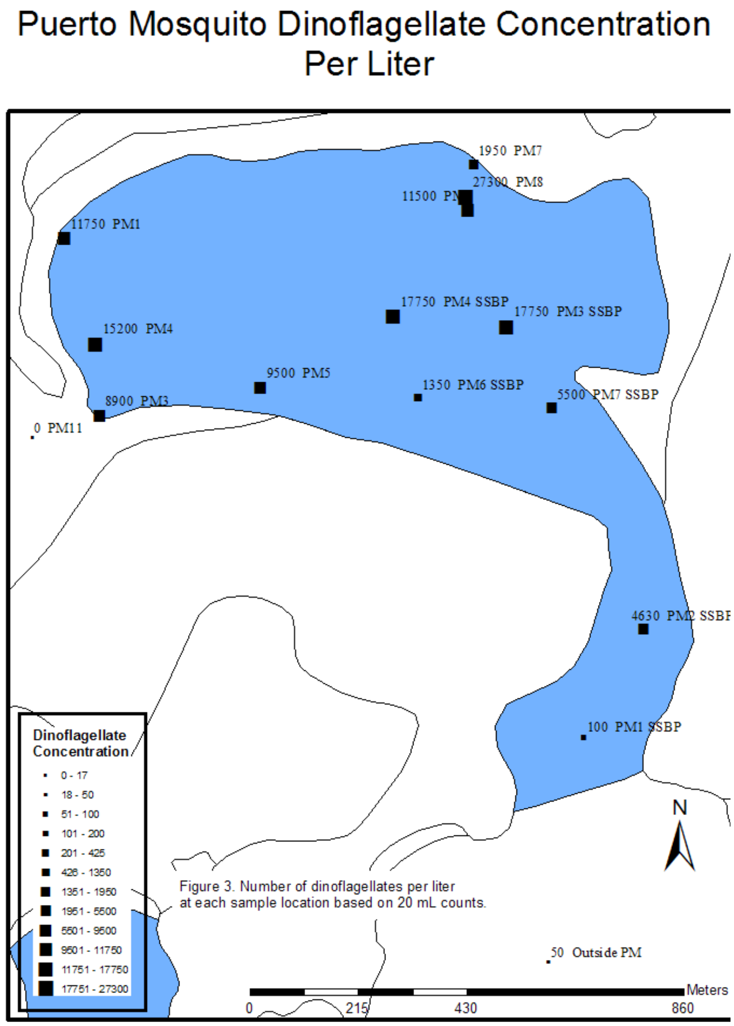

Figure 3. Number of dinoflagellates per liter at each sample location based on 20 mL counts.

DISCUSSION

The spatial arrangement of the dinoflagellate count data clearly displays lower concentrations of dinoflagellates in the bay mouths for Puerto Mosquito and Puerto Ferro in comparison with the rest of the bay. The mouth experiences more seawater flushing, which affects the pH, temperature, salinity, O_2, and nutrient budget of the mouth water, resulting in a lower abundance of dinoflagellates than the more stable interior of the bay. A total of 4 dinoflagellates were counted in Bahia Tapon; 3 dinoflagellates at BT4 and 1 dinoflagellate at BT1 SSBP. This data set is too small for accurate extrapolations to be made about the distribution of the dinoflagellates in this bay. Lying outside the VNWR, Puerto Mosquito is threatened by increasing development pressures.

The spectacular bioluminescence in Puerto Mosquito will attract more development, in all likelihood disrupting the environmental conditions necessary to sustain such high abundances of dinoflagellates. Further studies of this bay are necessary to obtain more data to preserve the unique Puerto Mosquito habitat.

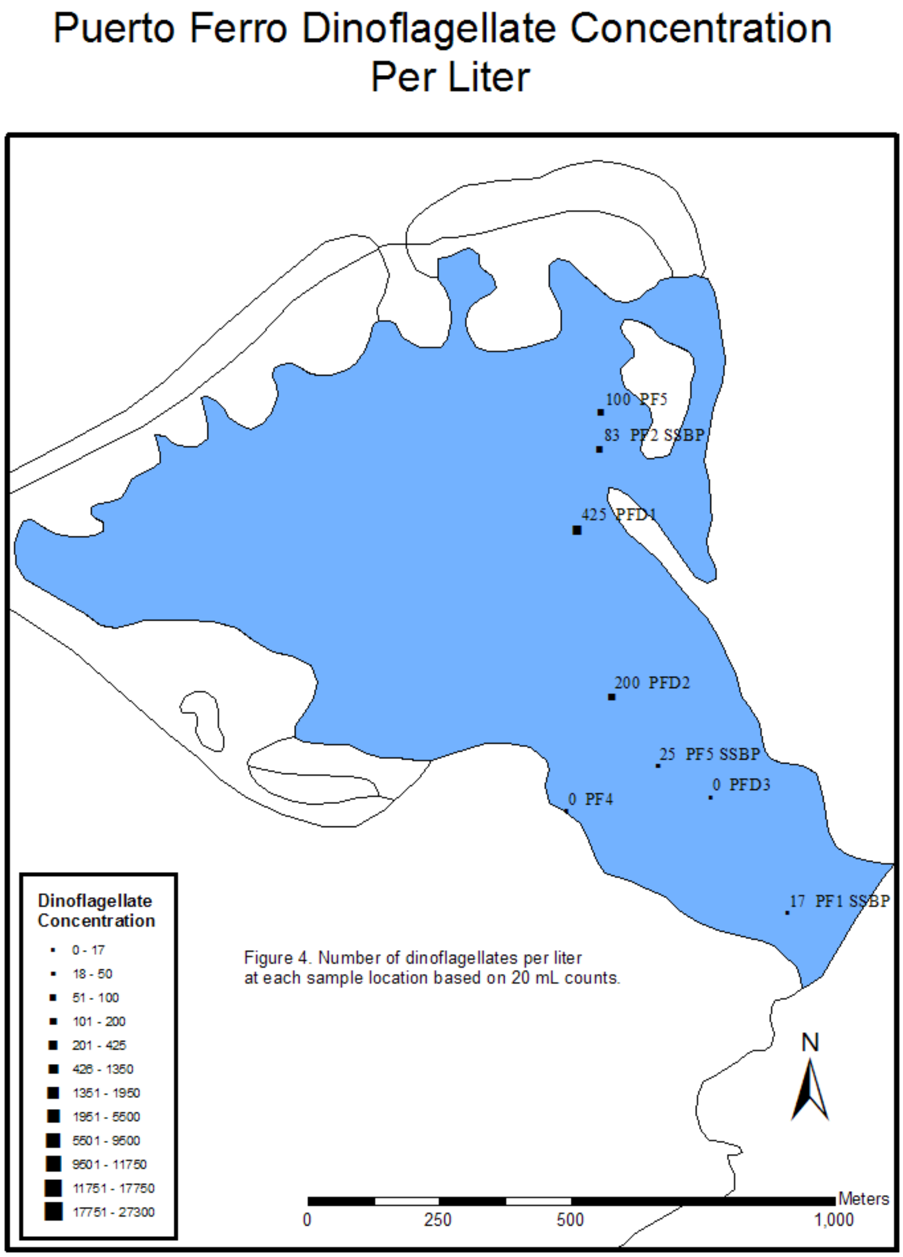

Figure 4. Number of dinoflagellates per liter at each sample location based on 20 mL counts.

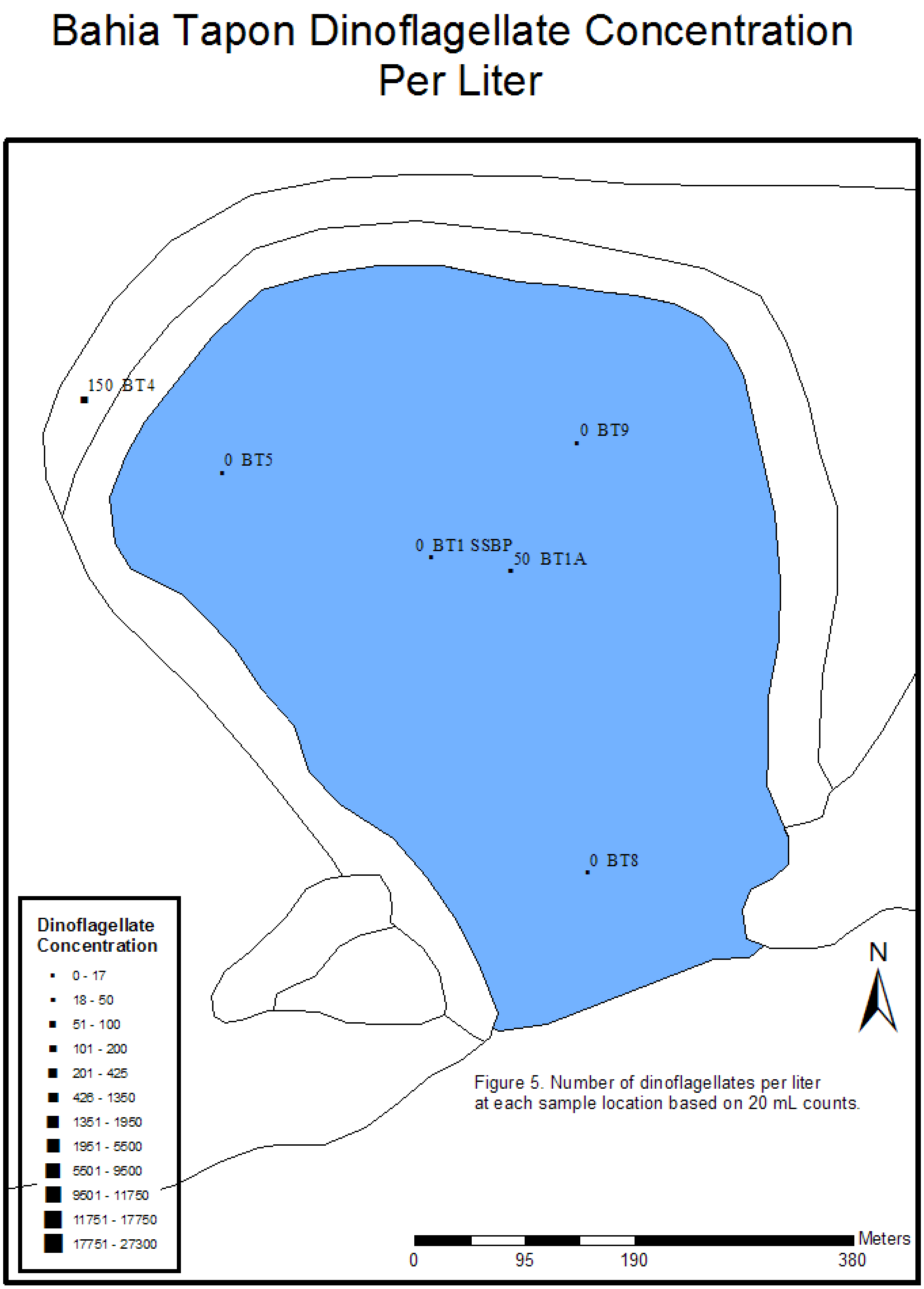

Figure 5. Number of dinoflagellates per liter at each sample location based on 20 mL counts.

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