Deciphering Bahía Fosforescente: New Approaches Towards the Understanding of this Unique Ecosystem

By

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Abstract

For decades, bioluminescent bays and lagoons have intrigued and puzzled researchers due to the accumulation of high cell densities of the bioluminescent dinoflagellate *Pyrodinium bahamense* var. *bahamense.* In recent years the general community have been concerned due to the perceived decrease in the abundance of this species and consequently the bioluminescence in one of the most important bioluminescent bay in Puerto Rico, Bahía Fosforescente. Additionally, temporal fluctuations with a non-bioluminescent dinoflagellate, *Ceratium furca* var. *hircus*, have been reported through the years. The lack of extensive and systematic monitoring studies over the past fifty years has been one of the major barriers in deciphering what mechanisms drives the fluctuations of species in this bay. The main goal of this research was to identify the links between the environmental and meteorological conditions, and the patterns of variability of *P. bahamense* and *C. furca* and the bioluminescence levels (BL) at Bahía Fosforescente. The specific objectives of this study were to: 1) determine the daily, spatial and seasonal variability of *P. bahamense* and *C. furca*, 2) identify the environmental factors that may drive the spatial and temporal (short-term and seasonal) fluctuations of these species, 3) determine the BL in the bay and to help evaluate the putative declining bioluminescence trend, and 4) identify the role of different environmental conditions on the BL. To achieve this, studies were conducted with a high temporal and spatial resolution and various environmental variables were measured (e.g. nutrients, dissolved organic matter fluorescence-DOMFl, chlorophyll, turbidity, pH, dissolved oxygen, salinity, temperature, wind speed and direction, rainfall). Furthermore, BL were recorded *in situ* with an *Underwater Bioluminescence Assessment Tool* (UBAT) and historical *P. bahamense* records were used to estimate BL and determine trends in bioluminescence over the years.

Results from each of the studies conducted in this dissertation underscore the importance of seasonal patterns as a significant factor modulating the abundance of *P. bahamense* and *C. furca*, and the BL at Bahía Fosforescente. Overall, seasonal changes in rainfall promoted variations in several environmental conditions, resulting in seasonal shifts in the abundance of each dinoflagellate in the system. *Pyrodinium bahamense* was the numerically dominant dinoflagellate during the wet season (average 2010: $7.8 \times 10^4 \pm 1.4 \times 10^4$ cells L^{-1} standard error; average 2012: $6.8 \times 10^3 \pm 6.5 \times 10^2$ cells L^{-1}), reaching in some occasions a bloom condition (i.e. 10^5 cells L^{-1}). In contrast, a shift towards high cell densities of *C. furca* was observed during the

dry season (average 2011: 1.2 x 10^4 ±2.8 10^3 cells L^{-1}; average 2013: 2.3 x 10^4 ±2.2 x 10^3 cells L^{-1}). The high cell densities of *P. bahamense* during the wet season appeared to be related to nutrient inputs (November 2010 - PO_4: 0.09-1.92 μmol L^{-1}; N+N: 0.34-7.67 μmol L^{-1}; NH_4^+: 2.9-29.4 μmol L^{-1}) and probably other land/watershed derived materials after rainfall events, which are important for the growth and bloom formation of this species. In contrast, the capacity of *C. furca* to prey over other planktonic organisms was suggested to be important to maintain their populations during periods of low nutrients concentrations (March 2011 - PO_4: 0.17– 0.5 μmol L^{-1}; N+N: 0.2–1.23 μmol L^{-1}; NH_4^+: 0.2-7.8 μmol L^{-1}) and a minor runoff influence. A multivariate analysis (i.e. Distance based redundancy analysis-dbRDA) further confirmed that the seasonal variability in the dinoflagellates composition was better explained by salinity, DOMFl, pH and silicates, environmental variables that are strongly modulate by pluviosity. Even though changes in the daily and spatial distribution of these organisms were also observed, each dinoflagellate maintained their seasonal dominance. Winds and wind-derived currents were suggested to be important in the short-term spatial fluctuations of both dinoflagellates, with the highest concentrations mainly observed in the north, northeast and southeast regions of the bay.

The seasonal shifts in the dinoflagellates composition were accompanied by changes in the BL. Maximum BL (average: 4.3 x 10^{11} ±4.9 x 10^{10} photons sec^{-1} L^{-1}) characterized the wet season and were related to high concentrations of *P. bahamense* (average: 2.5 x 10^4 ±6.3 x 10^3 cells L^{-1}). A two-fold reduction in BL relative to the wet season was observed during the dry season (average: 1.8 x 10^{11} ±3.1 x 10^{10} photons sec^{-1} L^{-1}), but peaks in bioluminescence during this period were associated to the presence of heterotrophic bioluminescent dinoflagellates such as *Protoperidinium* spp. (average: 1.4 x 10^3 ±6.4 x 10^2 cells L^{-1}). An evaluation of BL based on publications over the past fifty years showed variations, but not a declining trend, thus opposing the notion of dwindling BL at the bay and supporting the idea that as during past decades, the present conditions remain favorable for the accumulation of abundant populations of bioluminescent dinoflagellates.

Summarizing, results from these studies provide substantial evidence to support that weather and the resultant environmental conditions are the principal factor controlling the alternating abundances of *P. bahamense* and *C. furca* at Bahía Fosforescente. This represents a scientific advance in understanding the population dynamics of two potentially harmful dinoflagellates species in Bahía Fosforescente. Results show the importance of high-frequency

monitoring to understand better the role of environmental forcing on the populations densities of these fast fluctuating organisms, especially in the present times of global climate change. Thus, a well-established long-term monitoring program will provide the best science based management approach to guarantee the role of this environment as an important habitat for protected species and for tourism.

Resumen

Durante décadas, las bahías bioluminiscentes han intrigado y desconcertado a los investigadores debido a la acumulación de altas densidades celulares del dinoflagelado bioluminiscente *Pyrodinium bahamense* var. *bahamense*. En los últimos años, ha existido una preocupación en la comunidad en general debido a la disminución percibida en la abundancia de esta especie, y en consecuencia en la bioluminiscencia, en una de las bahías bioluminiscente más importante de Puerto Rico, Bahía Fosforescente. Adicionalmente, se han reportado, a través de los años, fluctuaciones temporales con el dinoflagelado no bioluminiscente *Ceratium furca* var. *hircus*. La falta de estudios extensos y monitoreos sistemáticos durante los últimos cincuenta años han sido uno de los principales obstáculos en descifrar los mecanismos que promueven las fluctuaciones de estas especies en la bahía. El objetivo principal de esta investigación fue identificar las relaciones entre las condiciones ambientales y meteorológicas y los patrones de variabilidad de *P. bahamense* y *C. furca* y los niveles de bioluminiscencia (BL - por sus siglas en inglés) en Bahía Fosforescente. Los objetivos específicos de este estudio fueron: 1) determinar la variabilidad diaria, espacial y estacional de *P. bahamense* y *C. furca*, 2) identificar los factores ambientales que promueven las fluctuaciones espaciales y temporales (diarias y estacionales) de ambos dinoflagelados, 3) determinar los BL en la bahía y evaluar la supuesta tendencia de disminución en la bioluminiscencia, e 4) identificar el rol de diferentes condiciones ambientales en los BL. Para lograr esto, se llevaron a cabo estudios con una alta resolución temporal y espacial y se midieron diversas variables ambientales (nutrientes, fluorescencia de la materia orgánica disuelta-DOMFl, clorofila, turbidez, pH, oxígeno disuelto, salinidad, temperatura, velocidad y dirección del viento, precipitación). Además, los BL se registraron *in situ* utilizando un batifotómetro conocido como UBAT (por sus siglas en inglés) y se utilizaron datos históricos de *P. bahamense* para estimar los BL y determinar tendencias en la bioluminiscencia durante los últimos años.

Los resultados de cada uno de los estudios realizados en esta disertación demostraron la importancia de los patrones estacionales como un factor significativo que modula la abundancia de *P. bahamense* y *C. furca* y los BL en Bahía Fosforescente. En general, los cambios estacionales en precipitación promovieron variaciones en diversas condiciones ambientales, lo cual resultó en cambios estacionales en la abundancia de cada dinoflagelado en el sistema. *Pyrodinium bahamense* fue el dinoflagelado numéricamente dominante durante la estación

húmeda (promedio 2010: 7.8 x 10^4 ±1.4 x 10^4 células L^{-1} error estándar; promedio 2012: 6.8 x 10^3 ±6.5 x 10^2 células L^{-1}), alcanzando en algunas ocasiones una condición de floración (10^5 células L^{-1}). Por otro lado, se observó un cambio hacia altas densidades celulares de *C. furca* durante la estación seca (promedio 2011: 1.2 x 10^4 ±2.8 10^3 células L^{-1}; promedio 2013: 2.3 x 10^4 ±2.2 x 10^3 células L^{-1}). Las altas densidades celulares de *P. bahamense* durante la estación húmeda estuvieron relacionadas con los aportes de nutrientes (noviembre 2010 - PO_4: 0.09-1.92 μmoles L^{-1}; N+N: 0.34-7.67 μmoles L^{-1}; NH_4^+: 2.9-29.4 μmoles L^{-1}) y, posiblemente con la entrada de otros materiales terrestres debido a las escorrentías causadas por la lluvia, los cuales son importantes para el crecimiento y las floraciones de esta especie. Por otro lado, se sugiere que la capacidad de *C. furca* de alimentarse sobre otros organismos planctónicos es uno de los factores principales que le permiten mantener sus poblaciones durante períodos donde las concentraciones de nutrientes son bajas (marzo 2011 - PO_4: 0.17– 0.5 μmoles L^{-1}; N+N: 0.2–1.23 μmoles L^{-1}; NH_4^+: 0.2-7.8 μmoles L^{-1}) y con una menor influencia de escorrentías. Un análisis multivariado (dbRDA, por sus siglas en inglés) confirmó además, que la variabilidad estacional en la composición de dinoflagelados se explica mejor por la salinidad, DOMFl, pH y silicatos, variables ambientales fuertemente moduladas por diferentes patrones en precipitación. A pesar de que también se observaron cambios en la distribución diaria y espacial de estos organismos, cada dinoflagelado mantuvo su dominancia estacional. Se sugiere que el viento y las corrientes resultantes son factores importantes relacionados a las fluctuaciones diarias y espaciales de ambos dinoflagelados, con las concentraciones más altas observadas principalmente en las regiones norte, noreste y sureste de la bahía.

Los cambios estacionales en la composición de dinoflagelados estuvieron acompañados por cambios en los BL. Niveles máximos en bioluminiscencia (promedio: 4.3 x 10^{11} ±4.9 x 10^{10} fotones seg^{-1} L^{-1}) caracterizaron a la estación húmeda y se relacionaron con altas concentraciones de *P. bahamense* (promedio: 2.5 x 10^4 ±6.3 x 103 células L^{-1}). Una disminución de dos veces en los BL, en relación con la estación húmeda, se observó durante la estación seca (promedio: 1.8 x 10^{11} ±3.1 x 10^{10} fotones seg^{-1} L^{-1}), pero picos en bioluminiscencia durante este período se asociaron a la presencia de otros dinoflagelados bioluminiscentes heterótrofos tales como *Protoperidinium* spp. (promedio: 1.4 x 10^3 ±6.4 x 10^2 células L^{-1}). Una evaluación de los BL basada en publicaciones de los últimos cincuenta años demostró variaciones, pero no una tendencia de disminución, oponiéndose de este modo a la noción de baja en los BL en la bahía y

apoyando la idea de que como durante las décadas pasadas, las condiciones actuales siguen siendo favorables para la acumulación de abundantes poblaciones de dinoflagelados bioluminiscentes.

En resumen, los resultados de estos estudios proporcionan evidencia sustancial para apoyar que el clima y las condiciones ambientales resultantes son el factor principal que controla la alternancia en la abundancia de *P. bahamense* y *C. furca* en la Bahía Fosforescente. Esto representa un avance científico en la comprensión de la dinámica poblacional de dos dinoflagelados potencialmente nocivos en la Bahía Fosforescente. También demuestra la importancia de hacer monitoreos de alta frecuencia para comprender la función de las condiciones ambientales en las poblaciones de estos organismos que fluctúan rápidamente, especialmente en esta época de cambio climático global. Por lo tanto, el establecimiento de un programa de monitoreo a largo plazo proporcionará el mejor enfoque de gestión, basado la ciencia, para garantizar el rol de este ecosistema como un hábitat importante para especies protegidas y para garantizar un turismo sustentable.

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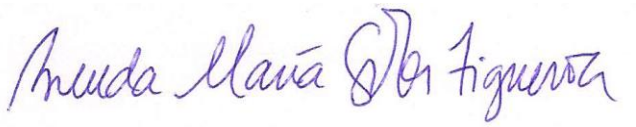

En una noche oscura. Grave el alma.
Por los cerrados cielos ni una estrella.
Un horizonte de betún. Adentro
sin forma o voz, un algo, una quimera.
En el silencio sin color se escucha
la ola espaciarse en la invisible arena.
Súbitamente se ilumina el ámbito.
Un repentino enjambre de luciérnagas,
un estrellado cielo en la bahía
prende a la vez sus mil fosforescencias.
Desde el fondo del mar salen a flote
algas de verdes ojos sirenas.
Y yo me arrojo al mar, ¡En luz me ahogo!
¡Insomne mar ardiente! ¡La Parguera!

Juan Antonio Corretjer

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who inspired my passion for the study of Bioluminescent Bays

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the Luminescence in my life

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...y vi estrellas en el mar.
Y mi corazón latió tan fuerte y se sintió tan vivo,
que jamás volvió a ser el mismo. –Anabakaena

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Chapter 1

General Introduction

Coastal marine ecosystems are under continuous pressure and experience alterations due to complex interactions between natural processes, human activities and climate change. Therefore, there is an urgent need to understand better how such pressures modify the normal functions of these ecosystems to establish effective management strategies. Bioluminescent Bays (BioBays) and lagoons, rare worldwide ecosystems, are an example of especially sensitive coastal systems for which the factors associated to their dynamic nature are poorly understood. A detailed examination of biological and environmental factors defining these systems should be a priority to local agencies and managers in order to safeguard the ecosystem services of these BioBays, such as habitat for protected species (i.e. dolphins and manatees), nursery for economically important species, tourism and recreation.

In Puerto Rico (PR), BioBays have intrigued and puzzled researchers for decades. These bays are characterized by the presence of high concentrations of the bioluminescent dinoflagellate *Pyrodinium bahamense* var. *bahamense* Plate (Margalef, 1957) that experience temporal and spatial fluctuations due probably to a combination of physical, chemical and biological factors (Seixas 1983, 1988; Walker, 1997; Soler-Figueroa, 2006). How these factors interact is not yet defined. Preliminary studies (Soler-Figueroa and Otero, 2011) suggest that low temporal resolution estimates of cell densities and nutrients should be interpreted carefully as daily changes encompass similar results, demonstrating the limitation of past low temporal resolution studies to identify the exact pattern of temporal fluctuations, underscoring the importance of increasing efforts to address short term observations to examine BioBays dynamics.

Similarly, although environmental parameters have been recognized as significant determinants of the abundance and distribution of these dinoflagellate species (Burkholder and Burkholder, 1958; Gold, 1965; Seliger et al., 1971; San Juan and González, 2000) limited studies are available that take a closer look to the relationship between the dynamic variations of these organisms and the fluctuations of environmental conditions at a wide range of timescales, encompassing days through months.

In recent years, special concerns have arisen in the scientific community, federal (i.e., *Fish and Wildlife Services* and *Sea Grant*), state (i.e. *Department of Environmental and Natural*

Resources of Puerto Rico) and private organizations (i.e., *Conservation Trust of Puerto Rico*), and the general public, related to apparent decreases in the observed bioluminescence at Bahía Fosforescente, La Parguera, a well-known tourist attraction on southwestern PR. Additionally, it was erroneously published that the entrance of the bay was widened and as a result the bioluminescent dinoflagellates disappeared from this bay (Dybas 2011). However, such decreases in bioluminescence are related to fluctuations in dinoflagellate abundances and, on some occasions, *Ceratium furca* var. *hircus* which is not bioluminescent (Sweeney 1981; Valiadi et al. 2014), comes to dominate the phytoplankton community (Seixas 1988; Walker 1997; Soler-Figueroa 2006). These shifts in the dinoflagellate species dominance could be considered an index of change of coastal ecosystem health triggered by increased boat traffic and associated pollution, and changes in watershed management trends and nutrient regimes. Yet, no systematic monitoring programs have been conducted in this bay to decipher the underlying mechanisms driving the fluctuations in the abundance of *P. bahamense* and *C. furca* and the presumed drop in bioluminescence has never been quantified.

Considering the importance of this bay for tourism, fisheries, endangered species, as a natural laboratory and for recreational and educational activities; and given the present and future climate-related changes as well as the constantly anthropogenic pressure that the bay has been experiencing through the years, it is essential to get a better understanding of the processes that control the dinoflagellate populations in the bay, with special emphasis on the bioluminescent dinoflagellate *P. bahamense*. Efforts described herein seek to study issues for which lack of information were underscored by academic sectors in PR (PR Sea Grant, 2011; http://seagrantpr.org/caribbean/sessions/academia-focus-on-conservation). These include monitoring of the dinoflagellate dynamics in bioluminescence bays and monitoring watershed processes and weather patterns. These efforts will provide watershed/coastal management support. The central issue in this study is to explore what causes the variations in the population densities of the dinoflagellates *P. bahamense* and *C. furca* in Bahía Fosforescente. Important scientific questions that need to be addressed include: 1) How do *P. bahamense* and C. *furca* populations fluctuates over time scales of days, weeks and seasons? 2) How meteorological and other environmental conditions regulate the presence and abundances of *P. bahamense*, and *C. furca*? 3) What are the actual bioluminescence levels and how these are modified by different

environmental conditions? In order to address these questions and the paucity of information available the objectives of this study are:

Objective 1: To determine the daily, spatial and seasonal variability of *P. bahamense* and *C. furca* at Bahía Fosforescente.

Objective 2: To identify the environmental factors that may drive the spatial and temporal (short-term and seasonal) fluctuations of these species.

Objective 3: To determine the actual bioluminescence levels at Bahía Fosforescente and evaluate the putative declining bioluminescence trend.

Objective 4: To identify the role of different environmental conditions on the bioluminescence levels at Bahía Fosforescente.

This dissertation is written in a manuscript format and consists of three main chapters each with its own abstract, introduction, materials and methods, results and discussion. The study performed in chapter two examines how short timescale changes in the environment influences the alternating abundance of *P. bahamense* and *C. furca* in Bahía Fosforescente, and determines if the fluctuations in the abundance are related to differences associated with seasonality. The main objective of the study was to explore the spatial and temporal (daily and seasonal) variability in the population densities of both dinoflagellates in the bay and evaluated the possible relationships between these species and various environmental cues. In the third chapter, the role of seasonality and environmental changes in the population dynamics of *P. bahamense* and *C. furca* was further analyzed by expanding the sampling efforts and including a larger array of environmental variables. The study describes the patterns of variability in *P. bahamense* and *C. furca* abundances at Bahía Fosforescente and identifies the underlying mechanisms behind those patterns. In chapter four bioluminescent measurements were conducted at Bahía Fosforescente, after more than fifty years (Clarke and Breslau 1960), in order to evaluate the bioluminescence trends at the bay. This study examines if seasonality and different precipitation regimes influence the dinoflagellate composition and how these changes are reflected in bioluminescence levels. Additionally, the temporal (short-term and seasonal) and spatial patterns in bioluminescence levels, and the possible relationships with the dinoflagellate composition were evaluated. The final chapter of this dissertation (Chapter 5) includes a general conclusion and provides future directions and management implications of the work.

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The Influence of Rain Regimes and Nutrient Loading on the Abundance of Two Dinoflagellate Species in a Tropical Bioluminescent Bay, Bahía Fosforescente, La Parguera, Puerto Rico

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Abstract *Pyrodinium bahamense* (bioluminescent) and *Ceratium furca* (non-bioluminescent), two potentially harmful algal bloom species (HABs), are the most abundant dinoflagellates in the tropical bioluminescent bay Bahía Fosforescente. Reductions in bioluminescence have been attributed to fluctuations in their abundances. It is unclear how short-term changes in the environment influence these fluctuations and how seasonality regulates their presence and abundance. Thus, temperature, salinity, nutrient concentrations, rainfall, wind velocity and direction, and counts of both dinoflagellates were examined at six stations, during five consecutive days, and during two climate extremes (i.e., the wet and dry seasons). During the wet season, *P. bahamense* abundances surpassed those of *C. furca*, reaching bloom conditions. Positive correlations were found between *P. bahamense* abundance, temperature, and salinity. The higher phosphates and nitrites+nitrates concentrations (PO_4^-, 0.09–1.92 μmol L^{-1}; N+N, 0.34–7.67 μmol L^{-1}) recorded during this season and their negative correlations with *P. bahamense* cell densities suggest efficient nutrient uptake and indicate that watershed-derived nutrients were important for the formation of blooms. *C. furca* cell densities were higher during the dry season, when nutrient concentrations were low (PO_4^-, 0.17–0.5 μmol L^{-1}; N+N, 0.2–1.23 μmol L^{-1}). Physiological characteristics and adaptive strategies of *C. furca*, such as mixotrophy, osmotrophy, and luxury consumption, may explain the higher abundances of this species under low-nutrient conditions. The spatial distribution of both organisms was similar during both seasons with higher cell densities in the east/southeast regions of the bay. This study provides new insights on the ecology of Bahía Fosforescente that are important for its management and broadens the knowledge on the factors that regulate the presence and abundance of two potentially HABs species.

Keywords Bioluminescent bays · *Ceratium furca* · Harmful algal blooms (HABs) · Nutrients · *Pyrodinium bahamense*

Introduction

Bahía Fosforescente, a bioluminescent bay in the southwest coast of Puerto Rico, is an ideal site to study how changes in the environment modify the phytoplankton composition, resulting in some occasions in the formation of dinoflagellate blooms. This bay is characterized by the presence of high concentrations of the bioluminescent dinoflagellate *Pyrodinium bahamense* var. *bahamense* (Plate 1906) among other phytoplankton, primarily dinoflagellates (Margalef 1957).

Previous studies reported a drop in the observed bioluminescence attributable to reductions in the abundance of *P. bahamense*, and these changes were associated with high cell densities of *Ceratium furca* var. *hircus* (recently named *Tripos furca* var. *hircus* (Schröd 1909); Gómez 2013), a non-bioluminescent dinoflagellate (Seixas 1988; Walker 1997; Soler-Figueroa 2006). The alternations between these organisms probably stem from a combination of physical, chemical, and biological factors and processes relevant to daily timescales; thus, monthly examinations of the dynamics of these organisms in the bay have been inadequate to understand these fluctuations and the responses of the organisms to environmental cues.

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The small size, nutritional requirements, rapid nutrient uptake and growth rates, and their vulnerability to grazing make phytoplankton susceptible to population fluctuations in response to environmental variability (Reynolds 1984). The generation times of phytoplankton range from hours to days; therefore, any change in the environment may lead to short-term changes in their growth patterns. Planktonic organisms in the bay may experience wide fluctuations in environmental factors, such as salinity and nutrients, especially during rainfall events. These temporal environmental shifts are more prominent at Bahía Fosforescente due to its small volume, narrow entrance, shallow depth, and low flushing rates (Margalef 1961; Seliger et al. 1971). However, it is unknown whether the variability in the abundance of *P. bahamense* and *C. furca* is related to different climate extremes (i.e., wet vs dry seasons). Thus, the main objective of this work was to examine how short timescale changes in the environment influence the alternating abundance of both dinoflagellates in Bahía Fosforescente and to determine if the fluctuations in the abundance are related to differences associated with seasonality. The study explores the spatial and temporal (daily and seasonal) variability of *P. bahamense* and *C. furca* in the Bahía Fosforescente and evaluates the possible relationships between these organisms and various environmental cues.

The bioluminescence displayed by *P. bahamense* has made this bay an economically important tourist attraction, and thus, understanding the relationship between this species and environmental patterns is important for effective management of the bay. In addition, since *P. bahamense* and *C. furca* have been categorized as harmful algal bloom species (HABs) in other coastal areas due to the presence of saxitoxin (Landsberg et al. 2006) and anoxia red tide-related events (Machida et al. 1999 in Baek et al. 2008a, b, c) respectively, results from this study contribute to the knowledge on the factors that regulate their presence and abundance.

Methods

Study Site

Bahía Fosforescente (Fig. 1) is located 3.2 km east of La Parguera, Lajas on the southwest coast of Puerto Rico (17° 58′ 30″ N; 67° 01′ 10″ W). The estimated area of the bay is 0.19 km^2 with an average depth of 3.5 m. The bay is of irregular shape, and its outlet to the coastal ocean is narrow (~100–150 m wide) and shallow. Although no river discharges into the bay, the infrequent watershed surface water flow is transported into the bay via three northern channels. Another potential source of surface water flow is from the *Rhizophora mangle* fringing forest that separates the bay waters from the coastal salt flats. The bay is characterized by high evaporation rates due to the arid conditions that prevail on the southwest coast of Puerto Rico (Margalef 1961).

Field Work

Water samples from six stations (referred to in the text as S1 to S6) were collected at ca. 0.1 m below the surface during five consecutive days during the wet (6–10 November 2010) and dry (12–16 March 2011) seasons, which were characterized based on local climatology (www.sercc.com/cgi-bin/sercc/cliMAIN.pl?pr5693). Triplicates of 7.7-L water samples were collected between 0800 and 0930 h using 9-L carboys. Each of the triplicates was filtered through a 25-μm mesh, concentrated to 50 ml and preserved with formalin (ca. 1 % final concentration) for later enumeration of *P. bahamense* and *C. furca*. In addition, duplicate water samples at each station were collected in 500-mL acid-washed polyethylene bottles for nutrient analysis. These water samples were kept frozen (−5 °C) until analysis. In situ temperature and salinity were determined at each station with a handheld thermometer and refractometer (Vee Gee), respectively.

Determination of *Pyrodinium bahamense* and *Ceratium furca* Cell Densities

Cell densities of *P. bahamense* and *C. furca* were determined using a Sedgewick-Rafter counting chamber on a CK40 inverted microscope (Olympus, Inc.) at 100× total magnification. Prior to cell counts, the volume of sample concentrates was adjusted when necessary to minimize cell clustering. These were gently homogenized, and two 1-ml aliquots (pseudoreplicates) for each of the triplicates were counted and averaged. Cell densities at each station are reported as the overall grand mean (±standard error) of the above averages.

Nutrient Analysis

Analyses for phosphate, nitrite + nitrate (N + N), and silicate concentrations were conducted according to the method of Parsons et al. (1984) using a UV-1601 double-beam spectrophotometer (Shimadzu). Ammonium concentrations were estimated immediately upon arrival to the laboratory using a Trilogy benchtop fluorometer (Turner Designs) according to the method of Holmes et al. (1999).

Meteorological Data

Precipitation and wind speed and direction data were obtained from a meteorological station maintained by the Department of Marine Sciences Bio-optical Oceanography Laboratory at Magueyes Island (http://bio-optics.uprm.edu/weather.html) located 3.2 km west of the sampling site.

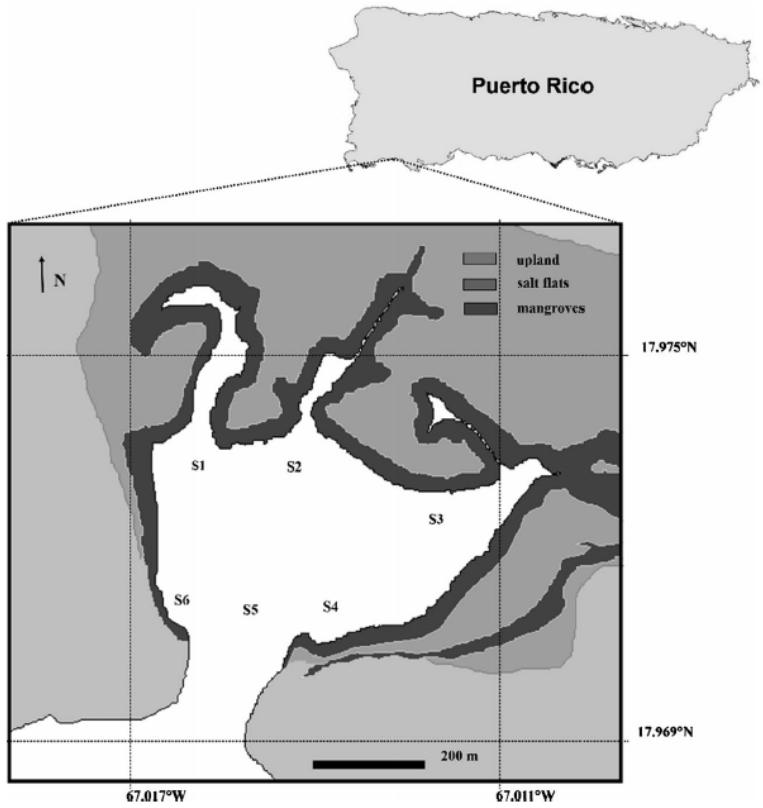

Fig. 1 Sampling stations at Bahía Fosforescente, La Parguera, Puerto Rico

Statistical Analysis

Since the data were not normally distributed, a series of non-parametric analyses were performed. Differences in daily and spatial variability of *P. bahamense* and *C. furca* cell densities were evaluated using a Kruskal-Wallis ANOVA (KW-ANOVA), while seasonal variability (i.e., dry and wet season) was evaluated using a Mann-Whitney *U* test. A Spearman rank correlation was used to evaluate relationships between cell densities and the physicochemical parameters. All statistical analyses were conducted using Sigma Plot 11.0.

Results

Environmental Parameters

The 1-week cumulative rain amount prior to sampling (1WR) during the wet season was 54.61 mm, while 12.95 mm of rain was registered during the dry season (Fig. 2). Surface water temperatures during the wet season averaged 28±0.16 °C, standard error, while salinity averaged 29±0.85 (Table 1). A peak in pluviosity (38.4 mm of rainfall) marked the first sampling day during the wet season. This peak was associated with lower temperatures and salinities at all stations (Table 1). The lowest salinity (15) at the time was observed at S1 in conjunction with the appearance of brown water coloration. During the dry season, the average surface water temperature was slightly lower than that during the wet season (27± 0.06 °C, Table 2). Precipitation was low 1 week prior to the dry season sampling, and this low level of rainfall resulted in a higher average surface water salinity (35±0.07, Table 2). The average wind speed during the wet season was 2.06± 0.22 m s^{-1}, and the wind direction was generally east-northeast (ENE). During the dry season, the prevailing wind pattern differed from that of the wet season sampling period with an average wind speed of 0.54±0.04 m s^{-1} and winds mainly from the north-northwest (NNW).

High nutrient concentrations were found at the beginning of the wet season sampling period (Table 1). During the fourth sampling day, high levels of ammonium at concentrations of 29.39±12.17 and 28.28 μmol L^{-1} were observed at S1 and S2, respectively, coinciding with 106 mm of rain on the third sampling day. Overall, the average ammonium concentration was higher than that of the other nutrients with a concentration of 14.69±1.3 μmol L^{-1} during the wet season. The average concentrations for phosphates, silicates, and N + N were 0.35 ±0.08, 7.18±0.63, and 1.51±0.29 μmol L^{-1}, respectively. The N:P ratio averaged 92.3±12.7, suggesting a strong P limitation during this period.

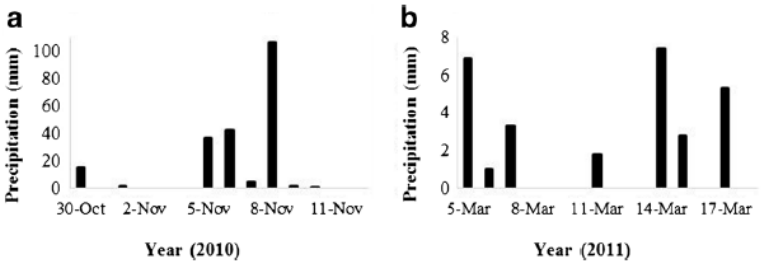

Fig. 2 Cumulative rain records in the week prior to sampling and during the sampling days in the **a** wet season (November 2010) and **b** dry season (March 2011). Note the differences in scale between the sampling periods

Table 1 Physicochemical variables measured at Bahía Fosforescente during the wet season in November 2010 (nutrient concentrations in μmol L^{-1})

Stations/days	T (°C)	Salinity	PO_4^-	s.e.	SiO_2	s.e.	$NO_2^- + NO_3^-$	s.e.	NH_4^+	s.e.	N:P
Station 1											
Day 1	26.0	15.0	1.9	0.2	17.9	1.5	7.8	0.4	24.0	0.9	16.5
Day 2	28.0	29.5	0.4	0.0	10.8	1.0	2.1	0.2	12.3	0.1	37.0
Day 3	28.0	32.0	0.1	0.0	5.1	0.3	0.6	0.1	18.6	1.5	147.2
Day 4	29.0	33.0	0.1	0.0	5.4	0.3	0.7	0.2	29.4	12.2	251.1
Day 5	28.0	31.0	0.2	0.0	8.0	0.1	1.4	0.1	15.9	0.1	72.0
Station 2											
Day 1	27.0	22.0	1.0	0.1	10.8	0.8	3.8	0.2	10.2	1.1	14.7
Day 2	28.0	29.5	0.4	0.1	10.1	1.8	2.3	0.6	14.3	1.1	46.1
Day 3	28.0	32.0	0.1	0.0	4.3	0.0	0.4	0.1	18.4	1.1	188.1
Day 4	28.5	32.0	0.2	0.0	6.3	0.2	0.8	0.0	28.2	0.0	120.5
Day 5	28.5	32.0	0.3	0.1	8.2	1.2	1.1	0.0	20.3	6.2	69.0
Station 3											
Day 1	25.0	25.0	0.3	0.0	5.8	0.3	1.3	0.2	7.9	0.8	28.9
Day 2	28.5	32.0	0.1	0.0	3.6	0.0	0.3	0.0	5.9	0.6	68.9
Day 3	28.5	32.0	0.1	0.0	4.4	0.1	0.7	0.0	21.9	0.8	205.1
Day 4	28.5	32.0	0.1	0.0	5.0	0.2	0.7	0.0	14.0	1.3	162.8
Day 5	29.0	32.0	0.1	0.0	5.7	0.1	0.7	0.0	2.81	1.04	26.8
Station 4											
Day 1	26.5	20.0	1.0	0.3	12.2	2.4	5.3	1.1	15.9	0.9	20.6
Day 2	28.5	30.0	0.1	0.0	5.8	1.1	0.8	0.2	7.7	0.6	77.1
Day 3	28.0	32.0	0.1	0.0	4.6	0.2	0.8	0.0	22.7	2.4	195.8
Day 4	28.5	32.0	0.1	0.0	5.0	0.4	0.7	0.0	16.6	1.0	157.5
Day 5	28.5	32.0	0.1	0.0	5.8	0.5	0.8	0.0	2.9	0.4	33.9
Station 5											
Day 1	27.0	24.0	0.8	0.1	10.1	0.0	3.5	0.0	10.6	1.8	17.7
Day 2	28.0	31.0	0.1	0.1	5.0	1.1	0.8	0.3	7.3	2.7	58.4
Day 3	28.0	31.5	0.1	0.0	5.1	0.5	1.3	0.0	24.4	1.3	197.7
Day 4	28.5	31.0	0.1	0.0	4.7	0.1	0.7	0.1	12.9	0.8	113.3
Day 5	28.5	31.5	0.1	0.0	5.9	0.1	0.8	0.0	4.4	0.4	43.1
Station 6											
Day 1	27.0	19.0	1.4	0.0	15.9	0.6	1.8	0.4	15.2	2.3	12.3
Day 2	28.0	29.0	0.3	0.1	7.6	1.1	1.2	0.1	10.7	0.3	47.6
Day 3	28.0	32.0	0.1	0.0	4.9	0.3	0.6	0.2	21.4	0.3	157.1
Day 4	28.5	31.0	0.1	0.0	5.0	0.3	0.7	0.0	14.4	4.3	116.2
Day 5	28.5	31.5	0.2	0.0	6.6	0.1	1.0	0.0	9.6	0.7	66.1

s.e. standard error

Table 2 Physicochemical variables measured at Bahía Fosforescente during the dry season in March 2011 (nutrient concentrations in μmol L^{-1})

Stations/days	T (°C)	Salinity	PO_4^-	s.e.	SiO_2	s.e.	$NO_2^- + NO_3^-$	s.e.	NH_4^+	s.e.	N:P
Station 1											
Day 1	27.0	35.5	0.2	0.0	4.7	3.2	0.5	0.2	0.3	0.0	3.6
Day 2	27.0	36.0	0.2	0.0	1.1	0.1	0.3	0.1	0.4	0.0	3.4
Day 3	27.0	36.0	0.2	0.0	4.3	0.3	0.5	0.0	2.0	0.1	10.9
Day 4	27.0	35.0	0.3	0.0	4.7	0.3	0.9	0.1	4.9	0.0	19.1
Day 5	27.5	35.0	0.3	0.0	5.3	0.9	1.0	0.1	4.4	0.0	21.4
Station 2											
Day 1	27.0	35.5	0.2	0.0	1.4	0.8	0.3	0.1	0.3	0.0	2.4
Day 2	27.0	35.0	0.2	0.0	5.2	0.6	0.6	0.0	0.3	0.1	4.9
Day 3	27.5	35.0	0.3	0.0	4.6	1.1	0.4	0.0	2.6	0.1	10.0
Day 4	27.0	35.0	0.3	0.0	2.9	0.1	1.0	0.2	4.9	0.0	19.7
Day 5	27.5	35.0	0.3	0.0	6.8	2.2	1.2	0.2	5.0	0.0	20.9
Station 3											
Day 1	27.0	35.0	0.3	0.1	0.6	0.0	0.2	0.0	0.4	0.0	2.4
Day 2	27.5	35.0	0.3	0.0	0.8	0.0	0.2	0.0	0.3	0.2	2.2
Day 3	27.5	36.0	0.5	0.0	7.3	3.2	0.2	0.0	2.7	0.1	5.9
Day 4	27.0	35.5	0.4	0.0	2.5	0.1	0.9	0.3	5.8	0.1	15.7
Day 5	27.5	35.0	0.4	0.0	5.2	0.0	1.1	0.0	7.4	0.3	22.9
Station 4											
Day 1	27.5	35.0	0.2	0.0	1.5	0.1	0.3	0.0	0.7	0.4	4.5
Day 2	27.5	36.0	0.4	0.0	1.2	0.2	0.2	0.0	0.2	0.1	1.2
Day 3	27.5	35.5	0.4	0.0	6.5	0.6	0.2	0.0	2.6	0.0	8.0
Day 4	27.0	34.5	0.5	0.0	2.8	0.0	1.0	0.0	4.7	0.1	11.6
Day 5	27.5	35.0	0.3	0.0	4.8	0.4	0.7	0.0	7.6	0.0	27.5
Station 5											
Day 1	28.0	35.0	0.3	0.1	1.2	0.0	0.3	0.0	0.3	0.0	2.1
Day 2	27.5	35.0	0.3	0.1	1.2	0.01	0.3	0.0	0.2	0.0	1.8
Day 3	27.5	35.0	0.3	0.0	6.6	1.3	0.4	0.2	3.2	0.0	11.9
Day 4	27.0	35.0	0.4	0.0	4.7	1.6	0.7	0.1	5.3	0.0	13.6
Day 5	27.5	35.5	0.3	0.0	4.6	0.3	0.9	0.0	5.3	0.3	22.0
Station 6											
Day 1	28.0	35.0	0.2	0.0	1.6	0.2	0.3	0.0	0.38	0	3.1
Day 2	28.0	35.0	0.2	0.0	3.1	1.7	0.5	0.0	0.15	0.03	3.6
Day 3	27.5	35.5	0.3	0.0	6.6	0.8	1.0	0.6	3.78	0.14	15.0
Day 4	27.0	35.0	0.4	0.0	10.3	6.5	1.0	0.1	4.38	0.09	15.3
Day 5	27.5	35.0	0.2	0.0	4.6	0.3	0.8	0.1	4.82	0	24.4

s.e. standard error

The dry season marked a period of consistently lower nutrient concentrations relative to the wet season (Table 2). Silicate and ammonium concentrations were higher than other nutrients with maximum values of 10.33±6.46 and 7.57± 0.04 μmol L^{-1}, respectively. The average concentrations for phosphates, silicates, and N + N were 0.30±0.02, 3.9±0.43, and 0.59±0.10 μmol L^{-1}, respectively. The N:P ratios averaged 11.0±1.5, and the bay phytoplankton productivity during the dry season appears to have been limited by N.

Pyrodinium bahamense and *Ceratium furca* Cell Densities

The daily variations in cell densities for both species of dinoflagellates were statistically significant during the wet season (*P. bahamense*: KW-ANOVA H=19.15, *df*=4, *n*=18, $p<0.001$; *C. furca*: KW-ANOVA H=27.78, *df*=4, *n*=18, $p<0.001$; Fig. 3a). The highest cell densities for *P. bahamense* were found on sampling day 4, while *C. furca* was more abundant on day 2. Both species also showed

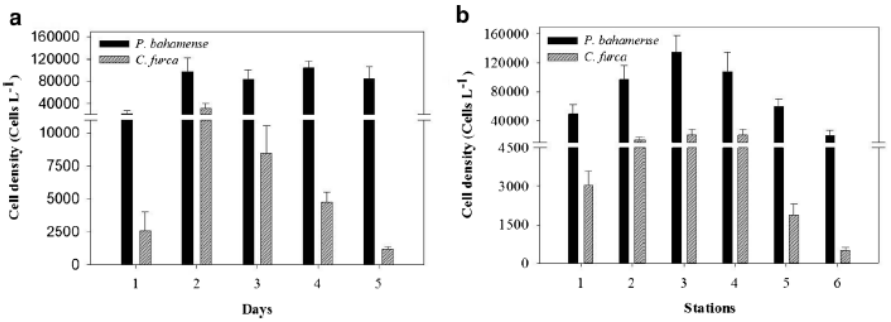

Fig. 3 Daily (**a**) and spatial (**b**) variations of *P. bahamense* and *C. furca* cell densities during the wet season in November 2010. *Bars* represent standard errors

statistically significant spatial variation in their cell densities (*P. bahamense*: KW-ANOVA H=31.37, *df*=5, *n*=18, $p<0.001$; *C. furca*: KW-ANOVA H=36.92, *df*=5, *n*=15, $p<0.001$; Fig. 3b). A *P. bahamense* bloom (defined as $>5.0\times10^4$ to 1.0×10^5 cells L^{-1}; Steidinger and Haddad 1981; Phlips et al. 2006) was detected at S3 and S4, with cell densities close to 10^5 cells L^{-1}. The *C. furca* cell densities were also higher at S3 and S4, albeit with lower concentrations than that of *P. bahamense*. Overall, the abundance of *P. bahamense* was higher than that of *C. furca* during the wet season (Mann-Whitney *U* test=846.5, *n*=90, $p<0.001$).

In contrast to the wet season, the daily variation in cell densities of the two species were not statistically significant (*P. bahamense*: KW-ANOVA H=6.15, *df*=4, *n*=18, $p=0.188$; *C. furca*: KW-ANOVA H=1.92, *df*=4, *n*=18, $p=0.751$; Fig. 4a) during the dry season. Spatially, statistically significant higher cells densities at S2, S3, and S4 were observed (*P. bahamense*: KW-ANOVA H=48.76, *df*=5, *n*=15, $p<0.001$; *C. furca*: KW-ANOVA H=54.37, *df*=5, *n*=15, $p<0.001$; Fig. 4b), even though the *P. bahamense* abundance was approximately 1/500 of that observed during the wet season and *C. furca* abundances remained practically unchanged between seasons. Thus, a shift toward the numerical dominance in abundance of *C. furca* over *P. bahamense* was observed during the dry season (Mann-Whitney *U* test=67.0, *n*=90, $p<0.001$).

Correlations Among *P. bahamense*, *C. furca*, and Environmental Parameters

During the wet season, significant positive correlations were found between *P. bahamense* and *C. furca* and between *P. bahamense*, temperature, and salinity (Table 3). A significant inverse correlation was found between *P. bahamense* and phosphates, silicates, and N + N. A negative correlation was observed between *C. furca* and phosphate. The only significant correlation during the dry season was a positive one between *C. furca* and phosphates.

Discussion

Temporal Patterns

Pluviosity showed a clear, seasonal pattern that followed the 1959–2012 climatology (www.sercc.com/cgi-bin/sercc/cliMAIN.pl?pr5693); however, 1WR prior to sampling was more intense than expected, based on average monthly rain data for both seasons. Overall, 50 % of the monthly rain fell in a short period of time, resulting in rains twice as intense as the normal monthly average. Even when the potential fertilization during our observations was higher than expected based on local climatology, seasonality still elicited differential

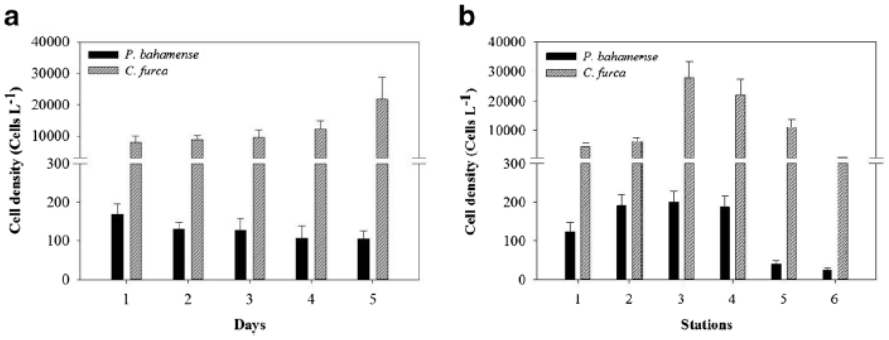

Fig. 4 Daily (**a**) and spatial (**b**) variations of *P. bahamense* and *C. furca* cell densities during the dry season in March 2011. *Bars* represent standard errors

Table 3 Spearman rank correlations between *P. bahamense*, *C. furca*, and environmental variables during the wet and dry seasons

		C. furca	PO_4^-	SiO_2	$NO_2^- + NO_3^-$	NH_4^+	N:P	T	S
Wet season	*P. bahamense*	**0.73**	**−0.66**	**−0.53**	**−0.57**	0.08	0.33	**0.61**	**0.53**
	C. furca		**−0.43**	−0.32	−0.3	0.03	0.25	0.21	0.25
Dry season	*P. bahamense*	0.12	0.00	−0.03	−0.17	−0.05	−0.08	−0.36	0.13
	C. furca		**0.42**	−0.11	−0.13	0.26	0.06	−0.21	−0.30

Bold data indicates $p<0.05$, $n=30$

fertilization since pluviosity during the wet season was four times greater than that during the dry season. This difference played a key role in nutrient and planktonic variability.

During the first wet season sampling day, the phosphate, N + N, silicate, and ammonium concentrations reached 2, 5, 2, and 4 times, respectively, the concentrations reported in previous studies (Smayda 1970; Soler-Figueroa 2006; Cedeño-Maldonado 2008). These nutrients were principally derived from the land/watershed, since negative correlations between salinity and silicate levels and between salinity and N + N concentrations were observed (data not shown). The importance of rainfall and its associated nutrient load was also sustained by a highly significant rain event (106 mm) on November 8, 2010; although, this event primarily impacted the ammonium concentration. Due to the high nitrogen influx (i.e., N + N and ammonium) observed during this season, the system was pushed toward phosphorus limitation. In contrast, the levels of rain recorded during the dry season were insufficient to trigger significant changes in nutrient concentrations. Thus, the concentrations observed during the dry season can be considered similar to the low background levels found during the wet season and reported previously (Smayda 1970; Soler-Figueroa 2006; Cedeño-Maldonado 2008).

Overall, the short-term environmental variability triggered during the wet season, increased concentrations of nutritional factors such as inorganic and organic nitrogen and phosphorus, and likely other fertilizing compounds, such as humic matter, that sustained blooms of *P. bahamense* and significant peaks in the abundance of *C. furca*, albeit with lower cell numbers than for *P. bahamense*. This notion is supported by the strong negative correlations between *P. bahamense* and phosphates and N + N and between *C. furca* and phosphates. These negative correlations support a rapid nutrient uptake during the bloom and suggest that the concentrations measured were actually the unassimilated fraction of nutrients (Sommer 1999).

Previous work has also revealed a direct link between pluviosity and increased numbers and bloom development of *P. bahamense*. In the Indian River Lagoon in Florida, blooms of this species have been associated with rainfall events that transported bioavailable phosphorus and nitrogen into the system (Phlips et al. 2004, 2006, 2011). Similarly, the release of humic substances from nearshore mangroves and terrestrial watersheds following rainfall periods has been suggested to be important in *P. bahamense* bloom formation (Usup and Azanza 1998; Morquecho et al. 2012; Usup et al. 2012). The nutrient influx from the watershed observed during the wet season promoted in addition an increase in the cell densities of *C. furca*, which reached high values particularly during the second sampling day. In Sagami Bay in Japan, high cell densities of *C. furca* were also observed during the rainy season and were associated with high nutrient concentrations, specifically high levels of nitrogen compounds (Baek et al. 2006, 2008c).

During the dry season, when nutrient levels and N:P ratios were the lowest, the cell densities of *P. bahamense* decreased by three orders of magnitude, while *C. furca* abundances were sustained. The differences in the physiological responses and adaptive strategies (Smayda 2002), nutritional preferences, uptake capacities, and nutritional status of the organisms (Anderson et al. 2002) play important roles in determining the species composition/dominance under different conditions. For instance, the capacity of *C. furca* for mixotrophy/phagotrophy and luxury consumption under nutrient-limiting conditions (Smalley et al. 2003; Baek et al. 2008b) may enable this species to maintain high numbers during the dry season. The capacity of *P. bahamense* for cyst formation when the environmental factors are not suitable (Anderson 1989) and the long-term viability of such cysts (Villanoy et al. 2006) favor the maintenance of a seed population that may flourish when increases in nutrient inputs occur after the dry season.

Our data suggests that temperature and salinity should have minimal effects on the patterns observed since these factors were always well within the range of tolerance of these species. It has been found that *P. bahamense* can form blooms at temperatures of 25 °C or higher and at salinities that range from 16 to 42 (Phlips et al. 2004). On the other hand, *C. furca* actively grows in temperatures that range from 20 to 30 °C and salinities from 17 to 34 (Baek et al. 2006; Baek et al. 2008a, b, c). Thus, this study suggests that precipitation regime and the resulting nutritional influx were probably the most important factors in modulating these populations.

Spatial Distribution

High cell densities of *P. bahamense* and *C. furca* were observed consistently in the east and southeast regions of the bay

(S3 and S4) in agreement with previous reports (Seliger et al. 1971; Seixas 1988). This finding suggests that wind-driven currents play an important role in defining the spatial distribution of dinoflagellate patches in the bay (Seixas 1988).

We found that the wind pattern observed during this study was different during both sampling periods and, therefore, expected sustained and contrasting differences in the horizontal distribution of both species. During the wet season, increased abundances were expected toward the southwest, while during the dry season, an increase in abundance was expected toward the southeast; however, no appreciable changes were observed with respect to the distribution of the examined dinoflagellates. In addition to wind, factors such as water currents and tidal flow (Steidinger 1973), phototaxis (Seliger et al. 1970, 1971), dial vertical migration (Anderson and Stolzenbach 1985), water residence time (Seliger et al. 1971; Phlips et al. 2006; Morquecho et al. 2012), and the vertical structure of the water column (Pannard et al. 2008) are important factors that define the spatial distribution of phytoplankton. Ultimately, the concentration, bloom dynamics, and spatial distribution of the species are the result of complex interactions between different biological and physical factors that occur over a wide range of temporal and spatial scales (Donaghay and Osborne 1997).

The strong correlation between *P. bahamense* abundance and *C. furca* abundance during the wet season (and less prominently during the dry season) indicates the presence of an ecosystem-wide mechanism that maintains alternating blooms of these species. The existence of bioluminescent systems such as Oyster Bay in Jamaica and Bahía Fosforescente has been suggested to be the result of accumulation mechanisms that tend to concentrate high cell densities of *P. bahamense* and other phytoplankton organisms (Margalef 1961; Seliger et al. 1970; Seliger et al. 1971). Longer water residence times or turnover rates are important factors that have enabled the development of high concentrations of *P. bahamense* in other ecosystems (Phlips et al. 2006; Morquecho et al. 2012). Additional longer studies examining the influence of wind and other factors affecting water circulation (i.e., tides and thermohaline circulation) and the effects of benthic-water column interactions (nutrient charging and cyst reservoirs) will provide additional insight into the complexity of these unique systems.

In summary, this study suggests the importance of seasonality and rain regimes as a significant factor modulating the natural fluctuations of phytoplankton at Bahía Fosforescente. Overall, the inherent adaptive strategies and physiological characteristics of organisms coupled with rain-induced changes in nutrient availability seem to promote changes in the phytoplankton community within days. However, other factors not considered here such as water currents, mixing with coastal waters, light availability, and top down control may also influence the abundance of the phytoplankton community. Longer termed studies could provide further evidence of the seasonality observed. Bay turnover should be also examined since it may constitute a significant driver of these populations. Finally, the effects of grazing by other planktonic species have yet to be examined.

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Chapter 3

Patterns of variability of two potentially harmful algal bloom forming dinoflagellates in a tropical bioluminescent bay, Bahía Fosforescente, La Parguera, Puerto Rico: The role of seasonality.

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Abstract

Blooms of potentially harmful dinoflagellates can be a recurrent phenomenon in some coastal tropical bays, such as Bahía Fosforescente. Previous reports have evidenced fluctuations of species abundance that often result in the dominance of *Pyrodinium bahamense* or *Ceratium furca*. However, previous works have not examined these fluctuations longer than daily periods. This work evaluates how the abundance of these two species varies over monthly periods within the northern Caribbean dry and wet seasonal extremes. A multivariable analysis was used to explore the influence of environmental variables (temperature, salinity, nutrients, dissolved organic matter fluorescence-DOMFL, pH, dissolved oxygen, Chl*a*, turbidity, rainfall, wind velocity and direction) in the dinoflagellates abundance. Seasonal changes accounted for 48% of the total dinoflagellate abundance variation. *Pyrodinium bahamense* was numerically dominant during the wet season with maximum cell densities of 4.8×10^4 cells L^{-1}. A 26-fold reduction in their cell densities were observed during the dry season and *C. furca* dominated, reaching a bloom condition (i.e. 10^5 cells L^{-1}). Seasonal variability in the dinoflagellate composition was associated with salinity, DOMFl, pH and silicates, environmental variables that are strongly modulated by pluviosity. The association of these environmental factors with pluviosity suggests that land-derived materials strongly modulate changes in abundance and species composition triggering different population behaviors associated to different strategies of nutrient acquisition. Winds and pelagic-benthic interactions are suggested as important in the daily and spatial variations of dinoflagellates within each season. This study suggests a robust link between possible environmental changes and the response of two worldwide potentially harmful algal bloom species.

Keywords: Harmful algal blooms (HABs), weather, *Pyrodinium bahamense*, *Ceratium furca*, seasonal variability

Introduction

Increases in the frequency and magnitude of harmful algal blooms (HABs) have been reported for many coastal and marine ecosystems over the last few decades (Hallegraeff 1993; Anderson 1997), with dinoflagellates being one of the most prominent and noxious group (Smayda 1997). Due to the apparent global expansion and possible deleterious effects of these organisms, attention has been focused on the factors controlling their bloom dynamics (Smayda 2002). Yet, the underlying mechanisms sustaining the proliferation and persistence of these organisms are not completely understood.

Blooms of potentially harmful dinoflagellates are a common and recurrent phenomenon in small and shallow tropical bays and lagoons such as, Bahía Fosforescente, located in southwestern Puerto Rico. *Pyrodinium bahamense* var. *bahamense*, *Ceratium furca* var. *hircus*, *Cochlodinium polykrikoides*, *Akashiwo sanguinea*, *Prorocentrum micans*, *Dinophysis caudata* and *Protoperidinium spp.* (Margalef 1961, Seliger et al. 1971, Hernández-Becerril and Navarro1996) are some of the species that have been observed in Bahía Fosforescente and could form blooms. Thus, this bay represents a unique environment to study the underlying mechanisms promoting the bloom capacity of dinoflagellates. The most conspicuous of these is *P. bahamense* since its bioluminescence (Margalef 1957) has made this site an economically important tourist attraction. Although *P. bahamense* has been characterized in other regions (e.g. Indian River Lagoon, Florida) as a potentially toxic species causing paralytic shellfish poisoning (PSP; Landsberg et al. 2006), no toxic events have been reported for this bay.

Alternation of dense populations of *P. bahamense* with those of the non-bioluminescent *C. furca*, a species known to cause anoxia related red tides (Horner et al. 1997; Machida et al. 1999; GEOHAB 2001), have been consistently reported over time for the bay (Seixas 1983, 1988; Walker 1997; Soler-Figueroa 2006), with concurrent changes in bioluminescence levels (Soler-Figueroa and Otero, unpublished). Aside from being a hotspot for tourism and recreation, Bahía Fosforescente is of ecological importance for fisheries and the habitat of marine protected species (i.e. dolphins and manatees). Thus, an increased awareness concerning the health and ecological status of this bay exists in the scientific and local community (PR Sea Grant, http://seagrantpr.org/caribbean/sessions/academia-focus-on-conservation) and uncertainties arise on whether the fluctuations in the phytoplankton composition (i.e. dinoflagellates) are linked to anthropogenic disturbances or to environmental forcing, as has been studied in other coastal

ecosystems (e.g. Cloern and Jasby 2010; Paerl et al. 2010). However, given the absence of a continuous monitoring program and the paucity of studies conducted, the underlying mechanisms driving the fluctuations in the abundance of *P. bahamense* and *C. furca* are still inadequately understood.

In tropical ecosystems, such as Bahía Fosforescente, shifts in environmental conditions are linked to seasonal climate extremes, specifically the wet and dry seasons (González 1965; Cintrón 1969; Sournia 1969; García and López 1989). Thus, external environmental forcing related to weather patterns can result in alterations of a wide range of environmental factors such as temperature, salinity, irradiance, precipitation, runoff and nutrient loading, which can alter the structure and function of these coastal ecosystems (Pearl et al. 2001; Wetz and Yoskowitz 2013). Due to the shallow depth, small volume and low flushing rates of Bahía Fosforescente (Margalef 1961; Seliger et al. 1971), environmental pulses can be more evident. Additionally, since generation times of phytoplankton range from hours to days, environmental shifts may trigger rapid changes in phytoplankton community composition and abundance, as previously reported for other aquatic ecosystems (Litaker et al. 1993; Pannard et al. 2008; Romero et al. 2013; Egerton et al. 2014). Thus, efforts should be conducted at timescales relevant to the growth of these organisms, together with the acquisition of relevant environmental data (Smayda 1998). These studies are not only necessary from the perspective of management of coastal ecosystems, but are also essential for our understanding of the bloom dynamics of *P. bahamense* (Usup et al. 2012) and *C. furca*, a species proposed to be used as a biological indicator of global climate change (Tunin-Ley et al. 2007, 2009; Tunin-Ley and Lemée 2013).

Recent studies at Bahía Fosforescente focused attention at short term population fluctuations of *P. bahamense* and *C. furca* within a seasonal context, finding a strong seasonality linked to pluviosity (Soler-Figueroa and Otero, 2015). However, the temporally limited nature of the study periods (i.e. five days during each seasonal extreme) and the scantiness of environmental data may have limited the ability to define the factors that drive the populations dynamics of these species. Thus, the present study expands its scope by studying how a suite of environmental variables, within similar seasonal extremes, are related with the patterns of abundance of both species in order to support implementation of sound coastal ecosystem management and to broaden our knowledge on environmental factors that influence two potential worldwide HABs species.

Methods

Study Site

The above goals were evaluated at Bahía Fosforescente (Fig. 1). This bay is located 3.2 km east of La Parguera, Lajas on the southwest coast of Puerto Rico (17° 58′ 30″ N; 67° 01′ 10″ W). This small and shallow bay (0.19 km^2; 3.5m average depth) is similar to others found in the Caribbean. The bay has three north inlets, is surrounded by *Rhizophora mangle* and has a narrow (~100 – 150 m wide) and shallow (ca. 3 m) outlet that connects to the ocean. There are no rivers discharging into the bay, but infrequent watershed surface water flows in via the three north inlets, especially during heavy rainfall events. Turbid waters characterizes this bay due to high phytoplankton concentrations, mangrove detritus, and wind-driven sediment resuspension (González 1965). A sandy substratum with small patches of *Thalassia testudinum* and some macroalgae cover the shallow borders of the bay, while fine muddy sediments dominate the central area (Ríos-Jara 1998).

Field Work

Six stations (Fig. 1) were sampled between 8:00 and 9:30 AM during 31 consecutive days, based on local climatology (Glynn 1973), during the wet (5 November – 5 December, 2012) and dry (1 – 31 March, 2013) seasons. Triplicate water samples were collected 0.1 m below the surface at each station using 9 L carboys. A portion of the sample (7.7 L) was filtered through a 25 µm mesh, suspended in filtered seawater (ca. 50 mL), and fixed with buffered formaldehyde (ca. 1% final concentration).

Duplicate water samples were collected at each station for nutrient and dissolved organic matter fluorescence (DOMFl) analysis in 500 ml acid-washed polyethylene bottles, and in 30 ml pre-washed and muffled (450 °C for 4 hours) dark glass bottles, respectively. Duplicate surface water samples were collected in 50 ml centrifuge tubes for pH determinations. Samples for nutrients and DOMFl analysis were kept at 5 °C until arrival to the laboratory. The DOMFl, ammonium, and pH (Oakton pH meter) determinations were conducted immediately upon the arrival to the laboratory (1-2 hours after collection). Water samples for other nutrient determinations (i.e. phosphates, nitrites+nitrates and silicates) were frozen (-80 °C) until the analysis.

Water temperature (°C), salinity, and dissolved oxygen (DO, mg L^{-1}) were measured at each station with a Pro Plus YSI probe. The instrument was calibrated prior to each sampling day

using certified conductivity standards (i.e. for the salinity measurements) and following the instruction manual (i.e. for the DO measurements). Chlorophyll *a* (Chl*a*) and turbidity were estimated at each station with a SCUFA II *in vivo* Chl*a* fluorometer/turbidimeter (Turner Designs), based on Otero (2009). Turbidity and Chl*a* are expressed as NTU and µg L^{-1}, respectively.

Laboratory work

Determination of Pyrodinium bahamense and Ceratium furca cell densities

A benchtop Flow Cytometer And Microscope (FlowCAM - FlowImaging; Sieracki et al. 1998) was used to determine the cell densities of *P. bahamense* and *C. furca*. Sample volumes were adjusted prior to the analyses to avoid cell clustering and water samples were filtered through 210 µm mesh to remove meso/macro zooplankton to avoid flow cell clogging. Samples were pumped through a 200 µm glass flow cell at a flow rate of 1.5 mL min^{-1} and viewed with a 4x objective. Dinoflagellate cells were analyzed in autoimage mode with an image recognition software (VisualSpreadsheet© Particle Analysis Software) and using a cell diameter threshold of 15-500 µm to improve the accuracy of counting. The cell flow was rinsed with distilled water between each sample analysis to avoid cross-contamination. *Pyrodinium bahamense* and *C. furca* cell densities were automatically classified and counted with biometrics filters previously created based on particle properties. Each automated classification was later screened and corrected for misclassifications.

To examine the accuracy of the FlowCAM classifications several samples were manually counted using a Sedgewick-Rafter counting chamber on a CK40 inverted microscope (Olympus, Inc.) at 100× total magnification. In average, the FlowCAM results accounted for more than 90% of *P. bahamense* and *C. furca* manual estimates. Averages and standard errors of cell densities were calculated from triplicate samples and are reported as cells L^{-1}.

Nutrients and DOMFl analysis

Phosphates (PO_4^{3-}), nitrites+nitrates (N+N), and silicates (SiO_2) concentrations were analyzed using the methodology of Parsons et al. (1984) with a UV-1601 double-beam spectrophotometer (Shimadzu). Ammonium (NH_4^+) concentrations were conducted using a Trilogy benchtop fluorometer (Turner Designs) based on the methods of Holmes et al. (1999).

Standard samples were run for each nutrient during the day of analysis and concentrations are expressed as µmol L^{-1}.

The DOMFl was measured using a Trilogy benchtop fluorometer (Turner Designs) fitted with an UV fluorescence cube (ex:em: 365:430 nm) as in Otero (2009) and based on Amador et al. (1990). Once at room temperature and filtered through pre-combusted GF/F filters, the fluorescence levels were measured in 2 ml pre-combusted glass vials. Calibrations were based on quinine sulfate (QS) standards prepared in 0.1 N sulfuric acid. Results are reported in ng QS ml^{-1}, which is proportional to the concentration of material derived from terrestrial sources, in this case run-off and mangrove systems, and the decomposition of detritus.

Meteorological Conditions

To make a detailed connection between the climatological conditions and the dinoflagellate abundances, a HOBO Micro Weather Station equipped with rain and wind (speed and direction) sensors was installed in the watershed immediately north of the bay. The logger was set to record data every minute and the data were retrieved once a week. Precipitation and wind records from a meteorological station (Davis Vantage Pro 2) located 3.2 km west of the sampling site and maintained by the Department of Marine Sciences Bio-optical Oceanography Laboratory at Magueyes Island (http://bio-optics.uprm.edu/weather.html) was also used to make comparisons and fill data gaps due to technical problems with the HOBO station. Results are expressed as the average of both stations since data were similar.

Data Analysis

To explore and visualize the multivariate patterns of spatial and temporal variability in the dinoflagellate composition (i.e. *P. bahamense* and *C. furca*) a Principal Coordinates Analysis (PCO) was performed. Vectors with the dinoflagellates cell densities were overlaid to identify better what drives the observed patterns of variability in the dinoflagellate composition. A PERmutational Multivariate ANalysis Of VAriance (PERMANOVA; Anderson 2001) was used to confirm statistically significant differences in dinoflagellate composition and estimate components of variations between seasons (fixed factor), among stations (fixed factor), and among days within each season (days nested in seasons and as random factors), and to test additionally for possible interactions between days and stations within each season. The PCO and PERMANOVA analyses were based on Bray-Curtis resemblance matrixes with the dinoflagellate cell densities log (x +1) transformed to reduce the influence of highly abundant or

rare species. Since PERMANOVA analysis showed that seasons alone accounted for 48% of the total variation in the dinoflagellate composition, a SIMilarity PERcentage Analysis (SIMPER; Clarke and Warwick 2001) was further performed to evaluate the dinoflagellate species that strongly contributed to the seasonal variability.

To explore which of the environmental factors are linked to the seasonal variation in the dinoflagellate composition a distance based redundancy analysis (dbRDA) was performed (McArdle and Anderson 2001), with the environmental factors overlaid according to their Spearman rank correlation with each axis. The analysis was based on the Bray-Curtis resemblance matrix of dinoflagellate cell densities and with the environmental variables used as predictors. Prior to the analysis the environmental data were log (x+1) transformed to reduce skewness and normalized to account for the differences in measurement units.

All statistical analyses were performed using PRIMER-e and PERMANOVA add-on software (Anderson et al. 2008).

Results

Physical and meteorological conditions

Surface water temperature during the wet season was 29.4 ±0.09 °C (mean ±standard error) and 27.9 ±0.14 °C during the dry season, with approximately 3 degrees increase during the last two weeks of sampling (Fig. 2). Surface water salinity during wet season ranged from 34.6 and 36.3 and from 37.2 to 37.9 during the dry season (Fig. 2). To account for the potential effects on our measurements of rain, we considered total precipitation for each seasonal sampling as the sum of rain falling two weeks prior to sampling and during sampling. Six times more rainfall (161 mm) fell during the wet season sampling period than during dry sampling campaign (28 mm; Fig. 3a and 3b). Decreases in salinities were observed following a November 28 rainfall event (40 mm), indicating the entrance of significant runoff into the bay during a short period of time (Figure 2). Mean daily wind speeds varied during the wet and dry seasons (Fig. 3a and 3b), ranging from 0.47 to 2.27 m s^{-1} and from 0.99 to 3.23 m s^{-1}, respectively. Wind direction during the wet season was frequently (ca. 32%) northwest (NW)/north-northwest (NNW), followed by a southeast (SE)/south-southeast (SSE) component (ca. 17%). In contrast, winds from the SE/SSE were more frequent during the dry season (ca. 26%), together with an east (E)/east-northeast (ENE) direction.

The pH ranged from 7.8 to 8.2 and from 8.0 to 8.3 during the wet and dry season, respectively, with lower values associated with the inner stations of the bay (Fig. 4). Dissolved oxygen concentrations during the wet season ranged between 2.9 and 5.1 mg L^{-1}, with the highest levels recorded at the outer stations of the bay (S4 – S6; Table 1). In contrast, DO concentrations during the dry season (range: 3.7 – 6.1 mg L^{-1}) were typically higher than those detected during the wet, and higher concentrations distributed similarly as during the wet season (Table 2). Turbidity was highly variable among days and stations within each season (wet range: 0.5 – 7.7 NTU; dry range: 1.4 – 11.4 NTU; Table1 and 2). Higher mean values were found during the dry season (3.62 ±0.72 NTU) than during the wet season (1.94 ±0.39 NTU). Overall, the highest turbidities were associated to the eastern portion of the bay (S3 and S3 – S4 during the wet and dry seasons, respectively).

Nutrients concentrations and DOMFl

Table 1 and 2 shows the daily and spatial nutrients concentrations (i.e. NH_4^+, N+N, PO_4^{3-} and SiO_2) during the wet and dry season. Nutrients reached the highest values during the wet season. Ammonium during the wet season reached the highest concentrations of all nutrients (range: 0.40 - 9.20 µmol L^{-1}), with maximum values mainly observed at S3. Nitrites+nitrates and SiO_2 concentrations during this season fluctuated between 0.23 and 2.98 and from 1.96 to 6.04 µmol L^{-1}, respectively. The maximum concentrations of both nutrients were markedly observed at S3 and S1, respectively, after the 40 mm of rain registered the previous day. Phosphate concentrations during this sampling period (range: 0.11 – 0.67 µmol L^{-1}) were commonly at their highest at S3, except from November 17 to 19 when maximum concentrations were detected at S1, coinciding with a *Cochlodinium polykrikoides* bloom (Table 1).

During the dry season, the highest concentrations of NH_4^+ (range: 0.08 - 4.96 µmol L^{-1}) were observed during the second sampling week (i.e. March 7 – 14) at all stations, but most notably at S3. Similarly, the highest concentrations of N+N (range: 0.08 and 1.45 µmol L^{-1}) and SiO_2 (range: 0.71 - 5.56 µmol L^{-1}) during this season were observed around the second sampling week. Phosphates concentrations fluctuated between 0.03 and 0.37 µmol L^{-1} during this season, with maximum values at S3 and S4. A noteworthy declining trend was observed for all nutrients, except PO_4^{3-}, during the last ten days of this sampling campaign (Table 2), coinciding with the appearance of several diatoms species (i.e. *Pleurosigma* spp., *Chaetoceros* spp., *Coscinodiscus* spp. and other unidentified diatoms).

Seasonal and spatial DOMFl fluctuations were significant (Table 1 and 2). The DOMFl was at its maximum during the wet season and ranged from 12 to 53 ng QS ml^{-1}. Increases in DOMFl during this season, mainly at S3 and S5, were detected after the November 28 rainfall event. During the dry season, DOMFL fluctuated between 9 and 32 ng QS ml^{-1}. The highest DOMFL were observed at the inner bay stations (i.e. S1 to S3) during both sampling periods (Table 1 and 2).

Chl a concentrations

Chlorophyll *a* concentrations during the wet season ranged from < 0.1 to 25.8 µg L^{-1} (Table 1). Peaks > 5 µg L^{-1} were detected during 7-18 November, especially at station S1, coinciding with blooms of *C. polykrikoides*. During the dry season, Chl*a* concentrations ranged between 0.35 and 52.08 µg L^{-1} (Table 2). Increases were more widespread during the last week of this sampling period and were associated with the occurrence of the benthic diatom *Pleurosigma* spp. and other diatom taxa.

Pyrodinium bahamense and Ceratium furca cell densities

The highest average cell densities of *P. bahamense* were found during the wet season with $6.8 \times 10^3 \pm 6.5 \times 10^2$ cells L^{-1} (Fig. 5). Cell densities of *C. furca* were in average lower than those of *P. bahamense* during this season ($2.0 \times 10^3 \pm 3.2 \times 10^2$ cells L^{-1}; Fig. 5). Daily and spatial variations were observed during this season in the population densities of both species (Fig. 6 and Fig. 7). Overall, the maximum concentrations were observed at the inner stations of the bay (i.e. *P. bahamense*: S1-S3; *C. furca* S2 and S3).

During the dry season, a drastic reduction in *P. bahamense* average cell densities were observed ($2.3 \times 10^2 \pm 0.23 \times 10^2$ cells L^{-1}; Fig. 8), representing only 4% of those detected during the wet season. The numerically dominant dinoflagellate during this season was *C. furca* (average: $2.3 \times 10^4 \pm 2.2 \times 10^3$ cells L^{-1}; Fig. 8), reaching in some occasions bloom conditions (i.e. 10^5 cells L^{-1}). Overall, *P. bahamense* abundance was approximately 1% that observed for *C. furca*. A trend in the daily fluctuations of both species was observed during this season. Increases in their population densities were observed around the second sampling week (i.e. especially at S4-S6), followed by a sudden reduction on March 20 (Fig. 9 and 10). Spatially, the highest cell densities of both species were mainly observed at S3 and S4.

Patterns of variability in the dinoflagellate composition

The patterns of variability in the dinoflagellate composition were identified using PCO analysis (Fig. 11). The main effect was that of seasons (i.e. accounting for a 55% of the total variation - Axis 1), with minor effects due to spatial and daily variability within each season. The wet season was represented towards the *P. bahamense* vector and the dry season towards the *C. furca* vector. Results from the PERMANOVA analysis confirmed the patterns observed in the PCO plot showing significant differences between seasons (PERMANOVA, $p = 0.001$), among days within each season (PERMANOVA, $p = 0.001$) and among stations during each season (PERMANOVA, $p = 0.001$, Table 3). Significant interactions between days (within seasons) and stations were also revealed (PERMANOVA, $p = 0.001$; Table 3). PERMANOVA analysis showed that seasons alone accounted for about 48% of the total variation (Table 3) and results of SIMPER analysis indicates that both species contributed equally (about 50%) to the observed dissimilarities between seasons (Table 4) even though the largest seasonal variation was observed for *P. bahamense*. Daily and spatial variations in the dinoflagellates only contributed ca. 10% of the total dinoflagellate variability (Table 3).

Environmental conditions and Pyrodinium bahamense and Ceratium furca variability.

The role of several environmental factors on the seasonal variations in dinoflagellate composition was assessed by dbRDA (Fig. 12). Along Axis 1, which explained ca. 39% of the total variability, seasons were separated. Strong correlations ($r > \pm 0.65$) along this axis were detected with salinity ($r = -0.84$), DOMFl ($r = 0.80$), pH ($r = -0.77$) and silicates ($r = 0.67$), suggesting their contribution to the seasonal difference in the dinoflagellate composition.

Discussion

This study was aimed at identifying the underlying mechanisms driving the alternations in abundance of two potentially HABs species at Bahía Fosforescente, *P. bahamense* and *C. furca*. Studies on dinoflagellate assemblages in this bay and available data on environmental conditions have been based on monthly samplings limiting their usefulness to decipher processes at time scales of days to weeks that may drive and control the population dynamics of these species. Different climate extremes can promote environmental pulsing processes that enable fluctuations in the dinoflagellate species abundance at periods unresolvable by monthly examinations (Smetacek and Cloern 2008). Daily samplings within the seasonal extremes

evaluated in the present work allowed for a better understanding of the environmental factors controlling *P. bahamense* and *C. furca* populations.

Daily and spatial variations in the dinoflagellate populations only contributed ca. 10% of the total variability. These variations were probably triggered by environmental forcing events, but these events did not result in alternations between *P. bahamense* and *C. furca* dominance within each seasonal extreme.

Wind pattern and the resulting wind-driven currents have been suggested to play important roles in defining the distribution of dinoflagellate patches in the bay (Seliger et al. 1971; Seixas 1988; Cedeño-Maldonado 2008; Soler-Figueroa and Otero 2015). Results from this study support these observations since *P. bahamense* and *C. furca* populations followed a similar spatial trend during both sampling campaigns. Overall, the highest concentrations were mainly observed in the north (S2), east (S3) and southeast (S4) regions of the bay, in agreement with previous observations (Seixas 1988; Soler-Figueroa and Otero 2015; Soler-Figueroa and Otero unpublished).

Wind patterns could also have been an important mechanism influencing the observed short term variations in the population densities of both species. Short term changes in phytoplankton biomass linked to wind-driven transport are described for other regions. These transport mechanisms effectively concentrate, redistribute or disperse the organisms (Yamamoto et al. 2002; Lenning et al. 2007; Anglès et al. 2008) providing a plausible mechanism explaining the fast daily changes in the spatial distribution of dinoflagellates observed during this study. The appearance of *Cochlodinium polykrikoides* at S1 during periods of northerly winds provides evidence of the importance in wind patterns since its only known source is towards the northwestern margin of the bay (Margalef 1961; Burkholder et al. 1967; Cintrón et al. 1970; Seliger et al. 1971; Seixas 1983; Cedeño-Maldonado 2008).

Short term changes in the dinoflagellates population densities could also have been influenced by wind-induced turbulent mixing and the resulting coupling between the pelagic and benthic compartments. Turbulent mixing events can facilitate resuspension of bottom sediments, especially in shallow ecosystems, and simultaneous acceleration of benthic nutrient release (Santschi et al. 1990; Warken et al. 2000). During periods of low precipitation and minor external nutrient inputs these internal nutrient pulses could be of great importance triggering phytoplankton growth (Fisher et al. 1982, Lagus et al. 2007; Guadayol et al. 2009; Cornwell et

al. 2014) and thus, promoting short term changes in their populations. This can be supported by the observed increases in *P. bahamense* and *C. furca* populations during the dry season, which followed previous increments in ammonium and N+N concentrations, not related to pluviosity. Likewise, it has been previously hypothesized that the high organic content and mangrove detritus of Bahía Fosforescente may result in ammonification processes, releasing nitrogen compounds from sediments that can trigger high densities of phytoplankton (San Juan and González 2000).

The influence of wind-induced turbulence mixing in the short term fluctuations of dinoflagellates was further demonstrated during the dry season with the appearance of several diatoms species (i.e. *Pleurosigma* spp., *Chaetoceros* spp., *Coscinodiscus* spp. and other unidentified diatoms) and concomitant reductions in *P. bahamense* and *C. furca* populations. Shifts from dinoflagellate dominance to diatom dominance during periods of strong vertical turbulent mixing have been extensively described and reported in the literature (Margalef 1978; Margalef et al. 1979; Jones and Gowen 1990; Badylak and Phlips 2004). Thus, it seems plausible that increases in winds speed during this period, with gusts ca. 7 m s^{-1} (data not shown), resulted in a sudden modification in the phytoplankton community due to the mixing of dinoflagellate cells over depth and simultaneous reductions in sedimentation losses of non-benthic diatoms.

Consistent with previous observations (Soler-Figueroa and Otero 2015; Soler-Figueroa and Otero, unpublished) our results showed that seasons and local weather play an important role in the patterns of variability of *P. bahamense* and *C. furca* at Bahía Fosforescente. Overall, seasonal changes in rainfall promoted variations in several environmental conditions that probably promoted shifts in the abundance of each dinoflagellate. Likewise, seasonal changes in phytoplankton composition have been described in many other coastal ecosystems, attributable to weather-related events, in which rainfall is the key factor by modifying water quality and delivering nutrients and other organic material (Pannard et al. 2008; Baek et al. 2009; Phlips et al. 2010; Phlips et al. 2012).

Salinity, DOMFl, pH and silicate concentrations, environmental variables that are significantly influenced by different precipitation extremes, were factors associated with the observed seasonal alternations in *P. bahamense* and *C. furca* abundances. *Pyrodinium bahamense* was consistently the numerically dominant dinoflagellate during the wet season, when rains were six times greater than those observed during the dry season. As indicated by the

lower salinities and pH, the bay during this season was characterized by inputs of terrestrial and watershed runoff that probably triggered increments in *P. bahamense* population density. The requirement for soil extracts to grow *P. bahamense* successfully in cultures (McLaughlin and Zahl 1961; Oshima et al. 1985), mainly as a source of selenium (Usup 1995), suggests the importance of land-derived materials in promoting bloom formation of this dinoflagellate.

The influence of land-derived materials during the wet season was also supported by the high DOMFl and silicates concentrations, which are well known tracers of terrestrial waters in the marine environment (Coble 1995; Corredor and Morell 2001, Otero 2009). Considering only the DOMFl, the influence of terrestrial inputs in the bay during this season seems to be substantial since values were on average five times higher than those reported by Otero (2009) for the La Parguera area. While silicates are not an essential element for the growth of *P. bahamense*, the DOM originated from the watershed could benefit the populations of this organism by providing nitrogen (Carlsson and Granéli 1993; Carlsson et al. 1993; Bushaw et al. 1996; Bronk et al. 2006) and other nutritional factors associated to humic substances (Prakash and Rashid 1968; Granéli et al. 1985). This is in agreement with the bloom dynamics of *P. bahamense* in other regions, which have been related to bioavailable nitrogen and phosphorus transported after rainfall events (Phlips et al. 2004, 2006, 2011, 2015) and to the release of humic substances from nearshore mangroves and terrestrial watersheds following rainfall periods (Usup and Azanza 1998; Morquecho et al. 2012; Usup et al. 2012). Thus, results herein suggest that land-derived nutrients inputs during the wet season, help support sustained growth of *P. bahamense*, confirming findings from previous studies (Soler-Figueroa and Otero 2015).

A different scenario was observed during the dry season, when nutrient supply from land was limited. Precipitation accounted for only 30% of that during the wet season resulting in higher salinity and pH, lower DOMFl and silicates, and a 26-fold decrease in *P. bahamense* cell densities. Low nutrient periods unfavorable for sustainable growth of this species have been linked to the production of dormant and temporary cyst stages (Anderson 1989; Corrales et al. 1995; Onda et al. 2014) that will allow for the fast recovery of the population when favorable conditions arise. Grazing may also have contributed to the *P. bahamense* decline although top-down control on *P. bahamense* populations are thought to be less important than bottom-up effects (Badylak and Phlips 2008). Higher selective grazing pressures on other phytoplankton groups (i.e. diatoms; Huntley 1982; Kim et al. 1989) seems to confirm that *P. bahamense*

populations are driven mostly by bottom-up effects. For example, *Acartia tonsa*, one of the most abundant zooplankter in the bay (Coker and González 1960; González and Bowman 1965; Olivares-Chicón 1989) has been shown to preferentially ingest diatoms over dinoflagellates (Kleppel and Burkart 1995; Paffenhöfer et al. 2005).

The onset of the dry season also resulted in a shift towards the dominance of *C. furca*, becoming blooms of up to 10^5 cells L^{-1} that visibly discolored the water. This high proliferation with minor runoff and external nutrient inputs, denoted the relevance of other environmental factors or adaptive strategies different to those of *P. bahamense*. Turbulent mixing associated with seasonal winds, promoted nutrient releases from resuspended sediments, as a result of bacterial degradation of organic matter. This effect could indirectly promote *C. furca* growth by supporting its mixotrophic capabilities (Smalley et al. 2003) by stimulating the proliferation of heterotrophic protists that in turn serve as prey for *C. furca* (Smalley et al. 1999; Smalley and Coats 2002). This seasonal ecosystem change is supported by the high turbidities detected during this season, together with the high abundance of several tintinnid species and other heterotrophic (i.e. *Protoperidinium* spp.) and mixotrophic (i.e. *Dinophysis caudata*) dinoflagellates, in contrast with the wet season.

The limited range of temperature, salinity and pH observed during this study suggests that there was no significant influence on the physiology of *P. bahamense* and *C. furca* that could explain their population's seasonal shifts. The range of all these environmental variables was well within the range of conditions in which both species actively grow. Blooms of *P. bahamense* have been observed at 25 °C or higher (Phlips et al. 2006; Sastre et al. 2013), at salinities between 16 and 42 (Phlips et al. 2004, 2006), and its optimum pH for growth is near pH 8.0 (Blackburn and Oshima 1989). Likewise, the upper pH growth limit for *C. furca* is about 9 (Søderberg and Hansen 2007), its optimal temperature range is 20-34 °C and its salinity tolerance is 17 to 42 (Baek et al. 2006; Baek et al. 2008a, b, c; Sastre et al. 2013). Thus, under the present climate conditions, variations in the precipitation regime and the resulting differences in watershed derived materials are probably the main seasonal factors modulating *P. bahamense* and *C. furca* populations at Bahía Fosforescente.

In summary, this study confirmed the role of seasonality in the patterns of variability of *P. bahamense* and *C. furca* populations at Bahía Fosforescente. Different precipitation regimes resulted in different environmental conditions which in turn promoted seasonal shifts in

dinoflagellates dominance. Short-term variations in the dinoflagellate populations were also revealed, probably related to wind patterns and wind-induced pelagic-benthic interactions. This is the first study conducted at the bay with a high spatiotemporal resolution and yielded vital information to develop a robust link between environmental forcing and the response of the dinoflagellate populations.

Although this study provided new insights on processes underlying the population dynamics of *P. bahamense* and *C. furca* at Bahía Fosforescente, much research is still needed. For example, information on the whole phytoplankton community in the bay is limited. Under some circumstances, diatoms could be an important component of the phytoplankton community representing a potential source for light and nutrient competition with the extant dinoflagellate assemblages. Thus, future studies should include the examination of the whole phytoplankton community and explore possible interactions among different groups. Other factors that are yet to be defined include the possible top-down control over the dinoflagellate populations and the pelagic-benthic interactions as a source of nutrients and cysts reservoirs.

In conclusion, this study shows the importance of high frequency monitoring to understand better the impacts of environmental forcing in this coastal ecosystem. The findings that the dinoflagellate populations at Bahía Fosforescente are strongly modulated by weather and runoff have profound management implications and warn of the sensitiveness of this and other similar coastal ecosystems to changes in watershed practices and to climate change. Thus, a well stablished long-term monitoring program will provide the best science based management approach to guarantee the role of this environment as important habitat for protected species and for tourism.

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Tables

Table 1. Physicochemical variables measured at Bahía Fosforescente during the wet season from November 5 to December 5, 2012.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 1								
Day 1	3.46	23.98	0.17	5.21	0.90	2.81	1.82	2.12
Day 2	3.60	29.35	0.16	5.15	1.25	2.43	1.60	2.15
Day 3	3.61	30.16	0.27	5.63	1.08	1.43	9.43	1.55
Day 4	3.82	24.39	0.34	4.50	1.23	1.94	1.45	1.33
Day 5	4.21	30.42	0.20	4.40	1.09	2.30	5.94	2.76
Day 6	3.47	21.72	0.20	5.24	1.19	2.82	2.46	1.68
Day 7	3.83	21.83	0.13	4.96	1.02	2.09	2.16	1.22
Day 8	3.80	20.79	0.14	4.88	0.97	2.40	2.96	1.61
Day 9	3.24	27.14	0.20	5.67	1.25	2.45	4.00	1.39
Day 10	3.74	24.48	0.15	5.55	1.34	2.23	1.28	1.68
Day 11	3.18	24.20	0.17	5.42	1.31	3.51	1.38	1.25
Day 12	3.71	25.19	0.18	5.17	1.29	2.44	3.01	2.61
Day 13	4.58	26.02	0.50	4.85	0.86	0.87	21.49	1.24
Day 14	4.43	20.65	0.67	4.89	0.87	1.06	25.83	1.18
Day 15	4.23	20.14	0.35	4.81	0.60	1.13	3.79	3.44
Day 16	3.51	21.93	0.20	5.05	0.68	1.86	-	-
Day 17	3.93	17.05	0.21	5.43	0.55	1.73	1.74	1.69
Day 18	3.92	17.81	0.20	6.04	0.72	2.32	0.98	1.29
Day 19	4.15	16.06	0.13	5.27	0.78	2.11	0.45	1.33
Day 20	3.70	18.07	0.14	5.00	1.11	2.49	2.19	1.28
Day 21	3.99	15.44	0.16	5.22	1.03	1.99	1.93	1.51
Day 22	3.48	20.32	0.22	5.33	1.54	2.78	2.20	1.45
Day 23	3.52	17.59	0.27	4.70	1.11	2.04	1.98	1.61
Day 24	3.58	14.89	0.19	3.33	1.01	2.49	1.56	1.59
Day 25	3.70	20.51	0.16	5.55	0.94	0.40	3.08	0.83
Day 26	3.68	16.59	0.16	3.82	0.54	1.02	0.98	0.94
Day 27	3.61	17.36	0.20	3.68	0.58	1.48	2.07	1.52
Day 28	3.43	23.65	0.19	4.73	0.58	1.04	4.87	1.48
Day 29	3.21	18.29	0.22	3.87	0.63	1.90	2.17	1.64
Day 30	2.98	21.46	0.20	4.41	0.73	1.39	2.35	1.28
Day 31	3.38	18.99	0.18	3.23	0.75	2.18	2.17	1.14

Table 1 cont. Physicochemical variables measured at Bahía Fosforescente during the wet season from November 5 to December 5, 2012.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 2								
Day 1	4.02	32.00	0.15	5.17	0.98	3.06	0.97	1.70
Day 2	3.94	25.99	0.15	4.41	1.19	2.67	1.38	1.47
Day 3	3.91	17.50	0.15	4.62	1.47	2.10	0.71	1.45
Day 4	4.09	20.20	0.20	3.89	1.34	2.03	0.70	1.28
Day 5	4.47	21.25	0.17	4.22	1.12	2.18	1.49	1.06
Day 6	3.35	26.47	0.22	4.68	1.28	3.25	2.02	1.10
Day 7	4.53	19.10	0.13	4.43	0.95	1.63	1.60	1.11
Day 8	3.73	21.30	0.16	5.15	1.29	2.41	1.38	1.22
Day 9	4.20	23.74	0.16	5.09	1.16	2.02	2.36	1.37
Day 10	3.35	21.11	0.18	5.03	1.47	3.75	2.83	0.87
Day 11	3.78	20.53	0.18	4.70	1.37	3.00	1.13	0.89
Day 12	4.27	20.34	0.18	4.78	1.06	1.99	2.95	1.35
Day 13	4.76	19.34	0.16	4.13	0.88	1.05	2.20	1.21
Day 14	4.74	18.88	0.16	4.12	0.66	1.11	1.23	1.02
Day 15	4.50	16.18	0.14	4.13	0.45	0.91	0.97	1.05
Day 16	4.12	17.99	0.23	4.70	0.35	1.21	-	-
Day 17	3.73	25.09	0.21	5.42	0.82	1.70	0.97	0.99
Day 18	3.95	20.75	0.21	5.27	0.87	1.73	-	-
Day 19	3.95	15.99	0.12	4.84	1.36	2.63	0.41	0.81
Day 20	3.64	16.24	0.16	4.59	1.01	2.43	2.59	2.23
Day 21	4.34	14.83	0.17	4.15	0.94	1.78	2.05	1.20
Day 22	3.89	15.83	0.23	4.67	1.30	2.39	3.37	1.32
Day 23	4.40	15.23	0.19	3.81	1.05	1.90	1.52	1.19
Day 24	4.08	15.87	0.20	3.42	1.07	1.66	4.64	1.66
Day 25	4.38	14.30	0.13	2.23	0.76	1.31	0.64	0.66
Day 26	4.11	14.68	0.18	2.72	0.70	1.95	1.10	1.27
Day 27	3.80	18.91	0.23	2.94	0.64	2.13	1.61	1.42
Day 28	4.70	21.41	0.26	3.28	0.63	1.96	2.67	1.18
Day 29	3.42	23.80	0.25	3.54	0.47	1.93	1.69	1.31
Day 30	3.27	19.14	0.22	2.81	0.52	1.50	3.88	1.59
Day 31	3.65	21.32	0.18	2.11	0.64	1.76	2.74	0.76

Table 1 cont. Physicochemical variables measured at Bahía Fosforescente during the wet season from November 5 to December 5, 2012.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 3								
Day 1	3.06	22.11	0.36	5.39	0.90	9.20	2.22	6.48
Day 2	3.06	21.92	0.35	5.08	1.02	7.48	3.34	6.07
Day 3	2.91	26.70	0.42	4.75	1.15	6.57	3.72	7.74
Day 4	3.45	21.41	0.43	4.64	1.02	6.83	2.89	5.34
Day 5	4.80	20.44	0.32	4.63	1.09	2.73	2.62	4.95
Day 6	3.93	20.15	0.36	4.92	0.82	2.41	3.43	3.66
Day 7	4.11	23.92	0.29	4.84	0.91	3.57	2.71	4.78
Day 8	3.98	18.70	0.24	5.06	1.00	3.80	1.29	2.53
Day 9	4.02	20.96	0.26	5.43	1.07	2.78	3.45	2.74
Day 10	3.55	21.23	0.20	5.38	1.23	2.99	3.03	1.51
Day 11	3.91	23.62	0.22	4.85	1.15	2.41	1.48	1.86
Day 12	3.84	18.24	0.22	5.13	0.98	2.51	1.49	2.66
Day 13	4.19	20.63	0.22	4.65	0.89	2.64	2.88	4.03
Day 14	4.02	20.63	0.28	4.76	0.50	3.00	4.20	5.16
Day 15	4.02	17.97	0.29	5.22	0.58	2.79	2.67	2.83
Day 16	3.72	20.41	0.39	5.17	0.41	2.31	-	-
Day 17	4.10	17.27	0.25	5.12	0.52	1.70	1.60	2.18
Day 18	3.77	16.92	0.32	5.92	0.62	3.03	-	-
Day 19	4.40	18.53	0.15	4.82	0.64	1.87	2.72	1.80
Day 20	4.05	19.16	0.25	4.80	0.84	2.51	3.02	2.91
Day 21	4.06	19.00	0.28	4.76	1.17	1.95	3.33	3.41
Day 22	4.35	16.18	0.25	4.33	1.10	1.99	3.71	3.00
Day 23	3.88	15.66	0.37	4.66	1.12	2.58	2.01	3.51
Day 24	4.50	14.23	0.22	3.19	0.84	1.99	1.43	2.15
Day 25	4.42	26.37	0.22	3.91	2.68	2.83	0.44	0.58
Day 26	4.77	18.03	0.18	2.71	0.98	1.97	0.43	0.65
Day 27	4.06	15.42	0.35	3.06	0.66	1.56	4.19	3.32
Day 28	5.06	21.37	0.23	2.98	1.13	1.87	1.93	1.09
Day 29	4.55	16.17	0.31	2.45	0.36	1.04	19.17	4.93
Day 30	4.20	18.89	0.25	2.14	0.37	0.73	2.76	1.96
Day 31	3.78	17.92	0.27	1.96	0.52	0.95	2.32	2.04

Table 1 cont. Physicochemical variables measured at Bahía Fosforescente during the wet season from November 5 to December 5, 2012.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 4								
Day 1	4.40	52.50	0.28	5.13	0.71	2.85	1.62	4.42
Day 2	4.27	29.00	0.16	4.43	1.06	2.53	0.98	1.68
Day 3	4.76	27.00	0.15	3.93	1.15	1.48	1.45	1.56
Day 4	4.27	24.87	0.19	3.77	1.20	1.95	1.39	1.23
Day 5	4.92	20.68	0.18	4.03	1.23	1.83	2.14	1.90
Day 6	4.65	25.38	0.19	4.39	0.75	1.69	0.66	1.22
Day 7	3.56	20.36	0.27	4.92	0.95	4.13	4.86	4.83
Day 8	4.45	27.60	0.13	4.72	0.88	1.77	1.28	1.91
Day 9	3.95	19.05	0.14	4.94	1.18	2.49	1.23	1.37
Day 10	3.69	19.88	0.14	5.15	1.47	2.23	0.54	1.59
Day 11	3.75	15.89	0.14	4.79	1.41	1.70	0.26	1.38
Day 12	4.42	23.26	0.17	4.54	1.18	1.98	1.07	1.26
Day 13	4.85	21.59	0.17	3.96	0.87	0.95	-	-
Day 14	4.73	17.87	0.14	3.99	0.63	0.88	2.08	3.62
Day 15	4.64	19.72	0.16	4.17	0.40	0.77	1.09	1.22
Day 16	4.56	22.30	0.18	4.44	0.26	0.91	-	-
Day 17	4.24	16.16	0.19	5.30	0.36	1.70	0.92	1.27
Day 18	4.51	17.75	0.19	5.04	0.51	1.86	-	-
Day 19	4.54	16.61	0.11	4.23	0.59	1.71	0.18	0.80
Day 20	3.92	17.61	0.21	4.82	0.80	2.60	1.98	2.06
Day 21	4.26	16.23	0.32	4.82	0.85	3.38	1.74	3.22
Day 22	3.94	18.97	0.23	5.14	1.05	2.49	1.23	2.69
Day 23	3.94	13.38	0.21	3.76	1.15	2.03	0.76	1.64
Day 24	4.49	16.17	0.16	2.93	0.83	1.74	0.83	1.21
Day 25	4.42	19.95	0.16	3.25	1.50	2.22	0.18	0.65
Day 26	4.86	16.67	0.17	2.53	0.72	1.67	0.52	0.83
Day 27	4.75	15.17	0.21	2.69	0.49	1.21	1.06	1.27
Day 28	5.05	21.79	0.20	2.99	0.77	1.92	1.16	0.74
Day 29	4.37	17.21	0.30	2.63	0.33	1.53	2.09	2.80
Day 30	4.29	16.14	0.26	2.31	0.41	0.90	2.20	2.80
Day 31	3.65	17.84	0.33	2.40	0.48	1.77	3.52	4.02

Table 1 cont. Physicochemical variables measured at Bahía Fosforescente during the wet season from November 5 to December 5, 2012.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 5								
Day 1	4.79	21.06	0.17	4.67	0.84	2.05	1.72	2.88
Day 2	4.35	24.09	0.16	4.20	1.15	1.83	0.97	1.65
Day 3	4.72	19.61	0.13	3.78	1.22	1.16	0.65	1.45
Day 4	4.66	18.83	0.18	3.68	1.01	1.06	0.80	1.27
Day 5	5.12	23.90	0.17	3.88	1.10	1.70	0.57	1.05
Day 6	4.52	18.63	0.15	4.76	0.94	1.53	0.26	1.99
Day 7	4.03	16.00	0.14	4.49	1.16	1.71	2.53	3.58
Day 8	4.07	15.52	0.15	4.94	1.11	2.05	0.25	2.33
Day 9	4.27	17.81	0.14	4.84	0.98	1.65	0.89	1.94
Day 10	4.20	14.88	0.12	5.07	0.92	1.49	0.60	3.30
Day 11	4.51	16.06	0.12	5.05	1.01	1.43	0.36	1.44
Day 12	4.58	17.55	0.16	4.51	1.24	1.39	0.59	2.05
Day 13	4.65	16.27	0.13	3.96	0.85	1.11	0.76	1.86
Day 14	4.46	17.68	0.13	4.08	0.73	0.89	1.54	2.68
Day 15	4.63	21.50	0.14	4.19	0.42	0.92	1.36	1.97
Day 16	4.66	18.37	0.17	4.35	0.37	1.00	-	-
Day 17	4.16	21.58	0.19	4.61	0.49	1.34	0.62	1.42
Day 18	4.40	16.23	0.17	4.84	0.55	1.41	-	-
Day 19	4.84	13.95	0.14	4.34	0.61	1.41	0.86	0.87
Day 20	3.79	15.13	0.18	4.48	0.92	2.14	0.69	1.92
Day 21	3.99	16.77	0.17	4.30	1.00	2.06	0.72	1.69
Day 22	3.98	14.33	0.21	4.68	1.12	2.55	0.52	1.67
Day 23	3.95	13.19	0.17	3.59	0.92	1.49	0.37	1.77
Day 24	4.36	14.25	0.14	2.39	0.88	1.90	0.41	1.25
Day 25	4.45	22.37	0.17	3.42	1.15	2.00	0.09	0.69
Day 26	4.81	13.60	0.15	2.53	0.54	1.04	0.50	0.86
Day 27	4.71	18.34	0.19	2.67	0.55	1.17	0.83	0.91
Day 28	4.87	17.34	0.19	2.40	0.51	0.72	0.86	1.08
Day 29	4.05	16.78	0.22	2.57	0.49	1.42	1.06	2.58
Day 30	4.17	15.50	0.20	2.42	0.65	0.91	1.37	2.79
Day 31	3.97	15.99	0.21	2.39	0.62	1.62	1.53	2.55

Table 1 cont. Physicochemical variables measured at Bahía Fosforescente during the wet season from November 5 to December 5, 2012.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 6								
Day 1	4.50	28.07	0.15	4.80	0.83	2.17	1.54	2.98
Day 2	4.60	39.15	0.14	4.19	1.09	1.63	1.42	2.21
Day 3	5.03	20.44	0.11	4.06	1.05	1.22	0.14	2.11
Day 4	4.83	25.20	0.18	3.82	1.09	1.36	0.56	1.74
Day 5	4.92	18.43	0.16	4.03	1.19	1.55	0.36	1.44
Day 6	4.31	16.83	0.16	4.75	1.03	1.31	0.33	1.90
Day 7	4.15	17.29	0.14	4.45	1.22	1.59	0.58	2.31
Day 8	4.08	16.09	0.16	5.07	1.19	1.73	0.28	2.20
Day 9	4.09	17.07	0.13	4.89	1.20	1.77	1.19	2.08
Day 10	4.20	14.16	0.12	4.71	1.12	1.24	0.25	1.47
Day 11	4.00	15.55	0.14	4.72	1.12	1.25	0.10	2.07
Day 12	4.40	18.01	0.16	4.33	1.07	1.50	1.16	1.87
Day 13	4.64	14.16	0.13	3.89	0.83	1.01	0.11	1.59
Day 14	4.70	16.73	0.12	4.08	0.63	0.79	0.51	1.53
Day 15	4.54	18.07	0.14	4.19	0.65	0.82	0.80	1.91
Day 16	4.14	15.92	0.18	4.25	0.61	0.99	-	-
Day 17	4.45	15.97	0.18	5.17	0.48	1.19	0.73	1.50
Day 18	4.66	15.00	0.19	5.28	0.23	1.53	-	-
Day 19	4.68	16.77	0.14	4.65	0.54	1.71	2.78	2.25
Day 20	3.68	18.79	0.12	4.43	0.77	2.13	0.78	2.06
Day 21	3.73	13.95	0.20	4.70	1.22	2.09	0.51	2.09
Day 22	4.14	14.19	0.17	4.21	1.17	2.07	0.52	1.99
Day 23	4.21	13.67	0.16	3.01	0.90	1.18	0.46	1.70
Day 24	4.22	12.12	0.14	2.25	0.99	1.23	1.14	1.40
Day 25	4.22	14.49	0.16	2.87	1.53	1.46	0.10	0.53
Day 26	4.28	14.94	0.17	2.85	0.70	1.38	0.51	1.39
Day 27	3.90	16.97	0.18	2.59	0.46	1.26	1.03	1.29
Day 28	4.21	17.70	0.21	2.74	0.43	0.63	1.70	1.12
Day 29	3.57	19.21	0.20	2.82	0.45	0.99	1.82	1.17
Day 30	4.02	16.82	0.20	2.50	0.53	0.85	1.55	2.43
Day 31	4.05	15.65	0.23	2.14	0.58	1.40	1.56	2.55

DO = Dissolved oxygen (mg L^{-1}); DOMFl = Dissolved organic matter fluorescence (ng QS ml^{-1}); PO_4^{3-} = Phosphate (µmol L^{-1}); SiO_2 = Silicate (µmol L^{-1}); N+N = Nitrites+nitrates (µmol L^{-1}); NH_4^+ = Ammonium (µmol L^{-1}); Chla (µg L^{-1}); Turbidity (NTU)

Table 2. Physicochemical variables measured at Bahía Fosforescente during the dry season from March 1 to 31, 2013.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 1								
Day 1	4.44	11.11	0.22	4.13	1.21	3.51	1.33	2.31
Day 2	4.29	32.23	0.22	4.33	1.22	1.99	2.51	2.61
Day 3	5.05	17.75	0.34	3.95	1.10	1.04	4.78	3.12
Day 4	4.85	20.72	0.23	3.39	0.90	1.33	2.10	1.78
Day 5	4.53	12.70	0.20	3.87	0.70	2.15	2.42	2.28
Day 6	3.74	16.74	0.20	4.50	0.78	2.01	2.98	2.91
Day 7	4.41	11.95	0.20	4.45	0.87	2.39	1.31	1.63
Day 8	3.90	13.43	0.23	4.63	1.01	2.92	0.58	1.71
Day 9	4.06	12.86	0.19	4.98	1.12	3.28	0.92	2.05
Day 10	4.18	12.93	0.18	5.56	1.39	4.07	13.79	4.74
Day 11	3.81	12.54	0.19	5.25	1.31	3.72	1.01	2.18
Day 12	4.73	10.83	0.17	5.23	1.03	2.13	1.11	1.53
Day 13	4.67	11.47	0.22	4.48	1.22	2.24	16.39	2.47
Day 14	4.84	11.54	0.23	4.49	1.13	1.63	4.98	2.02
Day 15	5.22	11.75	0.23	4.40	0.89	0.96	3.62	1.95
Day 16	5.21	11.15	0.19	2.89	0.51	1.34	2.13	2.35
Day 17	4.40	10.93	0.19	3.86	0.53	2.14	2.29	2.23
Day 18	4.12	10.83	0.20	3.47	0.59	2.13	13.98	4.49
Day 19	4.25	12.69	0.14	3.25	0.69	1.75	20.99	5.43
Day 20	4.54	12.34	0.13	3.41	0.78	0.88	1.17	1.85
Day 21	4.62	12.97	0.16	2.73	0.53	1.23	5.15	3.03
Day 22	4.64	13.37	0.14	2.44	0.79	2.02	3.33	2.30
Day 23	4.44	14.50	0.13	3.14	0.70	2.05	7.14	2.96
Day 24	4.51	12.06	0.13	2.44	0.47	1.92	2.67	2.59
Day 25	4.88	12.79	0.16	2.30	0.49	1.40	7.19	2.88
Day 26	4.70	12.41	0.17	1.92	0.26	0.61	2.68	2.55
Day 27	5.21	11.44	0.17	1.77	0.23	0.33	2.76	2.61
Day 28	4.99	11.76	0.14	1.89	0.32	0.60	3.57	4.03
Day 29	4.98	11.39	0.14	0.90	0.27	0.93	3.56	2.58
Day 30	4.60	12.51	0.15	1.74	0.28	0.84	5.86	2.32
Day 31	5.28	11.45	0.15	1.80	0.30	0.31	7.78	2.13

Table 2 cont. Physicochemical variables measured at Bahía Fosforescente during the dry season from March 1 to 31, 2013.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 2								
Day 1	4.40	11.19	0.22	4.31	1.22	3.61	1.15	2.12
Day 2	4.47	22.42	0.19	3.80	1.17	2.12	1.60	1.63
Day 3	4.49	13.74	0.26	3.99	1.26	1.81	3.12	2.60
Day 4	4.94	30.24	0.21	3.43	0.91	1.28	3.51	1.75
Day 5	4.95	16.35	0.21	3.29	0.59	1.63	3.34	2.45
Day 6	4.16	12.39	0.20	3.88	0.76	2.41	2.44	2.53
Day 7	4.68	10.70	0.25	4.06	0.83	2.20	1.46	2.38
Day 8	4.09	11.72	0.22	4.43	1.05	3.38	1.02	1.64
Day 9	4.36	11.88	0.17	4.62	1.23	3.24	1.26	2.26
Day 10	4.08	18.67	0.17	5.43	1.38	3.99	2.07	2.89
Day 11	4.02	11.96	0.17	4.87	1.33	4.12	1.18	1.73
Day 12	4.95	11.30	0.18	4.64	1.18	2.80	0.73	1.70
Day 13	4.95	13.00	0.21	4.31	1.28	2.74	1.93	2.44
Day 14	4.79	17.26	0.26	3.53	1.17	1.94	2.48	1.59
Day 15	5.48	11.28	0.23	2.86	1.07	0.91	5.26	5.23
Day 16	5.40	13.24	0.22	2.06	0.34	0.91	3.37	1.86
Day 17	4.79	11.88	0.21	2.12	0.49	1.74	2.77	2.21
Day 18	4.64	11.93	0.22	2.73	0.68	2.04	2.47	5.22
Day 19	4.65	10.98	0.13	2.65	0.71	1.89	2.61	2.00
Day 20	5.10	11.57	0.13	2.36	0.72	1.00	1.45	1.86
Day 21	4.82	12.47	0.13	2.09	0.56	1.27	1.08	1.57
Day 22	4.77	12.02	0.14	1.66	0.61	1.33	1.80	1.39
Day 23	4.88	12.36	0.12	1.54	0.47	1.64	2.62	1.53
Day 24	5.08	12.72	0.13	1.33	0.34	1.35	2.00	1.75
Day 25	5.14	11.80	0.17	1.13	0.31	0.80	3.70	1.94
Day 26	5.32	11.00	0.18	1.09	0.15	0.13	5.54	3.07
Day 27	5.65	11.40	0.17	0.90	0.12	0.23	3.07	2.23
Day 28	5.28	12.48	0.20	0.95	0.16	0.31	3.52	2.88
Day 29	4.83	11.86	0.16	0.96	0.16	0.56	5.97	2.53
Day 30	5.19	11.40	0.16	1.38	0.13	0.35	7.96	2.51
Day 31	5.28	11.57	0.16	0.90	0.15	0.25	5.07	1.91

Table 2 cont. Physicochemical variables measured at Bahía Fosforescente during the dry season from March 1 to 31, 2013.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 3								
Day 1	4.76	10.77	0.24	4.82	1.26	3.27	1.55	3.15
Day 2	4.77	10.90	0.25	4.51	1.33	2.24	2.06	3.27
Day 3	4.91	11.59	0.32	4.03	1.16	2.05	3.79	3.89
Day 4	5.50	11.20	0.30	3.70	0.75	1.02	5.65	4.36
Day 5	5.12	11.06	0.29	3.60	0.53	1.90	3.30	5.03
Day 6	3.80	12.27	0.37	4.30	0.90	4.13	4.01	6.33
Day 7	4.35	12.67	0.32	4.52	0.85	4.69	2.73	6.62
Day 8	4.27	11.29	0.32	4.84	1.09	3.98	1.69	4.14
Day 9	4.57	11.86	0.29	5.12	1.28	4.08	3.35	6.72
Day 10	4.19	19.83	0.23	5.29	1.30	4.05	2.68	4.55
Day 11	3.71	14.88	0.27	5.29	1.37	4.96	3.73	6.69
Day 12	4.34	13.27	0.25	4.89	1.47	2.89	6.63	7.36
Day 13	4.57	11.67	0.22	4.57	1.47	2.81	2.58	3.36
Day 14	4.49	15.07	0.25	4.24	1.35	2.27	4.55	5.38
Day 15	5.38	10.86	0.29	3.59	0.87	1.06	4.75	3.99
Day 16	5.08	10.51	0.22	2.15	0.47	0.83	4.51	2.83
Day 17	4.52	10.79	0.24	2.50	0.48	1.80	1.37	2.59
Day 18	4.70	9.93	0.21	2.55	0.57	1.92	2.68	3.14
Day 19	4.61	11.78	0.28	3.11	0.61	2.38	6.30	8.46
Day 20	5.06	10.93	0.19	2.61	0.80	1.00	1.81	3.38
Day 21	5.04	13.45	0.19	2.44	0.48	1.41	4.70	3.21
Day 22	4.80	10.92	0.20	2.53	0.57	1.78	2.57	2.98
Day 23	4.95	11.52	0.19	1.68	0.34	1.50	4.46	5.08
Day 24	5.13	12.23	0.21	1.73	0.53	1.91	3.31	2.93
Day 25	4.71	11.33	0.26	1.99	0.51	1.70	4.14	4.03
Day 26	4.72	11.11	0.28	1.74	0.33	1.16	4.42	3.95
Day 27	4.96	11.72	0.25	1.28	0.24	1.47	3.48	4.72
Day 28	4.88	13.61	0.24	1.12	0.23	0.53	4.95	5.03
Day 29	5.36	14.70	0.19	0.77	0.13	0.26	5.40	3.62
Day 30	4.86	11.88	0.20	0.94	0.21	0.66	11.55	9.28
Day 31	5.78	11.36	0.19	0.88	0.08	0.22	9.89	3.51

Table 2 cont. Physicochemical variables measured at Bahía Fosforescente during the dry season from March 1 to 31, 2013.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 4								
Day 1	4.97	10.93	0.32	4.70	1.13	2.96	1.76	5.19
Day 2	4.98	22.49	0.36	4.28	1.13	2.10	3.58	6.71
Day 3	5.06	12.14	0.30	3.95	1.04	1.70	3.16	4.39
Day 4	5.75	12.67	0.28	3.58	0.74	1.44	2.22	4.12
Day 5	4.84	11.66	0.26	3.62	0.60	2.31	1.53	4.69
Day 6	4.01	11.18	0.29	4.06	0.71	2.15	-	-
Day 7	5.03	16.22	0.26	4.40	0.59	2.04	0.64	3.63
Day 8	4.33	12.26	0.25	4.62	0.99	3.60	0.63	3.32
Day 9	4.46	12.18	0.27	4.88	1.12	4.21	2.93	6.71
Day 10	4.18	12.98	0.26	5.04	1.27	3.92	1.66	6.00
Day 11	4.25	11.36	0.23	5.13	1.11	3.87	1.49	3.84
Day 12	4.66	10.10	0.20	4.72	1.14	2.61	2.27	4.19
Day 13	4.76	10.95	0.30	4.31	0.90	3.15	3.83	9.92
Day 14	4.94	10.97	0.29	3.92	1.09	2.55	6.61	11.44
Day 15	5.72	10.54	0.33	3.10	0.84	1.38	7.55	10.07
Day 16	5.29	13.68	0.23	2.18	0.44	1.00	2.39	3.22
Day 17	4.77	10.52	0.23	2.35	0.39	1.79	32.39	9.82
Day 18	5.05	10.43	0.23	2.92	0.50	2.12	2.21	4.78
Day 19	4.71	10.40	0.15	3.19	0.62	2.36	1.84	3.01
Day 20	5.01	10.96	0.21	3.62	0.63	2.34	5.15	4.50
Day 21	5.07	12.01	0.26	2.46	0.40	1.80	3.22	4.93
Day 22	4.19	14.27	0.28	2.67	0.47	2.49	4.09	8.12
Day 23	5.48	11.46	0.15	1.75	0.36	1.36	1.59	3.04
Day 24	4.61	12.27	0.15	1.95	0.42	1.62	2.68	3.65
Day 25	5.00	13.64	0.26	1.54	0.29	1.29	21.25	7.51
Day 26	4.97	11.11	0.22	1.53	0.18	0.53	4.45	4.47
Day 27	5.38	11.36	0.25	1.31	0.15	1.00	3.77	5.20
Day 28	5.08	11.46	0.23	1.39	0.13	0.66	3.78	6.87
Day 29	5.37	10.88	0.21	0.85	0.09	0.29	3.65	5.77
Day 30	5.35	12.34	0.22	0.89	0.08	0.32	7.51	3.94
Day 31	6.12	11.60	0.16	0.71	0.08	0.08	9.19	1.82

Table 2 cont. Physicochemical variables measured at Bahía Fosforescente during the dry season from March 1 to 31, 2013.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 5								
Day 1	4.95	10.48	0.22	4.70	1.12	2.81	4.33	4.38
Day 2	4.81	12.08	0.21	3.96	1.14	1.67	2.70	2.67
Day 3	5.43	10.04	0.19	3.33	0.92	0.77	3.26	3.14
Day 4	5.81	10.45	0.17	2.93	0.69	0.78	1.66	1.62
Day 5	4.95	11.54	0.21	3.33	0.56	1.61	2.63	3.49
Day 6	4.57	21.39	0.17	3.46	0.62	1.53	-	-
Day 7	5.15	10.47	0.18	3.98	0.71	1.96	2.23	2.23
Day 8	4.26	14.21	0.23	4.33	0.90	2.89	0.45	2.49
Day 9	4.64	11.15	0.19	4.48	1.17	3.47	0.84	2.21
Day 10	4.09	12.16	0.21	4.53	1.23	2.73	1.31	4.59
Day 11	4.36	11.53	0.18	4.78	1.14	3.15	3.96	2.83
Day 12	5.37	13.50	0.18	4.37	0.97	2.52	1.70	2.35
Day 13	4.99	10.90	0.25	4.40	1.09	2.40	2.36	4.57
Day 14	5.01	14.95	0.29	3.89	1.05	1.85	5.11	5.68
Day 15	5.67	11.01	0.26	3.44	0.76	0.94	3.60	4.25
Day 16	5.37	12.93	0.25	2.24	0.47	1.17	4.61	4.17
Day 17	5.11	10.51	0.20	2.32	0.38	1.55	2.33	3.32
Day 18	5.12	11.55	0.17	2.74	0.54	1.80	2.01	2.50
Day 19	5.30	9.19	0.10	2.83	0.52	1.45	1.85	1.92
Day 20	5.71	9.41	0.12	2.54	0.70	1.00	1.94	2.14
Day 21	5.63	9.63	0.14	1.97	0.45	0.81	1.97	2.01
Day 22	5.36	10.54	0.15	2.78	0.47	1.20	1.34	2.09
Day 23	5.36	10.87	0.10	1.74	0.44	1.21	1.23	1.51
Day 24	4.97	11.37	0.15	1.72	0.34	1.49	2.24	2.79
Day 25	5.32	10.87	0.18	1.61	0.33	0.69	2.84	2.34
Day 26	5.49	12.27	0.14	1.14	0.10	0.16	3.04	2.25
Day 27	5.68	11.05	0.18	1.27	0.11	0.19	3.33	3.55
Day 28	5.22	10.86	0.19	1.26	0.11	0.43	3.55	4.96
Day 29	5.01	12.16	0.22	0.79	0.12	1.07	4.80	6.78
Day 30	5.39	11.36	0.24	1.45	0.12	0.85	6.45	5.85
Day 31	5.85	11.15	0.14	0.71	0.08	0.08	8.53	1.72

Table 2 cont. Physicochemical variables measured at Bahía Fosforescente during the dry season from March 1 to 31, 2013.

Station/Day	DO	DOMFl	PO_4^{3-}	SiO_2	N+N	NH_4^+	Chl*a*	Turbidity
Station 6								
Day 1	4.82	10.70	0.24	4.24	1.08	2.27	1.06	3.84
Day 2	4.96	10.05	0.22	3.93	1.08	1.79	1.96	3.59
Day 3	4.84	9.76	0.21	3.28	0.93	0.84	1.35	2.91
Day 4	5.65	10.35	0.19	3.10	0.77	0.63	0.90	2.55
Day 5	4.84	11.10	0.21	3.28	0.71	1.33	1.22	4.11
Day 6	4.58	11.52	0.21	3.76	0.90	1.31	-	-
Day 7	4.79	9.03	0.21	3.99	0.63	1.91	0.77	2.78
Day 8	4.34	12.84	0.22	4.13	1.25	2.01	0.52	3.43
Day 9	4.03	11.26	0.23	4.70	1.17	3.70	1.08	4.32
Day 10	4.09	12.86	0.16	4.86	1.27	3.31	23.51	6.32
Day 11	4.12	10.22	0.20	4.24	1.25	2.19	0.56	3.35
Day 12	4.96	13.58	0.17	4.14	1.01	1.94	0.69	3.08
Day 13	5.01	10.50	0.23	4.15	1.00	2.16	1.22	3.94
Day 14	5.05	9.81	0.22	3.66	0.87	1.47	1.33	3.15
Day 15	5.40	10.77	0.26	3.32	0.89	1.06	2.52	4.02
Day 16	5.62	10.46	0.25	2.30	0.49	1.16	52.08	9.29
Day 17	4.65	13.10	0.20	2.72	0.53	1.62	1.35	3.56
Day 18	4.67	19.56	0.20	3.09	0.72	1.87	1.15	3.23
Day 19	4.67	9.57	0.15	2.89	0.64	1.56	1.51	2.96
Day 20	5.21	11.71	0.12	2.69	0.78	1.12	0.35	1.93
Day 21	5.24	9.86	0.14	2.21	0.53	0.97	0.72	2.24
Day 22	4.99	10.30	0.14	2.60	0.72	1.24	0.85	2.91
Day 23	5.01	10.19	0.13	2.96	0.69	1.36	1.23	2.78
Day 24	4.95	11.30	0.14	1.55	0.29	1.23	1.12	1.75
Day 25	5.16	10.11	0.16	2.74	0.50	1.01	1.53	2.98
Day 26	5.27	9.76	0.13	2.08	0.43	0.51	1.63	2.88
Day 27	6.01	10.20	0.16	1.44	0.18	0.25	1.53	2.98
Day 28	5.35	12.30	0.15	1.57	0.16	0.62	1.47	3.81
Day 29	4.73	11.25	0.21	1.63	0.15	1.02	4.57	4.03
Day 30	5.37	11.14	0.14	2.04	0.15	0.56	2.86	2.70
Day 31	-	14.61	0.14	0.79	0.12	0.12	4.23	2.34

DO = Dissolved oxygen (mg L^{-1}); DOMFl = Dissolved organic matter fluorescence (ng QS ml^{-1}); PO_4^{3-} = Phosphate (µmol L^{-1}); SiO_2 = Silicate (µmol L^{-1}); N+N = Nitrites+nitrates (µmol L^{-1}); NH_4^+ = Ammonium (µmol L^{-1}); Chla (µg L^{-1}); Turbidity (NTU)

Table 3. PERMANOVA results comparing *P. bahamense* and *C. furca* abundances between seasons (Factor - Season), among days within each season (Factor - Days (Season)), among stations (Factor - Station), among stations within each season (Factor - Season x Station) and the interactions between days and stations for each season (Factor - Day (Season) x Station). Tests were based on 999 permutations using a Bray-Curtis resemblance matrix.

Source	df	SS	MS	Pseudo-F	P (perm)	Unique perms	Components of variation	% contribution
Season	1	98317	98317	146.13	0.001	999	175.46	48.08
Station	5	35359	7071.9	32.913	0.001	999	36.964	10.13
Day (Season)	60	40375	672.92	79.86	0.001	999	37.009	10.14
Season x Station	5	18737	3747.4	17.441	0.001	998	38.086	10.44
Day (Season) x Station	300	64506	215.02	25.518	0.001	997	68.996	18.91
Residual	742	6252.3	8.4263				8.4263	2.31
Total	1113	263760						

df - degrees of freedom; SS - sum of squares; MS - mean squares; Pseudo-F – pseudo-F ratio; P (perm) – permutation *p* value; unique perms – number of permutations.

Table 4. SIMPER analysis of dissimilarities between the wet and dry season based on *P. bahamense* and *C. furca* cell densities.

Groups Wet and Dry Average dissimilarity = 23.8						
Species	Wet Avg. Abund.	Dry Avg. Abund.	Avg. Diss.	Diss/SD	% Contrib.	% Cum.
P. bahamense	7.8	4.74	11.97	1.54	50.32	50.32
C. furca	6.24	9.15	11.82	1.37	49.68	100

Avg. Abund. = average abundance; Diss = dissimilarity; Contrib. = contribution; Cum. = cumulative

Figures

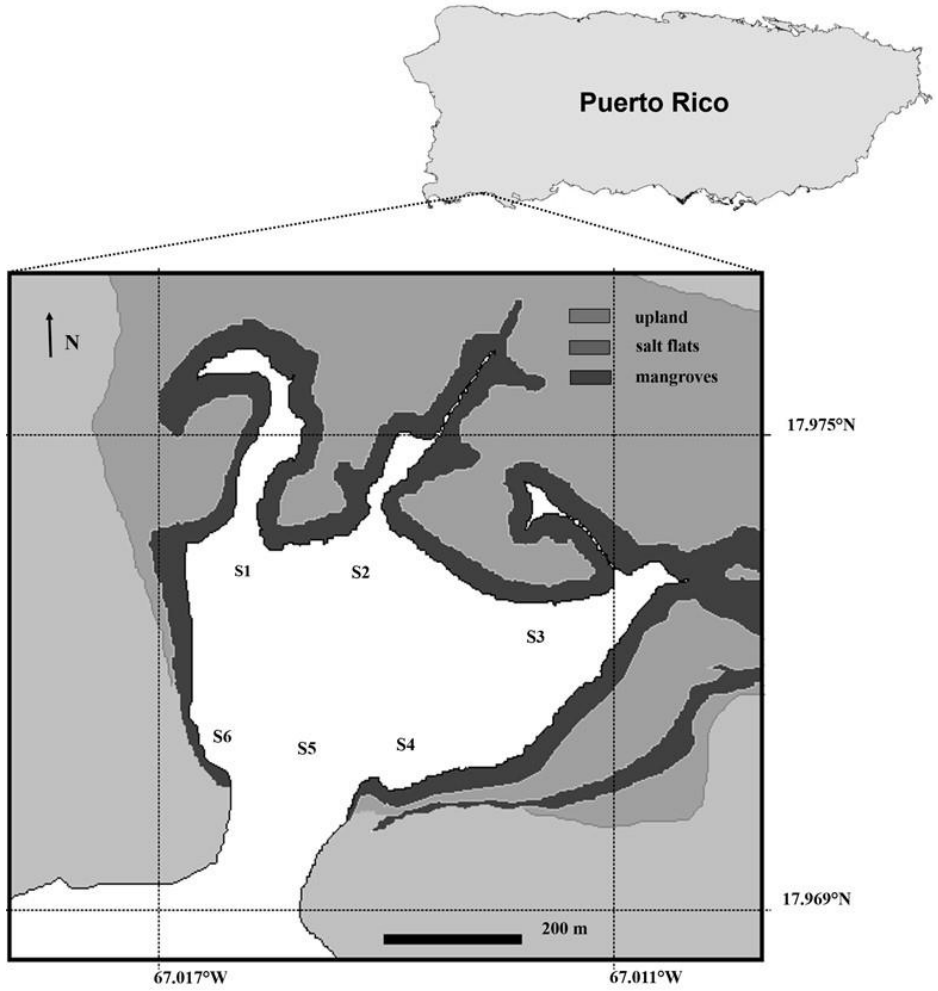

Fig. 1 Sampling stations at Bahía Fosforescente, La Parguera, Puerto Rico (Source: Soler-Figueroa and Otero 2015).

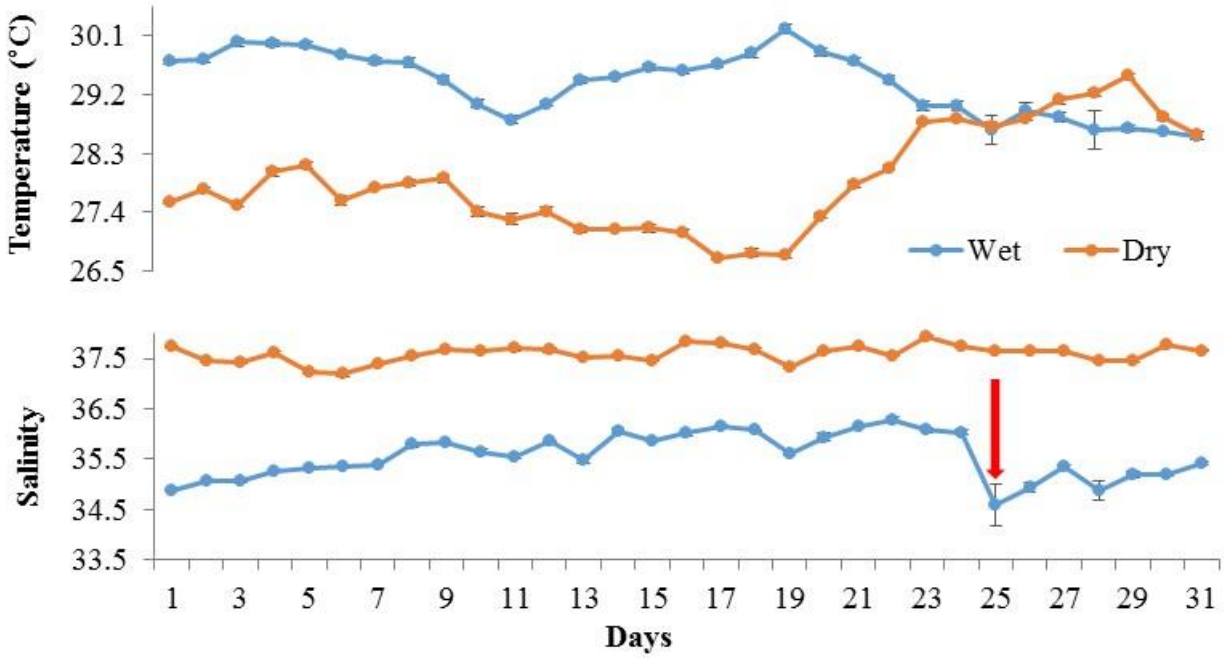

Fig. 2 Average daily temperatures and salinities measured at Bahía Fosforescente during the wet (November 5 – December 5, 2012) and dry (March 1 – 31, 2013) season. Arrow indicates a decrease in salinity after a 40 mm rainfall event on November 28, 2012. Bars represent standard errors of stations averages.

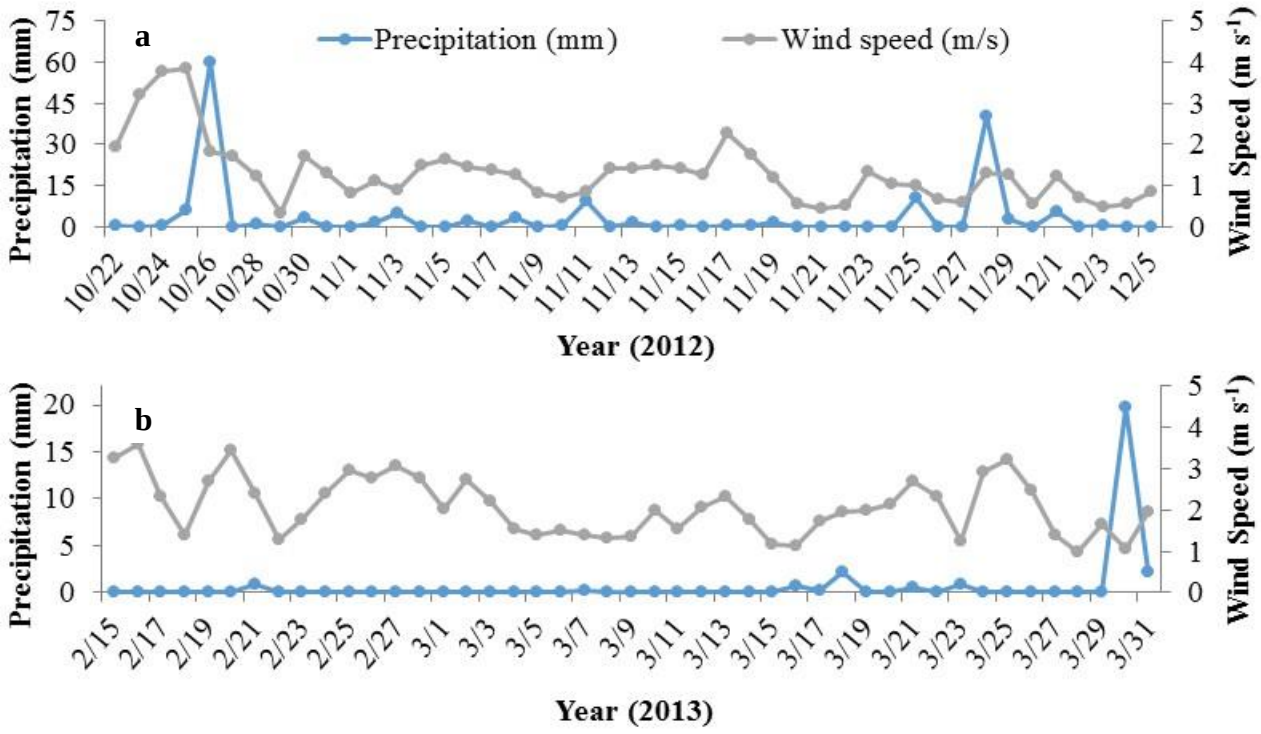

Fig. 3 Daily cumulative precipitation and daily average wind speeds recorded during the **a)** wet and **b)** dry seasons. Figures include the daily cumulative precipitation and winds speeds recording 2-weeks prior to each sampling campaign. Note the differences in precipitation scale between sampling periods.

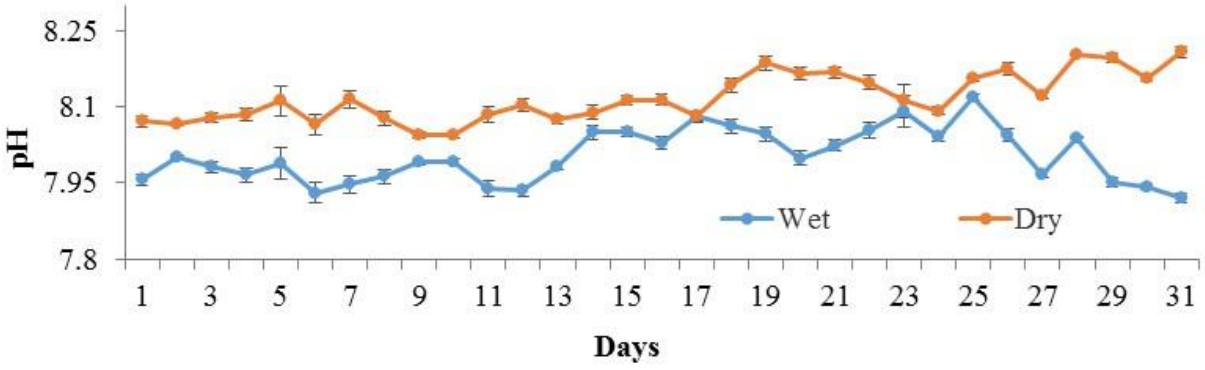

Fig. 4 Average daily pH measured at Bahía Fosforescente during the wet (November 5 – December 5, 2012) and dry (March 1 – 31, 2013) seasons. Bars represent standard errors of stations averages.

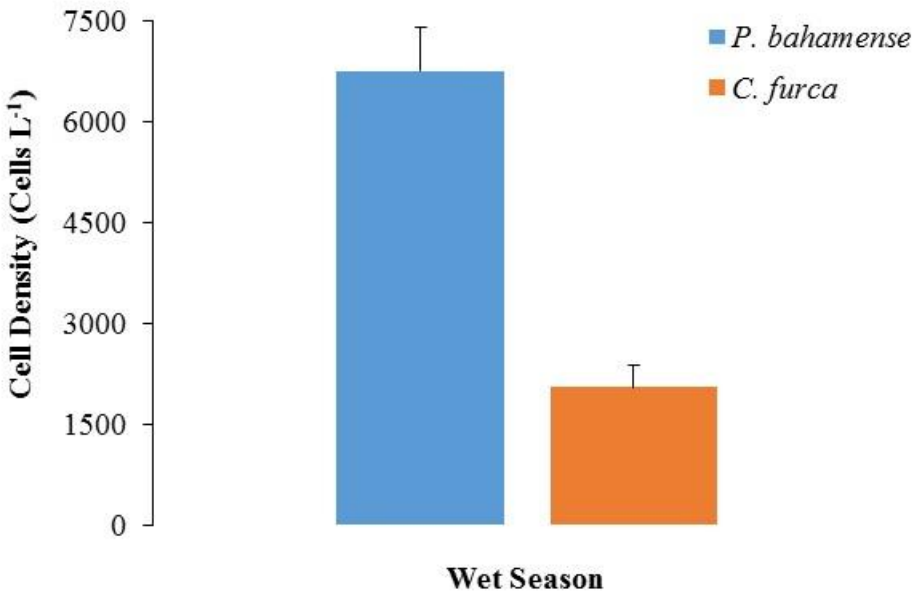

Fig. 5 Comparison between *Pyrodinium bahamense* and *Ceratium furca* cell densities during the wet season. Bars represent standard errors (n = 186).

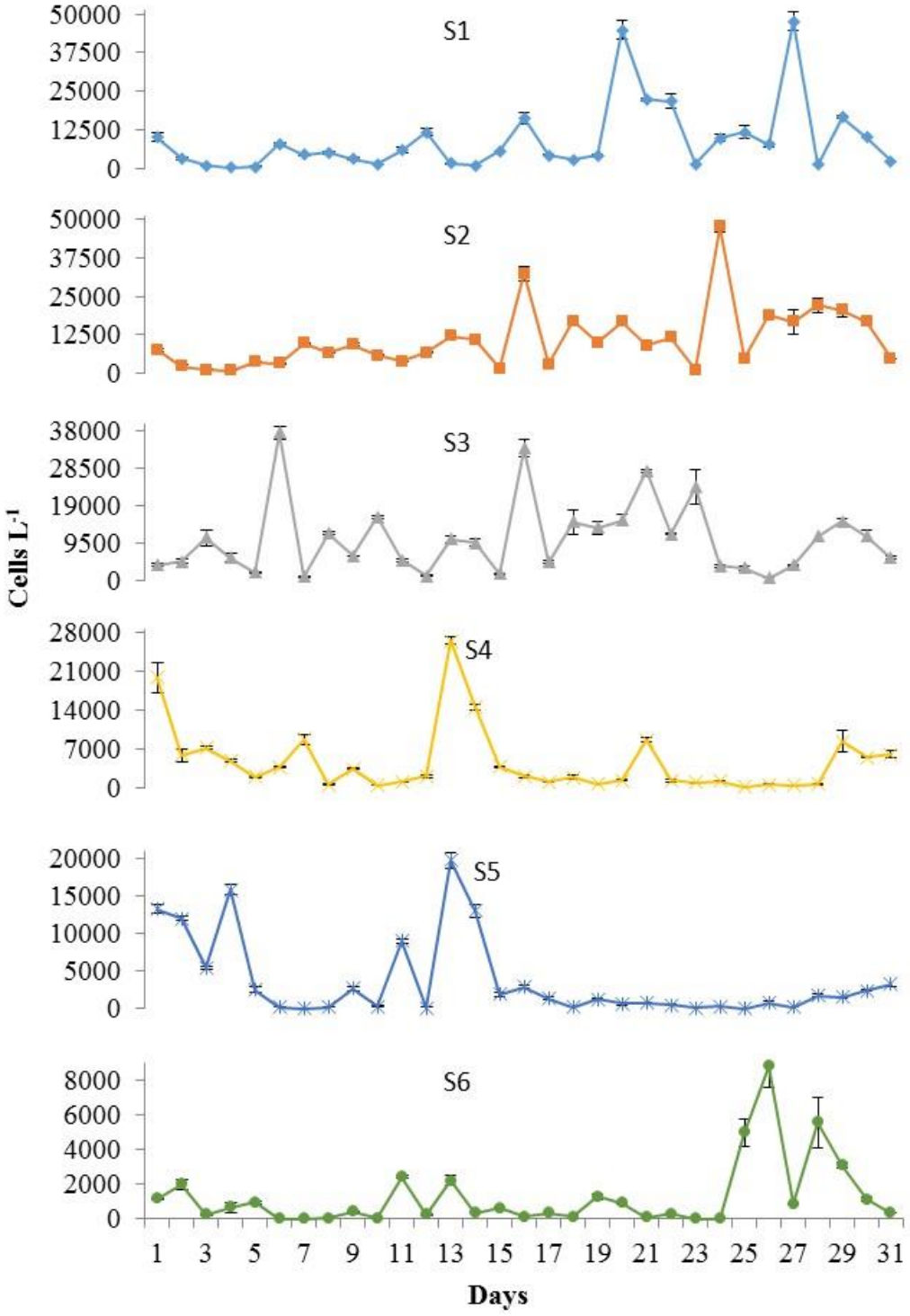

Fig. 6 *Pyrodinium bahamense* cell densities recorded at S1 to S6 during the wet season, from November 5 to December 5, 2012. Note differences in scale between stations. Bars represent standard errors of triplicate averages.

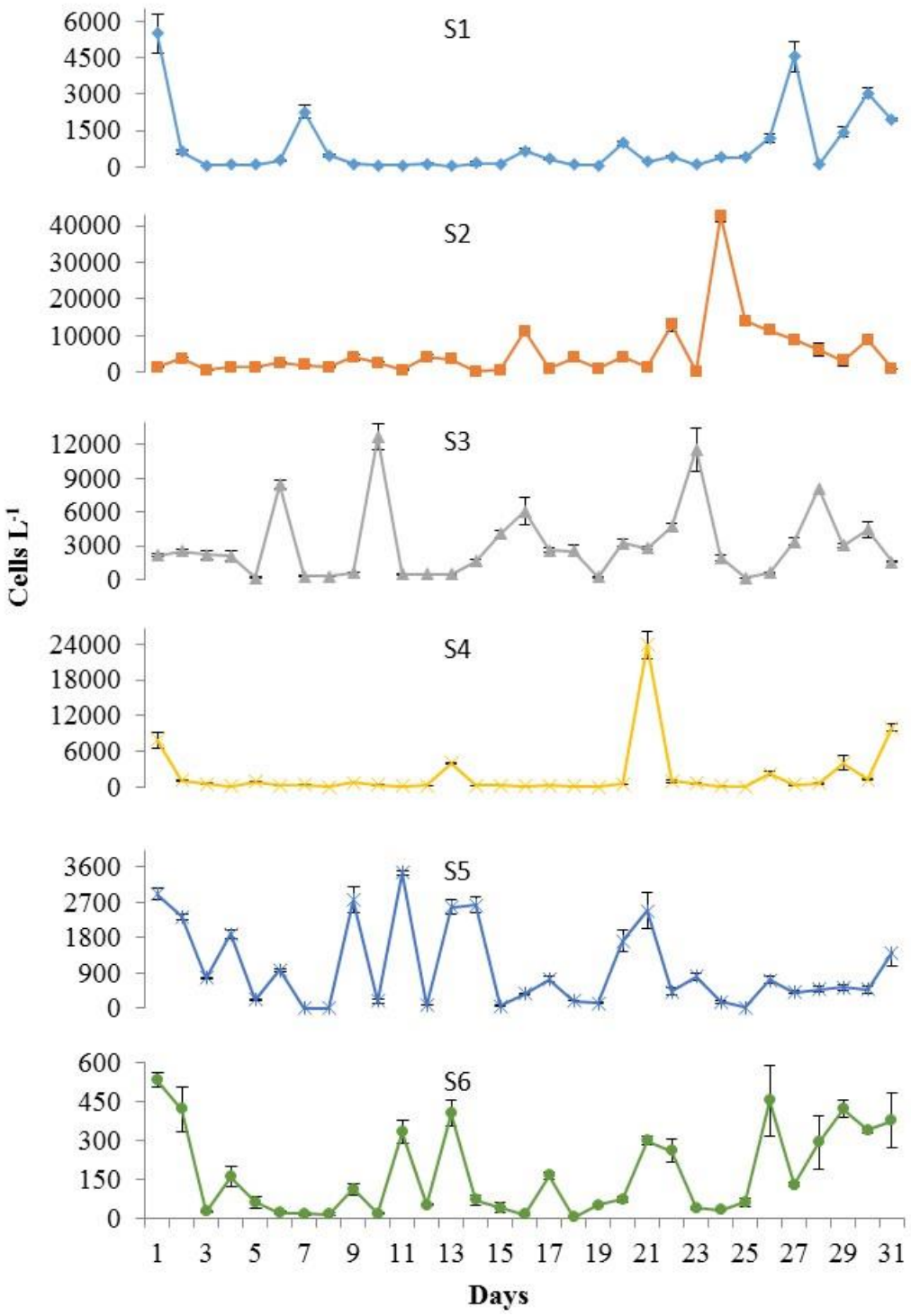

Fig. 7 *Ceratium furca* cell densities recorded at S1 to S6 during the wet season, from November 5 to December 5, 2012. Note differences in scale between stations. Bars represent standard errors of triplicate averages.

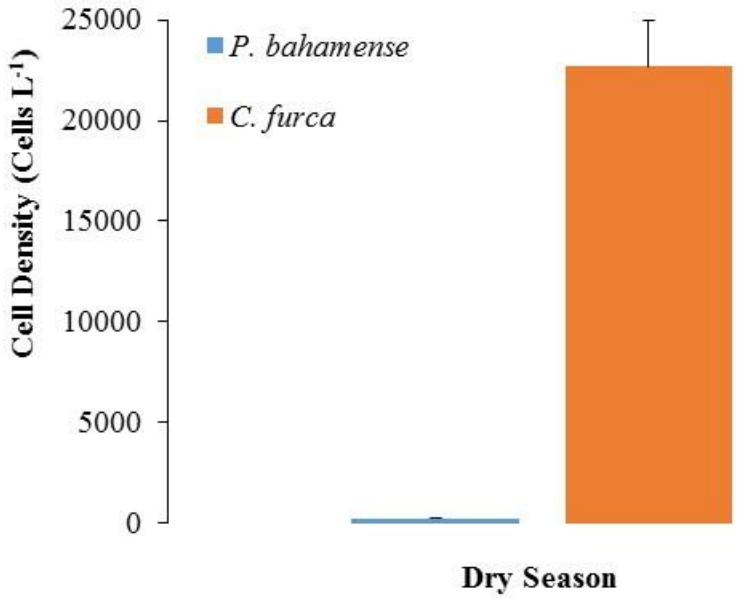

Fig. 8 Comparison between *Pyrodinium bahamense* and *Ceratium furca* cell densities during the dry season. Bars represent standard errors (n = 186).

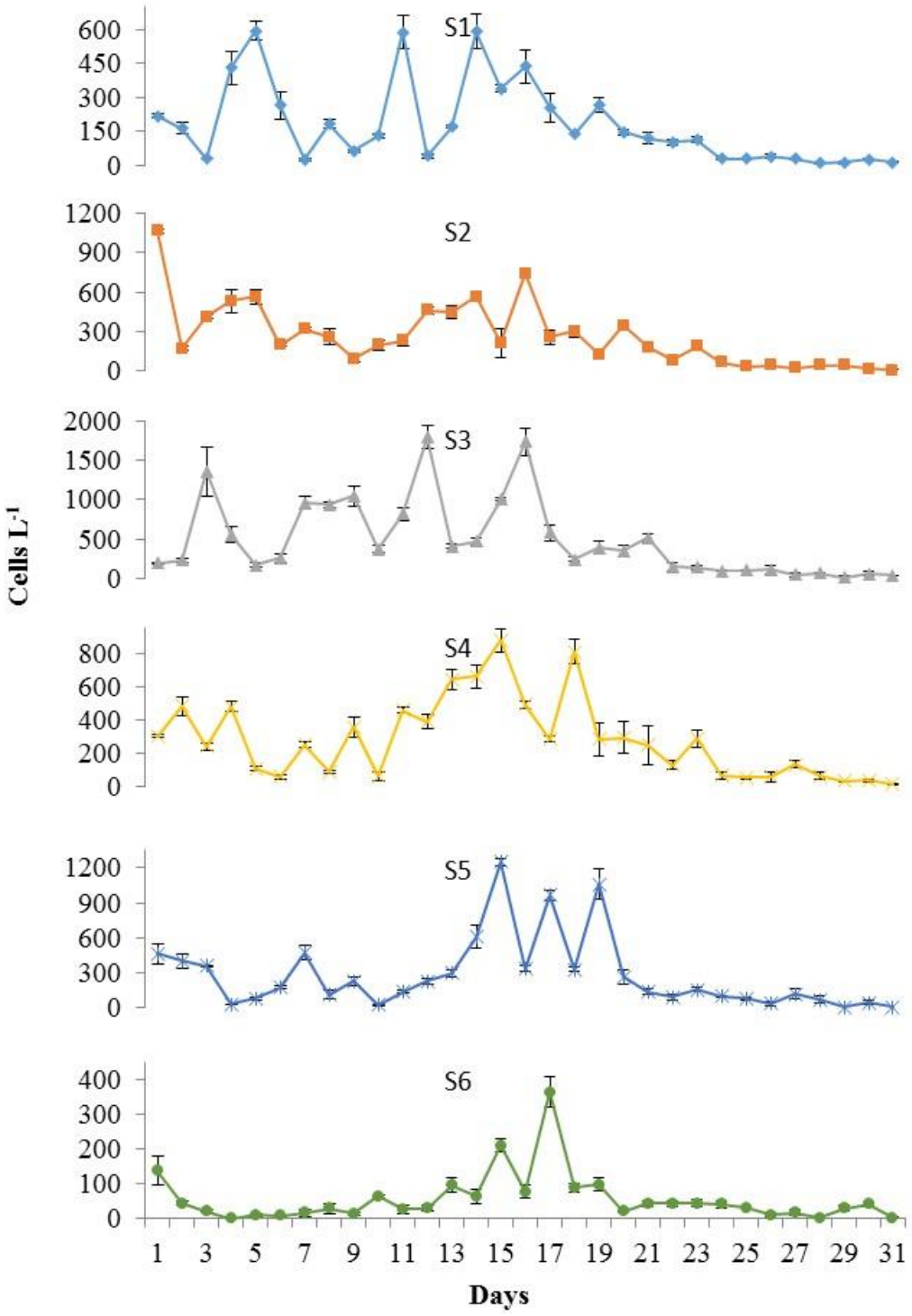

Fig. 9 *Pyrodinium bahamense* cell densities recorded at S1 to S6 during the dry season, from March 1 to 31, 2013. Note differences in scale between stations. Bars represent standard errors of triplicate averages.

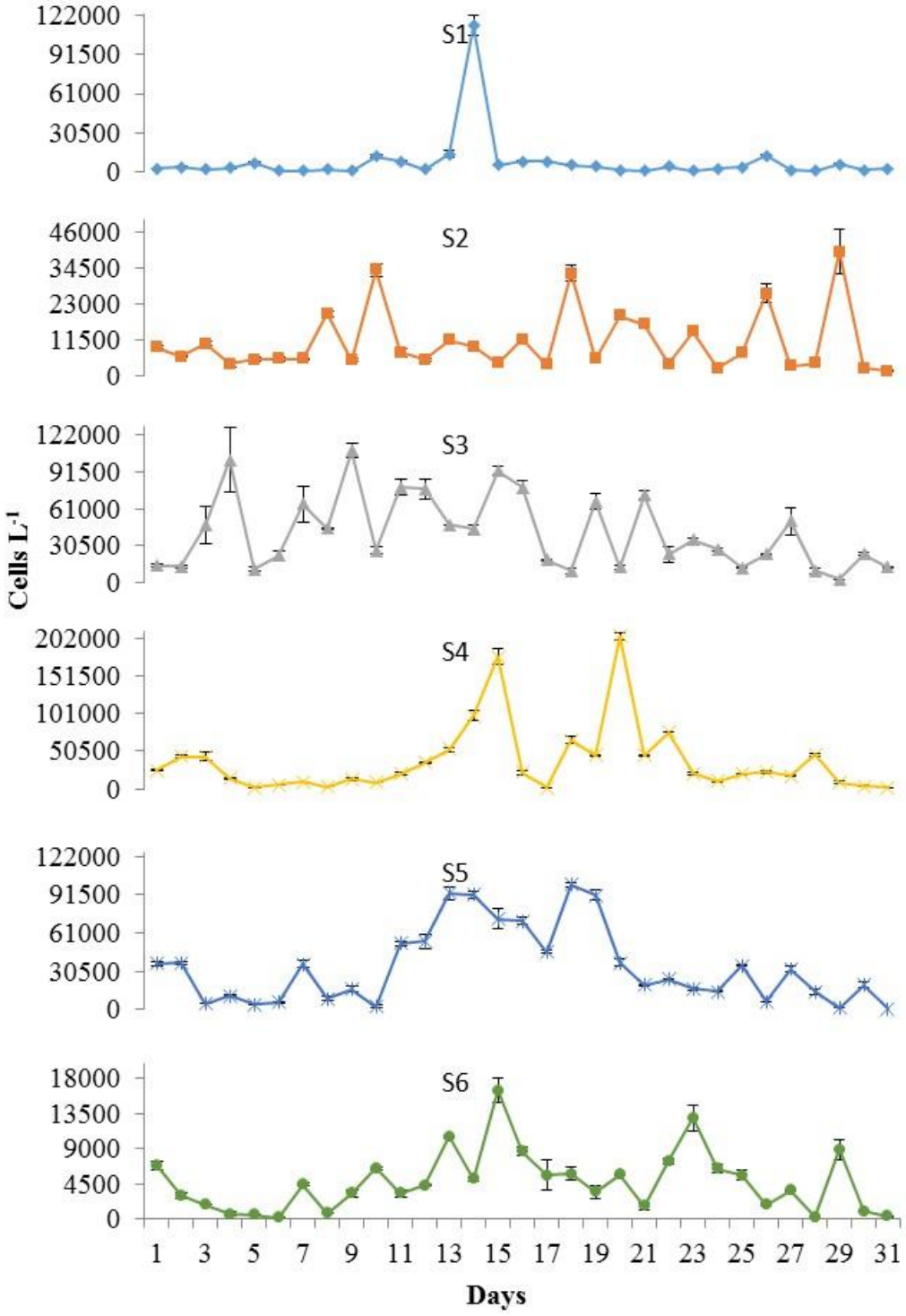

Fig. 10 *Ceratium furca* cell densities recorded at S1 to S6 during the dry season, from March 1 to 31, 2013. Note differences in scale between stations. Bars represent standard errors of triplicate averages.

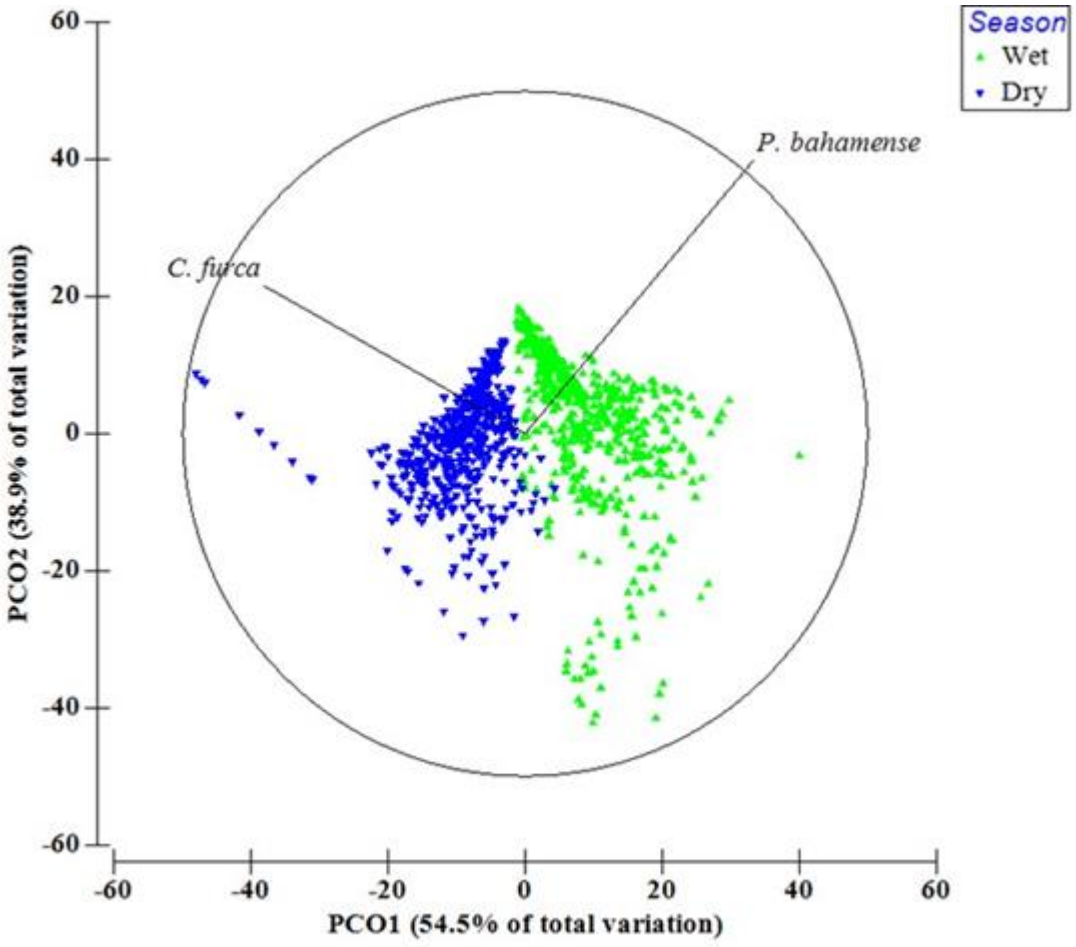

Fig. 11 Principal Coordinate Analysis (PCO) plot based on *P. bahamense* and *C. furca* abundances during the wet and dry seasons. Analysis was based on Bray-Curtis resemblance matrixes with dinoflagellate abundances log (x +1) transformed. Vectors with the dinoflagellate abundances indicate changes of direction.

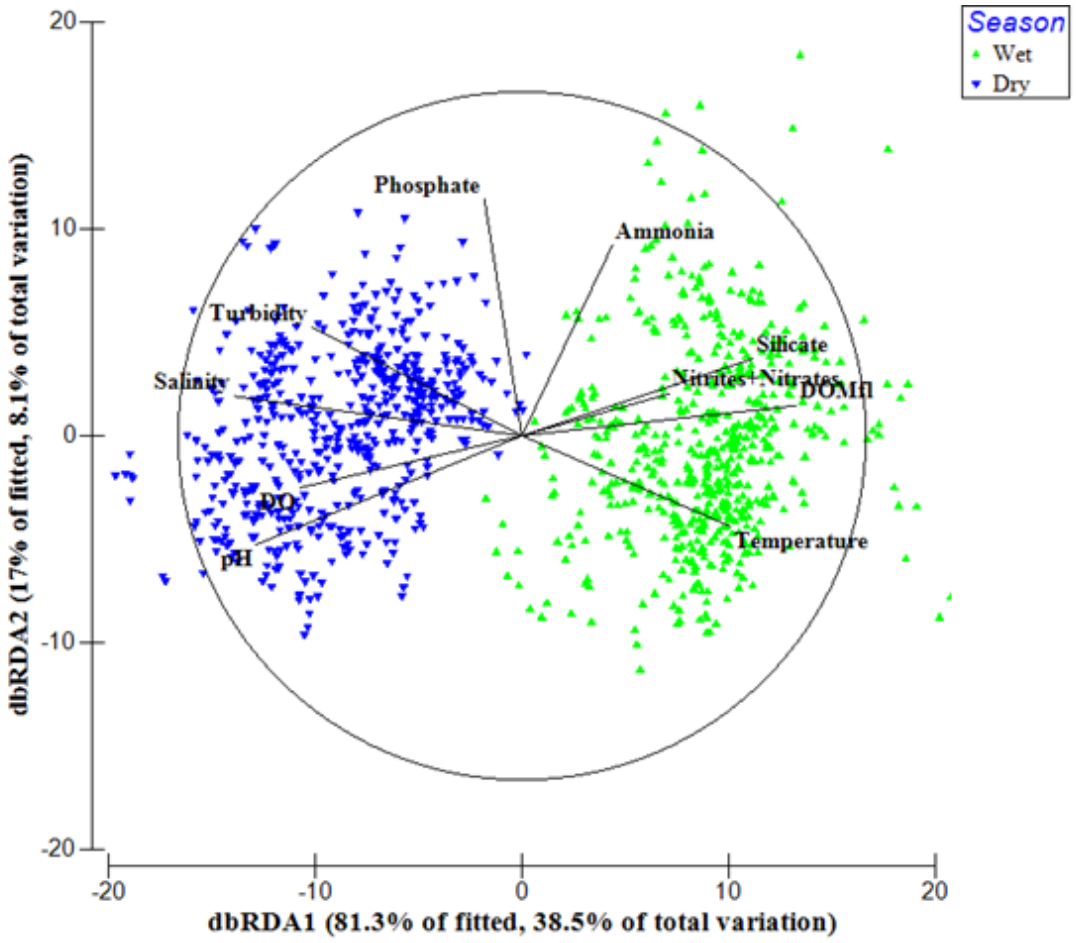

Fig. 12 Distance based redundancy analysis (dbRDA) plot based on *P. bahamense* and *C. furca* abundances during the wet and dry seasons, with vectors showing the Spearman correlations between environmental variables and dbRDA axis. Analysis was based on Bray-Curtis resemblance matrixes with dinoflagellate abundances log (x +1) transformed and environmental data log (x +1) transformed and normalized.

Chapter 4

Seasonal changes in bioluminescence levels and dinoflagellates composition in a tropical bioluminescent bay, Bahía Fosforescente, La Parguera, Puerto Rico.

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Abstract

Decreases in bioluminescence in Bahía Fosforescente have been associated with alternations between bioluminescent (*Pyrodinium bahamense*) and non-bioluminescent (*Ceratium furca)* dinoflagellates, two potentially harmful algal bloom species (HABs). Until now, these changes in bioluminescence have never been quantified, and their relationship with seasonality remains unclear. This study quantified bioluminescence levels (BL) in the bay with a high spatiotemporal resolution to evaluate the status of this phenomenon and to determine the link between seasonality on BL and dinoflagellate composition. Biweekly measurements, from August 2012 to July 2013, were conducted at six stations with an Underwater Bioluminescent Assessment Tool. The highest BL were observed during the wet season and were correlated with high cell densities of *P. bahamense*. *Ceratium furca* cell densities surpassed those of *P. bahamense* during the dry season, with concomitant reductions in BL. The presence of other bioluminescent dinoflagellates, such as *Protoperidinium spp.,* during the latter season, with abundances higher than those of *P. bahamense*, suggests their contribution to BL. It is suggested that environmental changes exerted by different meteorological conditions are linked to variations in BL by a modulation in dinoflagellate composition. Overall, higher BL were observed in the northern area of the bay, suggesting that wind-driven currents promoted the accumulation of organisms in this region. This study underscores how weather, changes in the abundance of these two potentially HAB species, and BL levels at Bahía Fosforescente are critically linked.

Keywords: Harmful algal blooms (HABs), bioluminescence, *Pyrodinium bahamense*, *Ceratium furca*, dinoflagellates, weather

Introduction

Dinoflagellates represent one of the most ubiquitous groups among the phytoplankton community in the marine ecosystem, occupying several ecological roles as primary producers, grazers, mutualists, parasites, and establishers of harmful algal blooms (HABs) (Gómez, 2012). Furthermore, dinoflagellates represent the only members of the phytoplankton community with the capability to emit light, contributing significantly to the bioluminescence that occurs on the surface of oceans and coastal waters (Tett, 1971; Kelly and Tett, 1978).

Much effort has been spent to understand the bioluminescence phenomenon caused by these organisms, including the cellular mechanisms of light emission (reviewed by Valiadi and Iglesias-Rodriguez, 2013), diurnal/circadian rhythms (Knaust el al., 1998; Akimoto et al., 2004; Marcinko et al., 2013a), influence of environmental factors on light production (Sweeny, 1981; Sullivan and Swift, 1995; Latz and Jeong, 1996; Li et al. 1996), patterns and distribution (Lapota et al., 1989; reviewed by Marcinko et al., 2013b), ecological function of light production (reviewed by Haddock et al., 2010 and Widder, 2010; Cusick and Widder, 2014), and *in situ* molecular detection (Valiadi et al., 2014). Even though these studies have provided valuable information on the ecophysiology of bioluminescent dinoflagellates, much remains to be learned about the population structure of these organisms in relation to environmental conditions (Valiadi and Iglesias-Rodriguez, 2013).

Bahía Fosforescente, a bioluminescent bay located in the southwest coast of Puerto Rico, represents an ideal natural laboratory to study the environmental regulation of phytoplankton composition, occasionally resulting in the formation of blooms of the bioluminescent dinoflagellate *Pyrodinium bahamense* var. *bahamense* (Margalef, 1957). For decades, the bioluminescence displayed by *P. bahamense* has made this bay an economically important tourist attraction. In contrast to other locales (i.e., Indian River Lagoon, Florida) (Landsberg et al., 2006), this species has not been associated with HAB's deleterious effects on marine species.

Dybas (2011) reported the drastic reduction or disappearance of bioluminescence in Bahía Fosforescente due to dredging at the entrance, supporting the notion of significant changes in the conditions favorable for the accumulation of abundant dinoflagellate populations. However, it is at best challenging to conclude that bioluminescence levels have dwindled because no systematic observation of this property has been conducted over the past 50 years. Recently, Soler Figueroa and Otero (2015) concluded that the abundance of *P. bahamense* is

highly influenced by patterns of pluviosity due to changes in nutrient availability at daily to seasonal scales. This pattern is consistent with the increased dominance (Seixas, 1988; Walker, 1997; Soler-Figueroa, 2006) of mixotrophic, non-bioluminescent, dinoflagellates such as *Ceratium furca* var. *hircus* (Sweeney, 1981; Valiali et al., 2014). Overall, these results suggest that bioluminescence is variable and modulated by weather conditions. However, precise measurements of bioluminescence and *P. bahamense* have yet to be conducted that would allow evaluation of bioluminescence levels (BL) over time.

To evaluate the bioluminescence trends of the bay, our study quantified the bioluminescence levels BL at Bahía Fosforescente after more than fifty years of no systematic observation (Clarke and Breslau, 1960). We examined whether seasonality and different precipitation regimes influence the dinoflagellate composition and how these changes are reflected in BL. In addition, the temporal (short-term and seasonal) and spatial patterns in BL and their possible relationships with dinoflagellate composition were evaluated. Results from this study broaden our knowledge of how different environmental conditions related to seasonality, influence the population structure of *P. bahamense* and *C. furca*, two potential HABs species (Landsberg et al., 2006; Baek et al., 2008). These data also provide valuable information on the actual conditions of Bahía Fosforescente, an important tourist industry and fisheries resource, which is vital for the establishment of effective management practices.

Methods

Study site

Bahía Fosforescente (Fig. 1) is located 3.2 km east of La Parguera, Lajas on the southwest (SW) coast of Puerto Rico (17° 58′ 30″ N; 67° 01′ 10″ W). The estimated area of the bay is 0.19 km^2 with an average depth of 3.5 m. The bay has an irregular shape with a narrow outlet to the coastal ocean (100–150 m) and shallow water (ca. 3.0 m). No river discharges into the bay but infrequent surface water flow is transported into the bay via three mangrove channels to the north. Dispersal of surface water flow is also possible through the fringing mangrove (*Rhizophora* mangle) forest that separates the bay waters from the coastal salt flats. The bay is characterized by high evaporation rates due to the prevailing arid conditions of SW Puerto Rico (Margalef, 1961).

Field work

Sampling was conducted during two sampling campaigns, from August 29 to December 11, 2012, and from March 5 to July 19, 2013. The wet (Aug – Nov 2012) and dry (Jan – the first two weeks of April 2013) seasons fell within these sampling days and were characterized based on local climatology (Glynn, 1973). Sampling was conducted mostly twice a week, depending on weather conditions. An Underwater Bioluminescence Assessment Tool (UBAT – WETLabs) (Orrico et al., 2010) was used *in situ* to quantify the mechanically stimulated bioluminescence levels (BL; photons s^{-1} L^{-1}). Bioluminescence levels were recorded at a depth of ca. 0.2 m for 3 minutes at each of the six stations (Fig. 1). Sampling was always conducted from 7:30 PM to 9:00 PM to minimize the effects due to the diel cycle of the bioluminescent organisms. No BL were recorded from 18 April-16 May 2012 due to equipment malfunction.

Near surface (0.1 m) water salinity and temperature were recorded at all stations using a Pro Plus YSI probe concurrently with BL determinations. In addition, at stations S1, S3, and S5, 7.7 L triplicate water samples were collected individually with 9 L carboys, filtered through 25 µm mesh, concentrated to 50 ml, and fixed with buffered formaldehyde (ca. 1 % final concentration) for dinoflagellate enumeration at a later time.

Determination of dinoflagellate cell densities

Cell densities of *P. bahamense*, *C. furca*, and *Protoperidinium spp.*, three of the most abundant dinoflagellates in the bay (Hernández-Becerril and Navarro 1996), were determined with a benchtop Flow Cytometer And Microscope (FlowCAM - FlowImaging; Sieracki et al. 1998). Prior to the analysis, water samples were filtered through a 210-µm mesh to remove meso/macro zooplankton while avoiding flow cell clogging. The sample volumes were also adjusted to avoid cell clustering. An image recognition software (VisualSpreadsheet© Particle Analysis Software) was used in auto-image mode while cells were pumped through a 200-µm glass flow cell at a flow rate of 1.5 mL min^{-1} and while being visualized using a 4× objective. In order to increase accuracy, a cell diameter threshold of 15-500 µm was selected. All inner surfaces in contact with the sample were rinsed with distilled water to minimize cross-contamination. The collection of images from each sample was automatically classified and counted with biometric filters previously created based on sample particle properties. *Protoperidinium* spp. images were classified manually. Each automated classification was later screened and manually corrected for misclassifications.

The accuracy of the FlowCAM classification was examined using several samples from the study site. Dinoflagellates were manually counted using a Sedgewick-Rafter counting chamber on a CK40 inverted microscope (Olympus, Inc.) at 100× magnification. On average, the FlowCAM results accounted for more than 90% of *P. bahamense* and *C. furca* manual estimates. Averages and standard errors of cell densities were calculated from triplicate samples and are reported as cells L^{-1}.

Meteorological data

A HOBO Micro Weather Station equipped with rain and wind (speed and direction) sensors was installed in the watershed immediately north of Bahía Fosforescente. The station logger was programmed to save data every minute, and these data were retrieved every 2 weeks. Comparisons of collected precipitation and wind records were conducted with the data from another meteorological station maintained by the Department of Marine Sciences Bio-optical Oceanography Laboratory at Magueyes Island (http://bio-optics.uprm.edu/weather.html), located 3.2 km west of the sampling site. Since the data were similar between both meteorological stations, results are expressed as the average of both stations.

Trends in bioluminescence levels

To examine general trends (i.e., increases/decreases) in bioluminescence over the years, estimates of BL were calculated based on averages of *P. bahamense* cell densities reported in previous studies (i.e., Clarke and Breslau, 1960; Seliger et al., 1971; Seixas, 1988; Walker, 1997; Soler-Figueroa, 2006; Soler-Figueroa and Otero, 2015) using the average BL to *P. bahamense* density ratio observed during this study.

Statistical analysis

Non-parametric analyses were performed since data failed parametric assumptions. The difference in BL among sampling dates and sites was evaluated using the Kruskal-Wallis Analyses of Variance (KW-ANOVA). This test was also used to examine whether the abundance of each dinoflagellate species differed among sampling dates, as well as to evaluate if the cell densities of *P. bahamense, C. furca,* and *Protoperidinium spp.* differed within each seasonal extreme. A Mann-Whitney U test was performed to evaluate seasonal differences in BL and species abundance (i.e., *P. bahamense* and *C. furca*). A Spearman rank correlation analysis was used to evaluate the relationships between the BL and bioluminescent dinoflagellate cell

densities (i.e., *P. bahamense* and *P. bahamense* + *Protoperidinium spp.*). All statistical analyses were conducted using Sigma Plot 12.0.

Results

Environmental parameters

A total of 321 and 50 mm of rain was recorded during the wet and dry seasons, respectively. Pluviosity peaked four times during the wet season, two of which were related to the passage of hurricanes Isaac and Sandy (Fig. 2). In contrast, pluviosity during the dry season was characterized by frequent, less intense rains during the latter portion of this period. Rains peaked again between the last two weeks of April and the first two weeks of May with 133 mm collected. The average surface salinity was 35.6 and 37.7 during the wet and dry seasons, respectively. The overall rain pattern was thus reflected in the salinity, especially during the peaks in precipitation, when decreases in surface salinity were observed (Fig. 2). These relationships underscore the importance of weather patterns in the potential fertilization of coastal waters and modulation of its productivity. In contrast to rain and salinity, the average seasonal temperature shift represented only slight shifts from 30.1°C to 29.4°C, but ranged between 29.5°C to 31.3°C and 26.8°C to 30.3°C during the wet and dry seasons, respectively (Fig. 2). Average monthly wind speed during the wet season fluctuated from 1.3 to 2.3 m s^{-1} (with peaks of greater than 4.5 m s^{-1} associated with the hurricanes mentioned earlier; Fig. 3). Wind speed usually dropped during the night, being frequently undetectable. Wind direction during this season was SE/SSE with the exception of November, when a strong NW component was observed. During the dry season, the average monthly speed fluctuated between 1.8 and 2.6 m s^{-1} while its direction was mainly SE-ESE. However, wind was highly variable during March.

Bioluminescence levels

The average BL during the wet season were 2.4 times higher than those observed during the dry season (Mann-Whitney U test = 39955.00, n = 396 *(wet)*, n = 195 *(dry)*, $p < 0.001$), with $4.3 \times 10^{11} \pm 4.9 \times 10^{10}$ photons sec^{-1} L^{-1}. This difference was present despite the high daily fluctuations observed throughout the study (K-W ANOVA H = 437.78, df = 46, n = 18, $p <$ 0.001; Fig. 3). Six overlapping homogeneous groups were found while examining daily BL using Dunn's multiple comparison test. We used this grouping to further evaluate if the BL ranked at the extremes were generally related to dry (i.e., lower ranks) or wet periods (i.e., higher ranks) of

the year. We found that 64% and 73% of the lowest and highest ranked BL fit the above assumption, thus confirming a robust link between these two weather extremes and BL that underlies the daily variability that is natural for these types of ecosystems.

Overall, the maximum BL during the wet season were recorded in October; however, BL decreased drastically after Hurricane Sandy (Fig. 3). In contrast, frequently low BL characterized the dry season, except on March 19 and 21, when the BL were similar to the wet season average. Bioluminescence levels peaked again in May with an average of $4.3 \times 10^{11} \pm 4.7 \times 10^{10}$ photons sec^{-1} L^{-1}.

Spatial variations in BL were also detected among stations within each season, with the maximum levels towards the north of the bay, at S1 and S3 and at S2 and S3 during the wet and dry season, respectively (Wet: K-W ANOVA H = 35.86, *df* = 5, *n* = 66, $p < 0.001$; Dry: K-W ANOVA H = 40.09, *df* = 5, *n* = 33, $p < 0.001$; Fig. 4).

Dinoflagellate cell densities

The average cell densities of each dinoflagellate species within each season were significantly different. *Pyrodinium bahamense* was numerically dominant during the wet season, with average cell densities of 16 and greater than 100 times those observed for *C. furca* and *Protoperidinium* spp., respectively (K-W ANOVA H = 391.90, *df* = 2, *n* = 207, $p < 0.001$). In contrast, the average cell densities during the dry season represented ca. 1% and 22% of those observed for *C. furca* and *Protoperidinium* spp., respectively (K-W ANOVA H = 223.71, *df* = 2, *n* = 99, $p < 0.001$).

Pyrodinium bahamense cell densities during the wet season were significantly higher than during the dry season (Wet vs Dry: Mann-Whitney U test = 5165.00, *n* = 207 *(wet)*, *n* = 99 *(dry)*, $p < 0.001$), averaging $2.5 \times 10^4 \pm 6.3 \times 10^3$ cells L^{-1}. During the dry season, average cell densities of this species represented ca. 1% of that observed during the wet season. Seasonal significant differences of *C. furca* were also revealed. Average densities of 1.9×10^4 cells L^{-1} were found during the dry season, while the density during the wet season averaged twelve times less abundant (Wet vs Dry: Mann-Whitney U test = 24396.00, *n* = 207 *(wet)*, *n* = 99 *(dry)*, $p < 0.001$).

Significant differences in dinoflagellate cell density across all sampling days were also detected (*P. bahamense*: K-W ANOVA H = 423.00, *df* = 55, *n* = 9, $p < 0.001$, Fig. 5a, 5b; *C. furca*: K-W ANOVA H = 385.56, *df* = 55, *n* = 9, $p < 0.001$, Fig. 6a, 6b; *Protoperidinium* spp. K-

W ANOVA H = 272.92, *df* = 52, *n* = 9, p < 0.001, Fig. 7a, 7b). Differences in *P. bahamense* cell densities occurred partly due to the bloom (≥ 5.0 x 10^4 cells L^{-1}) observed between October 9 and 23, which drastically decayed after the passage of Hurricane Sandy (Fig. 5a). Cell densities of this species remained low and increased again in May (Fig. 5b). Maximal cell densities of *C. furca* (5.4 x 10^4 ± 3.1 x10^4 cells L^{-1}) and *Protoperidinium* spp. (1.5 x 10^3 ± 3.1 x10^4 cells L^{-1}) were observed on March 12 and March 21, respectively.

Correlations between bioluminescent dinoflagellates and bioluminescence levels

A positive correlation was observed between *P. bahamense* cell densities and the BL recorded during all the sampling days. This correlation became slightly stronger when *Protoperidinium spp.*, which is also bioluminescent, was included (Table 1). During each sampling campaign, positive correlations were also observed between the bioluminescent dinoflagellates (*P. bahamense* and *P. bahamense* + *Protoperidinium spp.*) and the BL; however, the correlation was only stronger during the wet season (Table 1).

Trends in bioluminescence levels

The *P. bahamense* abundances from studies conducted during the past 50 years were used to calculate BL based on the average BL to *P. bahamense* ratio found during this study (Table 2). The highest BL were estimated during 1986, 2003, and 2010, which corresponds to years when blooms of *P. bahamense* (i.e., up to 10^5 cells L^{-1}) were reported (Seixas, 1988; Soler-Figueroa, 2006; Soler-Figueroa and Otero, 2015). Therefore, our results suggest that there is no particular increasing or decreasing trend in BL at Bahía Fosforescente. However, it must be noted that these BL estimates were based on studies with different sampling sizes, techniques, times, and sites. Additionally, we assumed that the average BL of this study only corresponded to *P. bahamense,* and contributions by other bioluminescent organisms were not considered.

Discussion

Temporal distribution in bioluminescence levels and dinoflagellate composition

The BL and dinoflagellate composition at Bahía Fosforescente exhibited a marked seasonality related to local meteorological conditions. Pluviosity during the wet season was six times greater than that observed during the dry season, undoubtedly resulting in the input of nutrients and other humic materials due to terrestrial and watershed runoff into the bay. This was

supported by the observed decreases in salinities after rainfall events. While nutrients were not evaluated over the course of this study, differences in nutrients due to seasonality were reported previously for this bay (Seixas, 1988; Soler-Figueroa, 2006; Cedeño-Maldonado, 2008; Soler-Figueroa and Otero, 2015). Therefore, it is suggested that nutrients increased during the wet season due to runoff promoted the observed high *P. bahamense* cell densities and bloom conditions, resulting in the total BL that were two times higher than those observed during the dry season.

The role of pluviosity as one of the principal modulators in the BL and *P. bahamense* populations in the bay was also supported by bioluminescence increases in May 2013. These increments were revealed after the rainfall events at the end of April and mid-May, suggesting that inputs of nutritional factors, after the observed periods of drought and probable low nutrient concentrations, promoted an increased growth of this species. Likewise, direct links between pluviosity and *P. bahamense* blooms have been observed in other regions, as well as associated with bioavailable nitrogen and phosphorus transported after rainfall events (Phlips et al., 2004; Phlips et al., 2006; Phlips et al., 2011). Moreover, the release of humic substances from nearshore mangroves and terrestrial watersheds have been linked to these phenomena (Usup and Azanza, 1998; Morquecho et al., 2012; Usup et al., 2012).

The onset of the dry season, during which rain accounts for only 15% of that observed during the wet season, resulted in a ca. two-fold reduction of BL relative to the wet season. This decrease was concomitant with a shift towards higher abundances of *C. furca,* a non-bioluminescent and mixotrophic dinoflagellate. These trends confirm our previous recent observations (Soler-Figueroa and Otero, 2015). The dominance of *C. furca* during the period of low pluviosity and watershed-derived nutrient enrichment was probably due to its ability to prey upon other planktonic species (Smalley et al., 2003; Baek et al., 2008). This shift towards other means of nutrient acquisition confers a competitive advantage over autotrophic dinoflagellates, such as *P. bahamense*, during the periods of lower nutrient availability (e.g., sustained low pluviosity).

The dry season was also characterized by high cell densities of heterotrophic bioluminescent dinoflagellates such as *Protoperidinium* spp., suggesting that these species likely contributed to the BL. Specifically, the peaks observed in March coincided with a seven-fold increase in the cell densities of these dinoflagellates compared to the wet season, while average

cell densities of *P. bahamense* decreased by two orders of magnitude. Although these peaks in bioluminescence reached levels similar to the mean values found during the wet season, the cell densities of *Protoperidinium* spp. accounted for only 4% of those observed for *P. bahamense* during the wet season. Thus, the data suggest that the specific bioluminescence potential of *Protoperidinium* spp. was higher than that for *P. bahamense*. This could be attributed to interspecies variability in bioluminescence capacities which, for heterotrophic dinoflagellates, is ten times greater than autotrophic dinoflagellates (Swift et al., 1995). Flash intensities of 2.8 x 10^8 photons sec^{-1} and 1.0 x 10^9 photons sec^{-1} have been reported for individual cells of *P. bahamense* (Seliger et al., 1969) and *Protoperidinium* spp. (Swift et al., 1995), respectively, which supports our findings. However, even though *Protoperidinium spp.* may have potentially contributed significantly to BL peaks observed in March, the weak correlations between BL and the bioluminescent dinoflagellates examined during the dry season suggest that other bioluminescent dinoflagellates could also contribute to the bioluminescence. One possible example is *Polykrikos sp*. The presence of this species in the bay has been reported (Seliger et al., 1971; F. Gómez, Spain, personal communication), but its counts could not be determined because the fixation technique used during the study was not appropriate for this (naked) type of dinoflagellate. Nevertheless, some deformed but still recognizable cells were observed during the sample processing.

In addition to cell densities, the physiological condition of the cells could have affected the BL observed. For example, cell size (Buskey and Swift, 1995) and the amount of luciferin or scintillions (reviewed by Valiadi and Iglesias-Rodriguez, 2013) can affect light emission in bioluminescent organisms. Furthermore, the availability of photosynthetically active radiation (Sullivan and Swift, 1995; Marcinko et al., 2013) and the feeding frequency (Latz and Jeong, 1996) could also play important roles in modulating light emission by autotrophic and heterotrophic dinoflagellates, respectively.

The meteorological conditions associated with seasonality played an important bottom up control on the dinoflagellate community composition and therefore on the BL. However, the dynamics underlying the dinoflagellate response to weather patterns need further investigation. During this study, episodes of heavy rainfall resulted in reductions in *P. bahamense* cell densities with concomitant decreases in BL. Non-significant increases in the abundance of other dinoflagellates were also observed. However, different scenarios due to rainfall events have been

reported for the bay, which included reductions in *P. bahamense* (and in the observed bioluminescence) with simultaneous dramatic increases in *C. furca* (Glynn et al. 1964; Gold 1965; Seliger 1988). The replacement of these two dinoflagellates by blooms of *Prorocentrum micans* and *Akashiwo sanguinea* (Seliger et al. 1971), or more recently a bloom of *P. bahamense* and significant increases in *C. furca* populations (Soler-Figueroa and Otero, 2015). These contrasting results may be explained by the intensity and frequency of pluviosity, as well as the length of drought periods before the rainfall events, which may differentially affect the water quality (e.g., temperature, salinity, nutrient regimes and composition, and water transparency) and hydrologic conditions (e.g., water circulation patterns and residence times). Such events could ultimately affect the distribution, abundance, and composition of phytoplankton organisms (Buyukates and Roelke 2005; Spatharis et al. 2007). Furthermore, nutrient types and concentrations, as well as the nutritional status of cells, can play important roles in the response of the organisms to nutrient enrichment (Anderson et al. 2002).

Spatial distributions in bioluminescence levels and dinoflagellate composition

Overall, high BL were mainly observed at S1, S2, and S3 probably due to the spatial displacement of bioluminescent dinoflagellates. This spatial distribution in bioluminescence suggests that the water currents resulting from the dominant SE-SSE and ESE winds led to the accumulation of these species in the northern area of the bay. Previous studies have reported similarly high BL (Seliger et al., 1962; Seliger et al., 1971) and high cell densities of *P. bahamense* (Seixas, 1988) in the northern and northeast regions of the bay (near what was defined as S3 in this study). The association of high cell densities of *P. bahamense* and *C. furca* previously reported in this region (Seixas, 1988; Soler-Figueroa and Otero, 2015), combined with the high BL and high cell densities of both organisms frequently observed during this study at S3 (data not plotted), suggests an ecosystem-wide, wind-driven accumulation mechanism that led to a concentration of the organisms in this area of the bay. However, the slower NW and NNW (or NE and ENE) wind component could periodically play a significant role in the bioluminescence and dinoflagellate spatial distribution, as observed during November. Under these wind “reversals”, dinoflagellates may be effectively flushed out of the bay, leading to low bioluminescence.

Trends in bioluminescence levels at Bahía Fosforescente

Decreases in bioluminescence have been frequently reported at Bahía Fosforescente along with concomitant shifts in *P. bahamense* and *C. furca* abundance (Seixas, 1988; Walker, 1997; Soler-Figueroa, 2006). However, short-term examinations of these fluctuations have seldom been conducted, and these analyses rarely examine how meteorological and physical parameters may be linked to BL. To our knowledge, this study is the first to use *in situ* measurements of BL and dinoflagellate counts to derive long-term estimates of the BL trends at Bahía Fosforescente. These estimates suggest the presence of variable but stable conditions in *P. bahamense* and BL over yearly cycles, without a net trend. Those cycles could have been associated with oscillations in precipitation regimens over the past fifty years (Fig. 8). Our results indicate a significant response by dinoflagellate populations and BL to weather patterns, thus suggesting the potential for change according to future climate variations. According to the *Puerto Rico Climate Change Council*, future decreases in precipitation are most likely, although with a higher risk of increased daily intensities (PRCCC, 2013). While this suggests possible alterations in the structure and function of this coastal ecosystem, the dynamics behind the response of the dinoflagellate composition and the overall phytoplankton community to future changes in weather patterns is uncertain and requires further investigation. Furthermore, in order to evaluate possible impacts of climate change over this and other sensitive coastal systems, as well as to improve our ability to separate the effect of natural patterns from anthropogenic influences, long-term monitoring programs should be implemented (Hays et al., 2005; Moore et al., 2008; Anderson, 2014).

At present, the perception of a BL decrease may be based on factors external to the bay. An increase in artificial light pollution over the years (Hölker et al., 2010; Davis et al., 2014) impairs the capacity of the human naked eye to discern the intensity of bioluminescence relative to the background. This effect is significant over long distances, especially due to scattering processes and cloud reflection (Kyba et al., 2011). This "bias" is avoided by the use of *in situ* photometers, such as the one used in this work. This type of instrumentation should be incorporated in future efforts to help define the trends in bioluminescence, which can be used as indicators of the environmental response and climate change in this type of system.

In summary, this study suggests that the clear seasonality in the BL at Bahía Fosforescente is related to pluviosity. Overall, seasonal differences in precipitation resulted in

modifications of the dinoflagellate composition, with simultaneous variations in bioluminescence. Phytoplankton organisms are subject to complex interactions among a wide range of environmental variables that influence their growth and abundance. The short generation times of these species are consistent with the fast shifts in bioluminescence observed during this study. Changes in the climate patterns in the near future are uncertain, but certainly the long-term pattern of bioluminescence in bays such as Bahía Fosforescente may serve as a sentinel of times to come.

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Tables

Table 1. Spearman rank correlations between the bioluminescent dinoflagellates and bioluminescence levels at Bahía Fosforescente.

		Bioluminescence levels
All sampling days	*P. bahamense*	$r = 0.74$, $n = 141$, $p < 0.001$
	P. bahamense and *Protoperidinium spp.*	$r = 0.82$, $n = 141$, $p < 0.001$
Wet Season	*P. bahamense*	$r = 0.89$, $n = 64$, $p < 0.001$
	P. bahamense and *Protoperidinium spp.*	$r = 0.89$, $n = 64$, $p < 0.001$
Dry Season	*P. bahamense*	$r = 0.46$, $n = 33$, $p < 0.05$
	P. bahamense and *Protoperidinium spp.*	$r = 0.52$, $n = 33$, $p < 0.05$

Table 2. Estimates of bioluminescence levels based on average cell densities of *Pyrodinium bahamense* from previous studies calculated using the average BL to *P. bahamense* density ratio observed during this study.

Date/Year	**Time**	**N**	***P.b.***	**s.e.**	**BL**	**s.e.**	**Study**
Feb '59	8-10 PM	1	0.76×10^4	n/a	1.7×10^{11}	n/a	Clarke and Breslau 1960
Jan-Dec '69	8-9 PM	28	2.3×10^4	4.9×10^3	5.1×10^{11}	1.1×10^{11}	Seliger et al. 1971
Feb '86-Jan '87	AM	27	2.4×10^4	11.3×10^3	5.4×10^{11}	2.6×10^{11}	Seixas 1988
May '95-Apr '96	AM	12	1.2×10^4	3.4×10^3	2.7×10^{11}	0.82×10^{11}	Walker 1997
Jan-Dic '03	7-8 AM	12	5.1×10^4	23.4×10^3	11.5×10^{11}	5.3×10^{11}	Soler-Figueroa 2006
Nov '10, Mar '11	8-930 AM	60	3.9×10^4	8.5×10^3	8.9×10^{11}	1.9×10^{11}	Soler-Figueroa and Otero 2015
Aug '12-Jul '13	730-9 PM	168	1.2×10^4	2.8×10^3	2.7×10^{11}	0.63×10^{11}	This Study

N = sample size, *P. b.* = *P. bahamense* (Cells L^{-1}), s. e. = standard error, n/a = not applicable, BL = Bioluminescence Levels (photons sec^{-1} L^{-1})

Figures

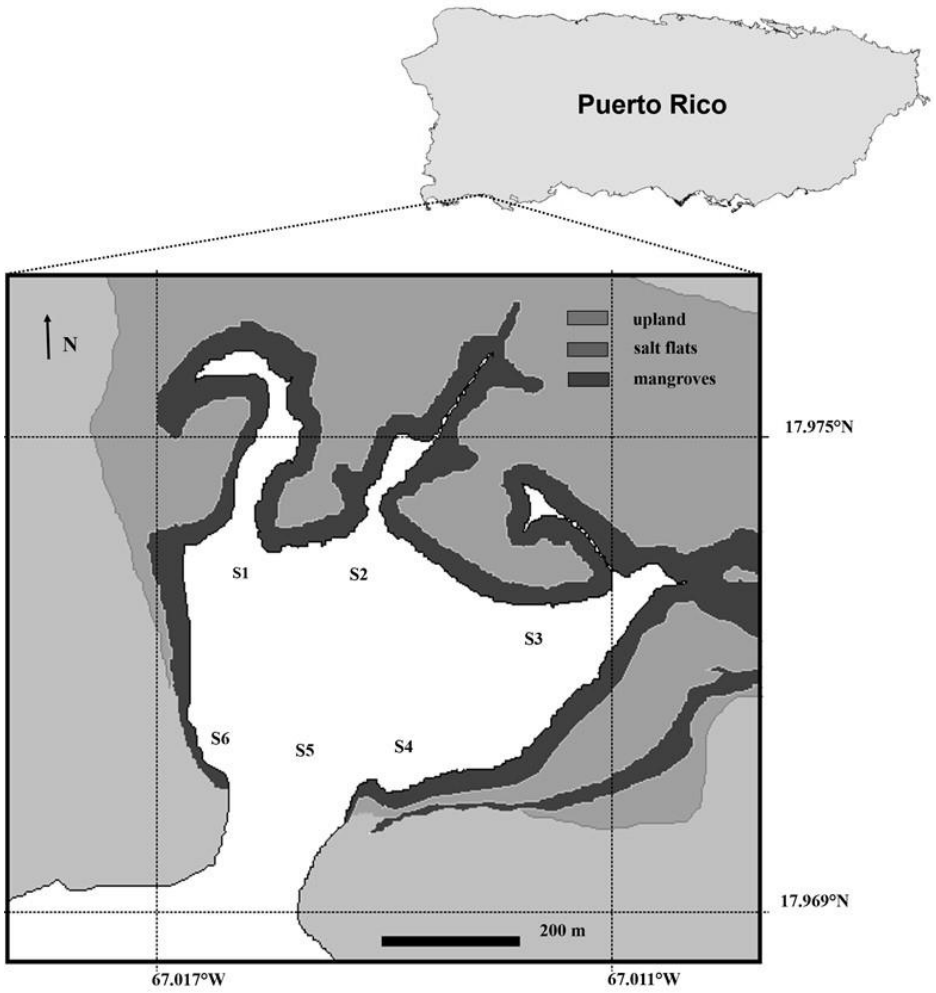

Fig. 1 Sampling stations at Bahía Fosforescente, La Parguera, Puerto Rico (Source: Soler-Figueroa and Otero 2015)

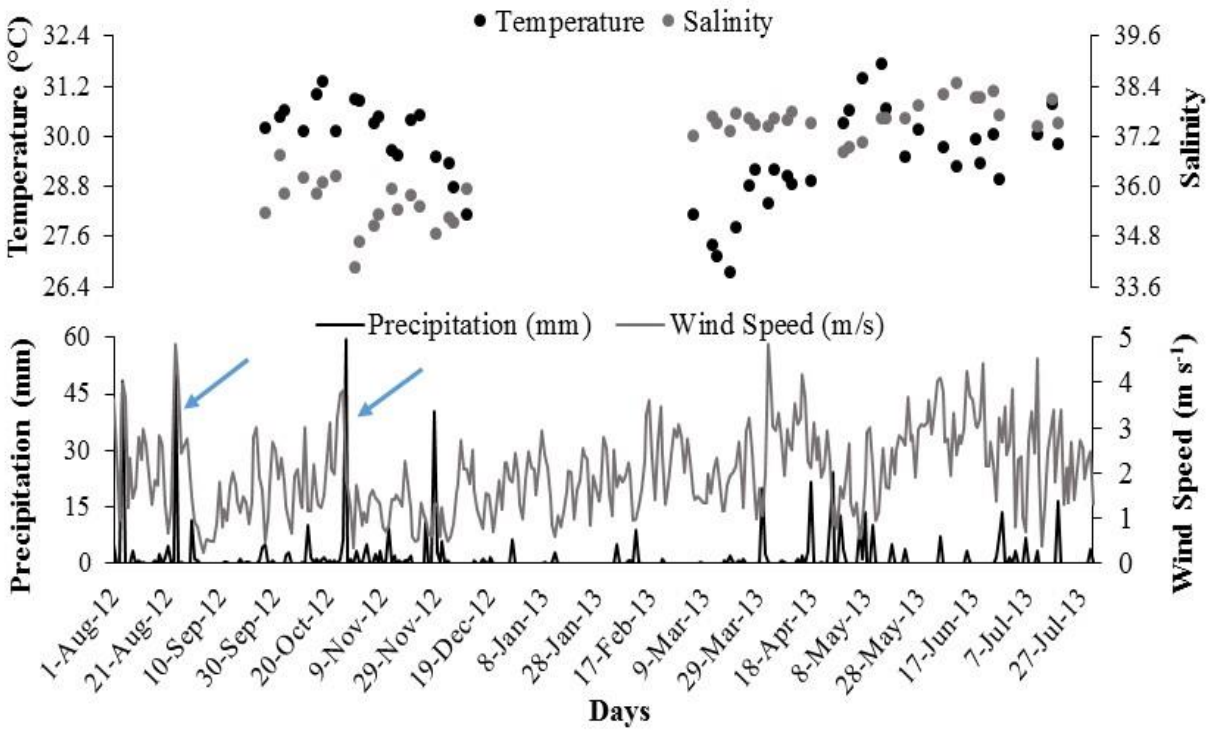

Fig. 2 Physical and meteorological conditions observed at Bahía Fosforescente. a) Temperatures and salinities recorded between September 27 and December 11, 2012 and between March 5 and July 19, 2013, b) Cumulative daily precipitation and average daily wind speeds recorded from August 2012 to July 2013. Arrows indicate the passage of Hurricane Isaac and Hurricane Sandy on August 24 and October 26, respectively.

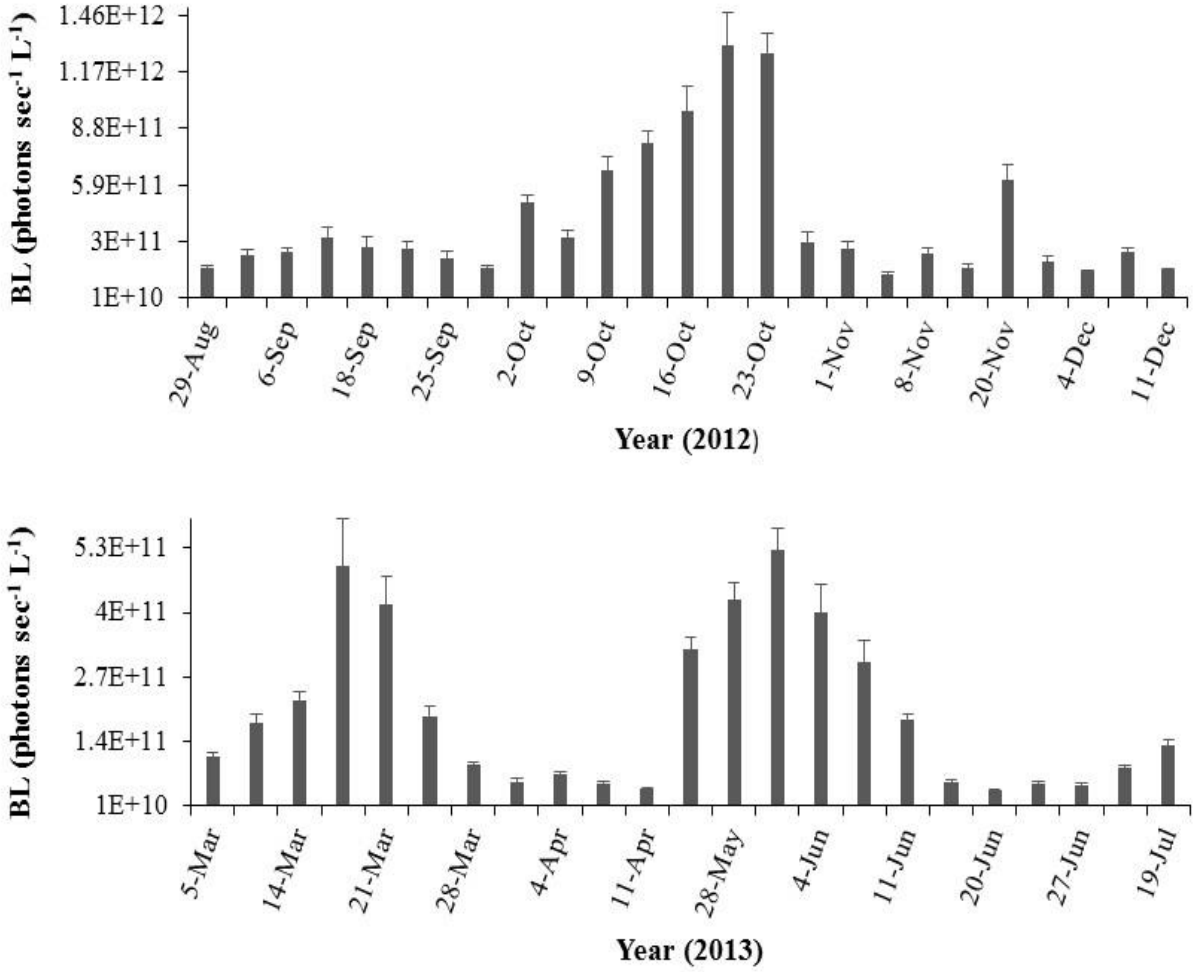

Fig. 3 Temporal variability in bioluminescence levels (BL) recorded at Bahía Fosforescente including the a) wet (August 29 – November 29, 2012) and b) dry seasons (March 4 – April 11, 2013). Note differences in scales. Bars represent standard errors.

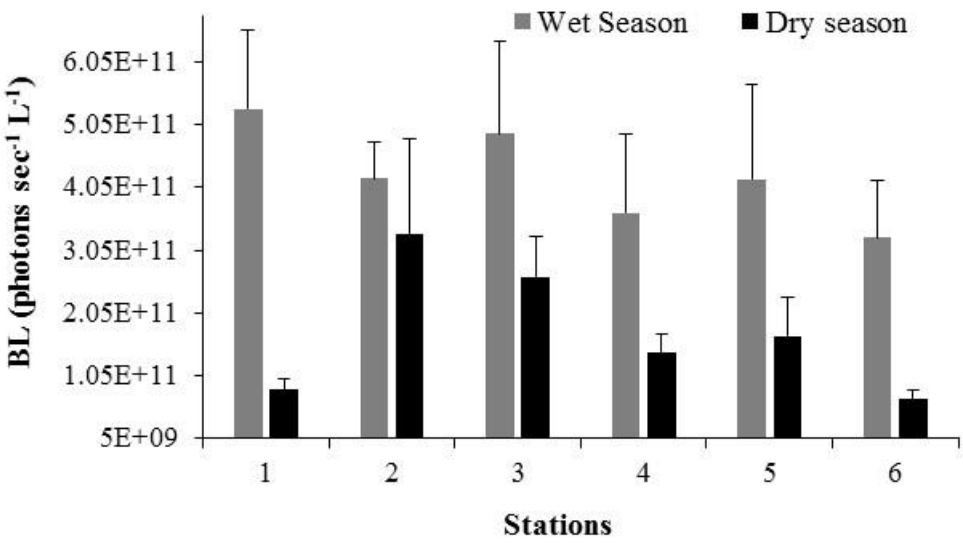

Fig. 4 Spatial variability in bioluminescence levels (BL) recorded at Bahía Fosforescente during the wet and dry seasons. Bars represent standard errors.

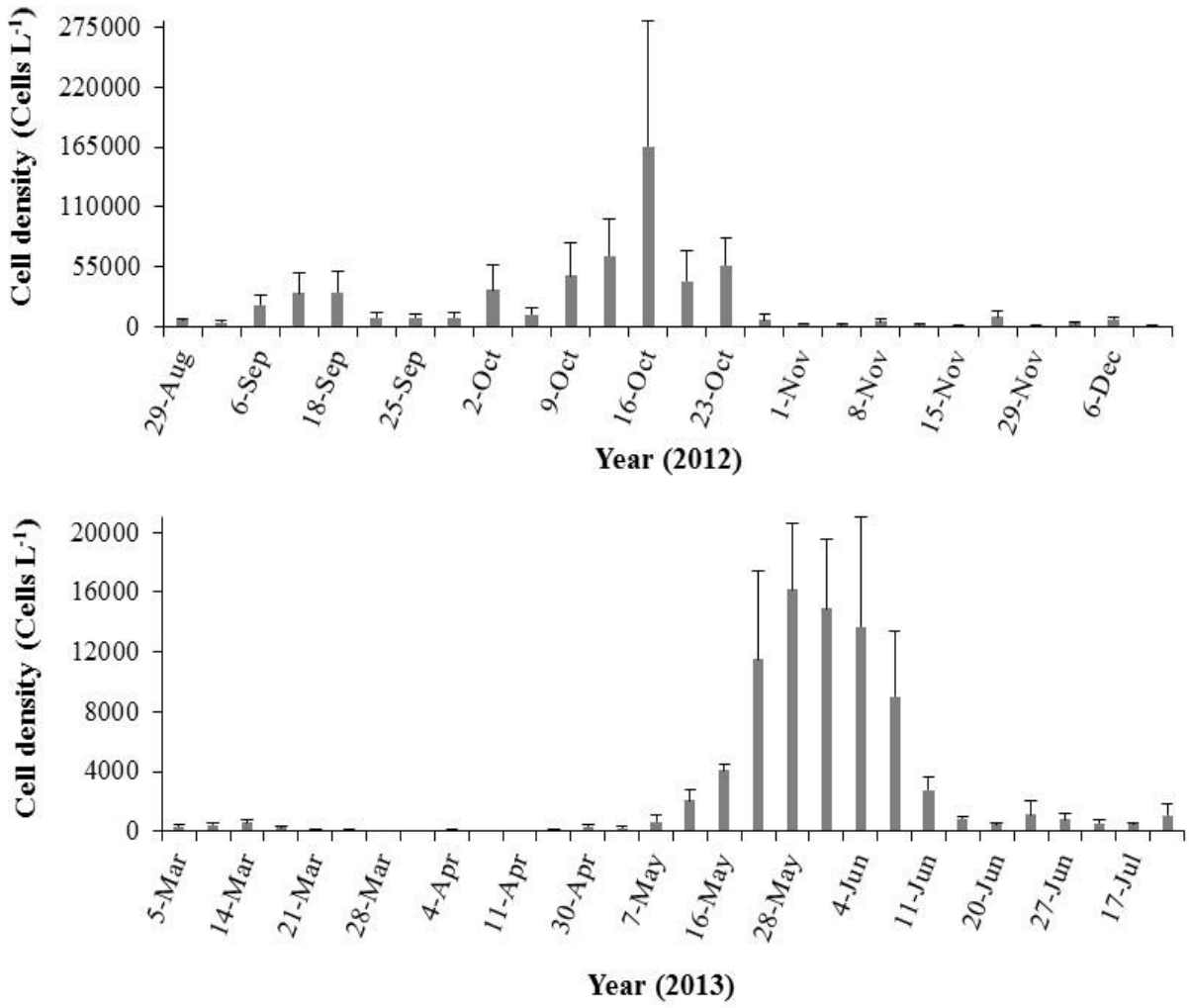

Fig. 5 Temporal variability in the cell densities of *P. bahamense* at Bahía Fosforescente including the a) wet (August 29 – November 29, 2012) and b) dry seasons (March 4 – April 11, 2013). Note differences in scales. Bars represent standard errors.

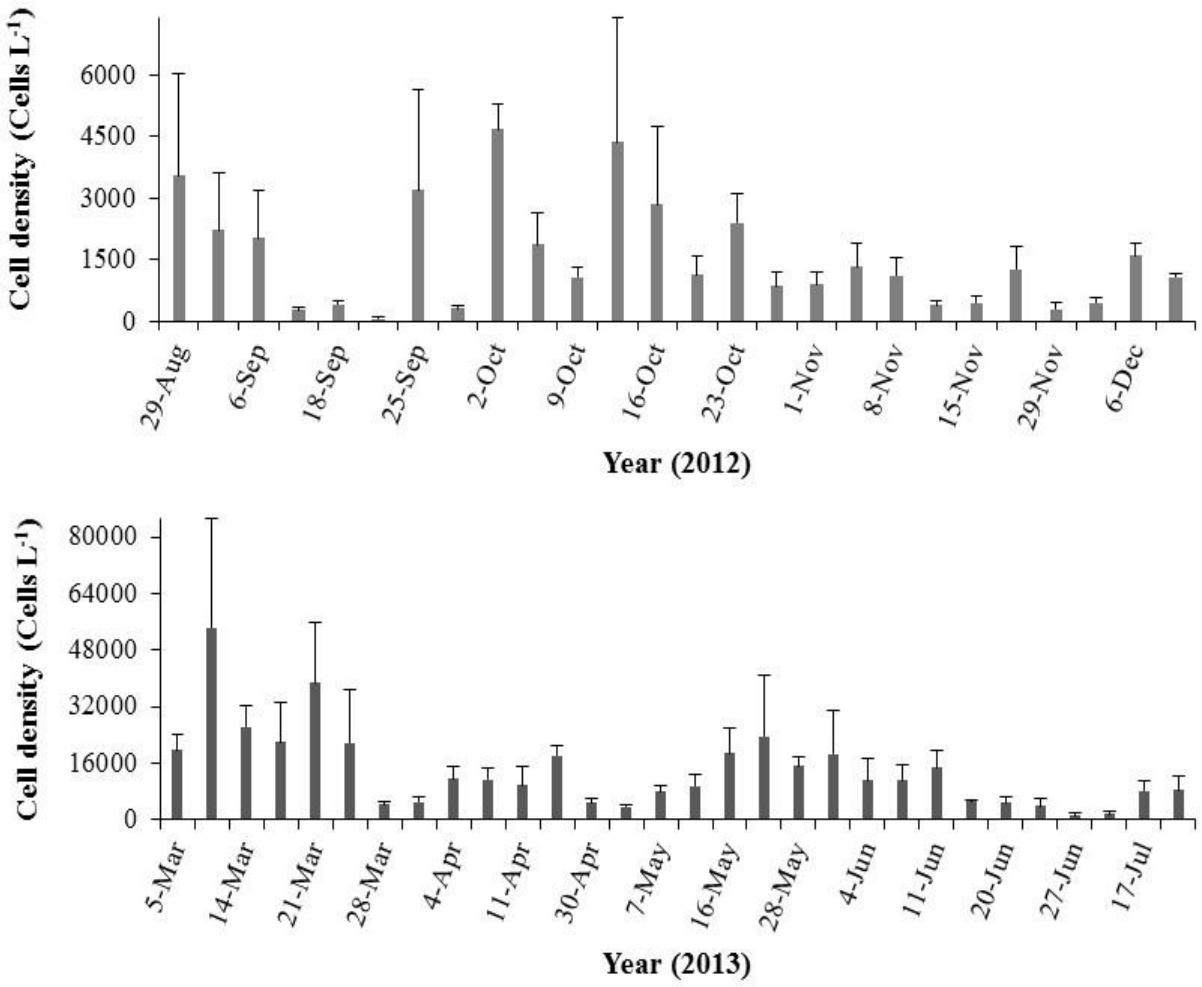

Fig. 6 Temporal variability in the cell densities of *C. furca* at Bahía Fosforescente including the a) wet (August 29 – November 29, 2012) and b) dry seasons (March 4 – April 11, 2013). Note differences in scales. Bars represent standard errors.

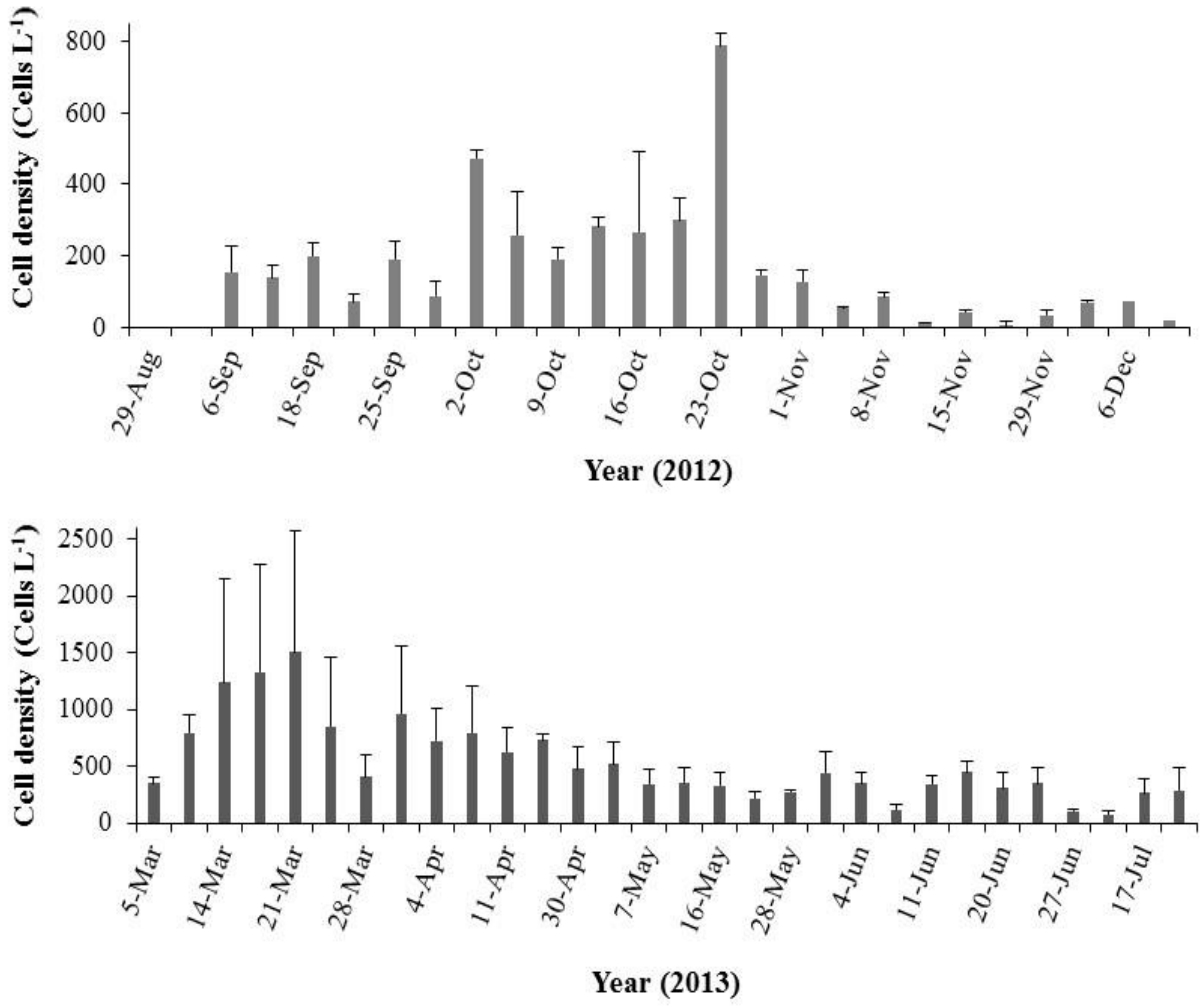

Fig. 7 Temporal variability in the cell densities of *Protoperidinium spp.* at Bahía Fosforescente including the a) wet (August 29 – November 29, 2012) and b) dry seasons (March 4 – April 11, 2013). Note differences in scales. Bars represent standard errors.

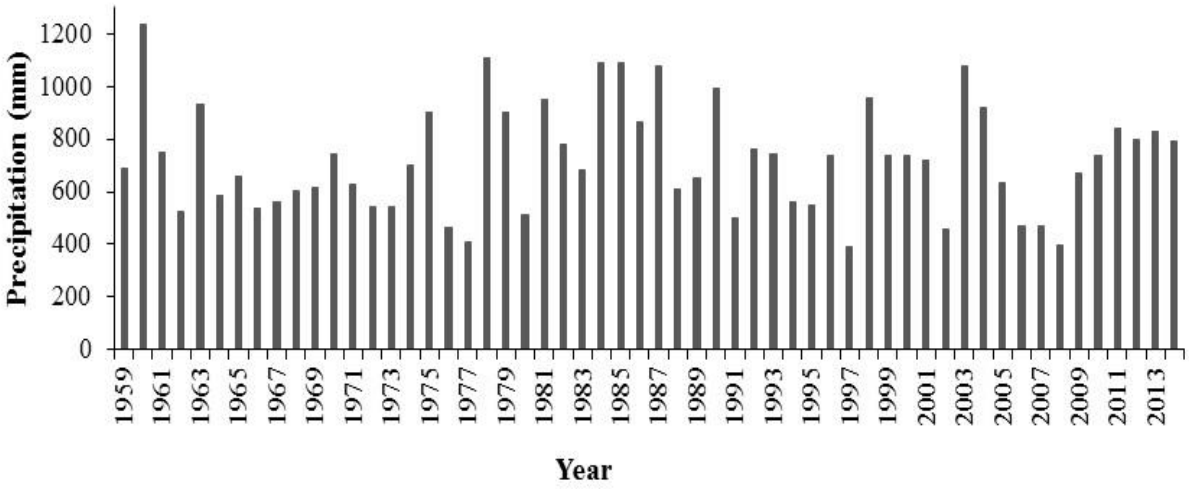

Fig. 8 Cumulative yearly precipitation for La Parguera area, from 1959 to 2014. Precipitation records were obtained from the Magueyes Island meteorological station operated by NOAA.

Chapter 5

General conclusion

Conclusions and future directions

The aim of this work was to determine what defines the variability in *Pyrodinium bahamense* and *Ceratium furca* at Bahía Fosforescente and to explore how the environmental factors and meteorological conditions drive the observed variations. Previous studies conducted in the bay have consistently reported the alternations in the abundance of both dinoflagellates species (Glynn 1964; Gold 1965; Seliger et al. 1971; Seixas 1983; Seixas 1988; Walker 1997; Soler-Figueroa 2006), with simultaneous reductions in the observed bioluminescence. However, the specific causes for these fluctuations in the dinoflagellate populations were unclear, and the presumed drop in bioluminescence over the years was never verified. One of the major barriers of past efforts, in deciphering the underlying processes driving the shifts in the dinoflagellate species dominance, is attributed to the low temporal resolution of such studies (i.e. single monthly measurements), which were ineffective in capturing the whole range of variability of this coastal ecosystem. Thus, the high frequency *in situ* monitoring approach employed in this study, encompassing days, weeks and seasons, allowed for a detailed examination of the environmental factors associated to the fluctuations in the dinoflagellate species composition and served to re-evaluate the presumed declining trend in the BL and the capacity of Bahía Fosforescente to maintain the populations of the bioluminescent dinoflagellates, and thus the bioluminescence.

Results from each of the studies above clearly indicate that the alternations in the abundance of *P. bahamense* and *C. furca* at Bahía Fosforescente are intimately linked to seasons, the local weather and the resulting changes in the environmental conditions. *Pyrodinium bahamense* was consistently the numerically dominant dinoflagellate during the wet season (average 2010: $7.8 \times 10^4 \pm 1.4\ 10^4$ cells L^{-1}; average 2012: $6.8 \times 10^3 \pm 6.5 \times 10^2$ cells L^{-1}) while *C. furca* dominated during the dry season (average 2011: $1.2 \times 10^4 \pm 2.8\ 10^3$ cells L^{-1}; average 2013: $2.3 \times 10^4 \pm 2.2 \times 10^3$ cells L^{-1}). This trend was observed during different years. The dominance of *P. bahamense* during the wet season appeared to be related to nutrients inputs (November 2010 - PO_4: 0.09-1.92 μmol L^{-1}; N+N: 0.34-7.67 μmol L^{-1}; NH_4^+: 2.9-29.4 μmol L^{-1}) and other land-derived and watershed materials after rainfall events, in agreement with the bloom dynamics of

this dinoflagellate in other regions (Usup and Azanza 1998; Phlips et al. 2004, 2006, 2011; Morquecho et al. 2012; Usup et al. 2012; Phlips et al. 2015). In contrast, the high cell densities of *C. furca* under periods of low precipitation, and thus, minimal terrestrial/watershed influence and lower nutrients concentration (March 2011 - PO_4: 0.17– 0.5 μmol L^{-1}; N+N: 0.2–1.23 μmol L^{-1}; NH_4^+: 0.2-7.8 μmol L^{-1}) may be attributed to the ability of this species to prey upon other planktonic species (Smalley et al. 1999; Smalley and Coats 2002; Smalley et al. 2003). Overall, the highest concentrations of both dinoflagellates were mostly observed in the north, northeast and southeast regions of the bay, within the context of short term spatial fluctuations influenced by winds and wind-derived currents.

The seasonal trend in the dinoflagellate composition at Bahía Fosforescente was also reflected in the bioluminescence. High levels characterized the wet season (average: 4.3×10^{11} $\pm 4.9 \times 10^{10}$ photons sec^{-1} L^{-1}) and strongly correlated with the high cell densities of *P. bahamense* (average: 2.5×10^4 ±6.3 x 103 cells L^{-1}). During the dry season, dramatic decreases in *P. bahamense* populations (average: 1.7×10^2 $\pm 0.4 \times 10^3$ cells L^{-1}) resulted in simultaneous reductions in the BL (average: 1.6×10^{11} $\pm 3.0 \times 10^{10}$ photons sec^{-1} L^{-1}. However, peaks in bioluminescence during this period, similar to the average levels of the wet season, were attributed to increases in the populations of heterotrophic dinoflagellates such as *Protoperidinium* spp., and probably other bioluminescent dinoflagellates such as *Polykrikos* spp. Estimates of BL, calculated using *P. bahamense* abundances from studies conducted during the past fifty years (i.e. Clarke and Breslau 1960, Seliger et al. 1971, Seixas 1988, Walker 1997, Soler-Figueroa 2006, Soler-Figueroa and Otero 2015), and based on the average BL to *P. bahamense* ratio of the study herein, explicitly showed variable but stable conditions in *P. bahamense* populations and BL, but no a net trend. It is suggested that variations in bioluminescence over the past fifty years could have been also influenced by yearly oscillations in precipitation regimens. This study provided the first evidence to support that BL at Bahía Fosforescente are not decreasing and the bay maintains conditions favorable for the accumulation of abundant bioluminescent dinoflagellate populations.

Although this study represents an important first step in deciphering the processes underlying the dynamics in *P. bahamense* and *C. furca* populations at Bahía Fosforescente, much research is still needed. For example, information on the total phytoplankton community in the bay is limited. As revealed by the second study conducted in this dissertation (Chapter 3), under

some circumstances, diatoms can be an important component of the phytoplankton community at the bay. Diatoms can represent a potential source for light and nutrient competition, altering the extant bioluminescent dinoflagellates assemblages and thus, modulating the bioluminescence levels at the bay. Thus, future studies should include the examination of the total phytoplankton community and explore possible interactions among different phytoplankton groups. Resuspended sediments, substantial populations of benthic taxa (i.e. *Pleurosigma* spp.), and low cell densities of *P. bahamense* and *C. furca* populations were also observed during the second study of this dissertation (Chapter 3), coinciding with periods of high wind velocities and recurrent boat traffic and other recreational activities associated with *Spring Break*. Resuspension events increase the potential for light limitation (Cloern 1987; Huisman and Weissing 1994), and can potentially affect the populations of light-dependent photosynthetic organisms, such as *P. bahamense*. Additionally, sediments resuspension may modify the nutrient regimens and the overall water quality of the bay promoting changes in the phytoplankton community composition (Harris 1983; Álvarez-Góngora and Herrera-Silveira 2006; Cloern and Jassby 2010). Thus, from a management perspective, the effects of wind-induced turbulence and boating activities on water mixing, sediment resuspension, light climate, nutrient fluxes and the response of the phytoplankton community at Bahía Fosforescente needs to be studied in detail.

Studies on the temporary and resting cysts of *P. bahamense* are strongly required, including their presence, distribution and the potential role of these cysts in the maintenance of a seed population of this species (Anderson 1989; Corrales et al. 1995; Morquecho et al. 2014; Onda et al. 2014). Such studies will provide important information to improve our understanding on the population dynamics of *P. bahamense* at Bahía Fosforescente. Other factors that are yet to be defined include the possible top down control over the dinoflagellates populations and the pelagic-benthic interactions as a source of nutrients and cysts reservoirs.

This is the first study conducted at the bay with a high spatiotemporal resolution that yielded vital information to help develop a robust link between environmental forcing and the response of dinoflagellate populations and BL at the bay. In conclusion, different precipitation regimens and the resulting changes in environmental conditions appeared to be the critical factors controlling the alternations in the abundance of *P. bahamense* and *C. furca* and the observed changes in bioluminescence at Bahía Fosforescente.

Management implications and recommendations

The fact that BL and the dinoflagellate populations at Bahía Fosforescente are strongly modulated by weather and runoff have profound management implications and warn managers of the sensitivity of this and other similar coastal ecosystems to changes in watershed practices and to climate change. At present it is unknown if the plasticity of the extant phytoplankton populations will cope with the more frequent rains and storms expected as a result of global climate change or to changes in land use pattern due to changes in human activities. These factors are capable of modifying the vertical structure of natural waters and the patterns of nutrient inputs, sediment loading, salinity and pollutant transport that may alter oxygen balance, light penetration and the overall bay productivity, thus potentially changing the capacity of the system to sustain the extant biological assemblages. Thus, our findings support the importance for establishing effective ecosystem based management for this important resource, especially considering the increased pressures exerted on this coastal system.

The technologies and instrumentation used in this work (i.e. FlowCAM and UBAT) demonstrated to be useful tools to conduct and implement high frequency monitoring programs, in this and other coastal ecosystems, to gain a better understanding of the role of environmental forcing on the populations of fast fluctuating phytoplankton organisms, which represent the base of the trophic food webs. Furthermore, the information gained by a continuous monitoring program will provide long-term data that serve to assess the response of the phytoplankton community to climate change (Hays et al., 2005; Moore et al., 2008; Anderson, 2014).

A balance between management/conservation practices and usage (i.e. tourist operations) should be encouraged to maximize sustainability. All bioluminescence bays and lagoons should be declared no-wake zones and no-anchoring zones to prevent destabilizing the bottom and sediment resuspension. Thus, the placement of speed signs and the installation of a mooring-buoy system are strongly recommended. These minimal efforts will provide a first step to safeguard and lessen any potential impact to these highly vulnerable and unique coastal ecosystems.

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