Population fluctuations of *Pyrodinium bahamense* and *Ceratium furca* (Dinophyceae) in Laguna Grande, Puerto Rico, and environmental variables associated during a three-year period

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Abstract: Fluctuaciones poblacionales de *Pyrodinium bahamense* y *Ceratium furca* (Dinophyceae) en Laguna Grande, Puerto Rico, y variables ambientales asociadas durante un periodo de tres años. Bioluminescent bays and lagoons are unique natural environments and popular tourist attractions. However, the bioluminescence in many of these water bodies has declined, principally due to anthropogenic activities. In the Caribbean, the bioluminescence in these bays and lagoons is mostly produced by the dinoflagellate *Pyrodinium bahamense* var. *bahamense*. Laguna Grande is one of the three year-round bioluminescent water bodies in Puerto Rico that are known to remain but *P. bahamense* var. *bahamense* density fluctuations have not been studied. In this study we describe water quality parameters and density fluctuations of the most common dinoflagellates in Laguna Grande, *P. bahamense* var. *bahamense* and *Ceratium furca*, over a three-year period. For this, three sampling stations were established in Laguna Grande from which water samples were collected in triplicate and analyzed for temperature, phosphates, nitrates, salinity, water transparency, fluorescence, and dinoflagellate densities, at the water surface and at 2m depth, from May 2003 to May 2006. The results showed a density fluctuation pattern for *P. bahamense* var. *bahamense,* where higher densities were observed mainly from April to September, and lower densities from October to February. Density fluctuations of *C. furca* were more erratic and a repetitive pattern was not observed. Densities of *P. bahamense* var. *bahamense* ranged from 0.48 to 90 978 cells/L and densities of *C. furca* ranged from 0 to 11 200 cells/L. The mean population density throughout the sampling period was significantly higher in *P. bahamense* var. *bahamense* (mean=18 958.5 cells/L) than in *C. furca* (mean=2 601.9 cells/L). Population densities of *P. bahamense* var. *bahamense* were negatively correlated with *C. furca* densities during the first year of sampling; however, they were positively correlated during the third year. Non-significant differences between surface and 2m depth samples were observed for temperature, phosphates, nitrates, salinity, fluorescence, and densities of *P. bahamense* var. *bahamense* and *C. furca,* suggesting a vertically mixed water column. Water transparency was positively correlated with salinity and negatively correlated with fluorescence. Fluorescence was negatively correlated with salinity. The mean population densities of *P. bahamense* var. *bahamense* and *C. furca* observed in this study were within the range of previous reports in other bioluminescent water bodies in Puerto Rico and Florida, USA. In order to conserve the continuous *P. bahamense* var. *bahamense* populations in Laguna Grande, as well as its bioluminescence, it is recommended to maintain the existing water flow levels in the 1.5km long inlet/outlet channel; to maintain unpolluted water quality parameters within the bay, the hydrographical basin and adjacent waters, and to preserve mangrove communities within the basin and adjacent areas. Results of this study may help to develop management plans aiming to conserve *P. bahamense*, its bioluminescence and the lagoon attraction. Rev. Biol. Trop. 61 (4): 1799-1813. Epub 2013 December 01.

Key words: *Pyrodinium bahamense, Ceratium furca,* Laguna Grande, Puerto Rico, bioluminescence, bioluminescent lagoons.

Bioluminescent bays and lagoons are economically important as tourist attractions (González-Sánchez, Muñoz-Salinas & Roset, 2012). The bioluminescence in many of these water bodies have declined or failed due to anthropogenic activities (e.g., Fire Lake, New Providence Island, Bahamas; Oyster Bay, Jamaica; Environmental Solutions, Ltd., 2005). There is some disagreement about the number of year-round bioluminescent bays in the world, but at least three of those remaining functional are found in Puerto Rico. Bioluminescent water bodies appear to be threatened due to poor water quality, habitat deterioration, light pollution (causes poor perception of bioluminescence) and unwise management. Conservation of these systems is important because they are rare and unique.

Caribbean bioluminescent bays and lagoons are usually bordered by highly productive mangrove communities (Seliger, Carpenter, Loftus, Biggley & McElroy, 1971), which release large quantities of nutrients into adjacent waters (Odum, McIvor & Smith, 1982; Lee, 1995). Nitrates, phosphates, B vitamins (Burkholder & Burkholder, 1958), humic substances (Prakash & Rashid, 1968; Carlsson & Granéli, 1993) and other nutrients, help to sustain the highly productive (Burkholder, Burkholder, Almodóvar, 1967) and persistent (Margalef, 1957; Soler-Figueroa, 2006) phytoplankton populations. Nutrients tend to accumulate in these water bodies since their mouths are narrow and shallow (Seliger, Carpenter, Loftus & McElroy, 1970; Zayas, 1979), resulting in low rates of water interchange with the ocean. The bioluminescence observed in these bays and lagoons is produced almost entirely by the photosynthetic dinoflagellate *Pyrodinium bahamense* Plate 1906 var. *bahamense* (Steidinger & Tangen, 1997), which often can reach very high densities (Margalef, 1961; Seliger et al., 1970, 1971; Soler-Figueroa, 2006; Phlips, Badylak, Bledsoe & Cichra, 2006).

Dinoflagellate bioluminescence is a defensive behavior that reduces grazing (Esaias & Curl, 1972; White, 1979) by affecting the behavior of animals that feed on them (Buskey & Swift, 1983; Buskey et al., 1983). In addition, it may serve as a "burglar alarm" to attract a secondary predator that threatens to eat the primary predator (Morin, 1983; Young, 1983).

Margalef (1961) proposed a general water circulation mechanism for Bahía Fosforescente (Puerto Rico), in order to explain the retention of *P. bahamense* var. *bahamense*, as well as other phytoplankton. Inshore winds, aimed towards the inside of bioluminescent bays, can also create hydrological conditions leading to high water retention times in the shallower innermost portions of these water bodies (Seliger et al., 1971). This could also contribute to the retention of nutrients and phytoplankton.

P. bahamense var. *bahamense* has been reported and/or studied in several bays and lagoons in tropical and subtropical Atlantic waters, including Fire Lake (near Nassau), Bahamas (Harvey, 1952); Oyster Bay, Jamaica (Seliger & McElroy, 1968; Seliger et al., 1970); and Tampa Bay (Steidinger, Tester & Taylor, 1980; Phlips et al., 2006; Badylak, Phlips, Baker, Fajans & Boler, 2007), Florida Bay (Phlips & Badylak, 1996; Phlips et al., 2006) and Indian River Lagoon (Badylak, Kelley & Phlips, 2004; Phlips et al., 2006), Florida.

The dinoflagellate, *Ceratium furca* (Ehrenberg) Claparède & Lachmann 1859 var. *hircus* (Steidinger & Tangen, 1997), can also reach very high densities in bioluminescent bays and lagoons in Puerto Rico, but is not bioluminescent. *C. furca* is cosmopolitan and found from cold temperate to tropical waters (Steidinger & Tangen, 1997). *C. furca* is mixotrophic and can act as a predator on ciliates, such as *Strobilidium* spp. (Smalley & Coats, 2002).

In Puerto Rico, population dynamics of *P. bahamense* var. *bahamense* and *C. furca* have been described in Bahía Fosforescente (also known as Bahía Bioluminiscente; Margalef, 1961; Seixas, 1983, 1988; Walker, 1997; Soler-Figueroa, 2006), Bahía Monsio José (Seixas, 1983), Puerto Mosquito (in the island of Vieques; Walker, 1997; Soler Figueroa, 2006) and Laguna Joyuda (Carvajal-Zamora, 1976). The thecate dinoflagellate species composition

of Bahía Fosforescente has also been described (Hernández-Becerril & Navarro, 1996).

Even though the presence of *P. bahamense* var. *bahamense* has been reported for Laguna Grande (Candelas, Cintrón & McKenzie, 1968; Zayas, 1979), a highly regarded (Departamento de Recursos Naturales de Puerto Rico, 1984) and one of the most frequently visited bioluminescent lagoon in Puerto Rico, no study has been done to describe the population fluctuations of *P. bahamense* at this site.

The goal of this study was to describe water quality parameters on density fluctuations of *P. bahamense* var. *bahamense* and *C. furca,* the two most abundant dinoflagellate species in Puerto Rico bioluminescent bays and lagoons, over a three year period, at surface and 2m depth sites in Laguna Grande, Puerto Rico. Concurrently, to gather basic environmental and ecological information in order to understand better the unique ecosystem. This will help to make more realistic and sound management plans in order to conserve the bioluminescence and water quality in the lagoon.

MATERIALS AND METHODS

Study site: Laguna Grande is a coastal lagoon located on the North-East coast of Puerto Rico. It is situated within a basin-type mangrove forest (*sensu* Lugo & Snedaker, 1974) and bordered by *Rhizophora mangle* (Linnaeus, 1753). The Lagoon is surrounded by the Cabezas de San Juan Natural Reserve, managed by the Conservation Trust of Puerto Rico (Fig. 1).

The average depth of Laguna Grande is 3m and its maximum depth is 5m. It occupies an area of about 50ha and contains about 662 000m^3 of water (Soler-López & Santos, 2010). The South-Eastern portion of Laguna Grande is connected to Las Croabas Bay by the Laguna Grande Channel, a 1.5km long channel (or tidal inlet) that averages about 5m across

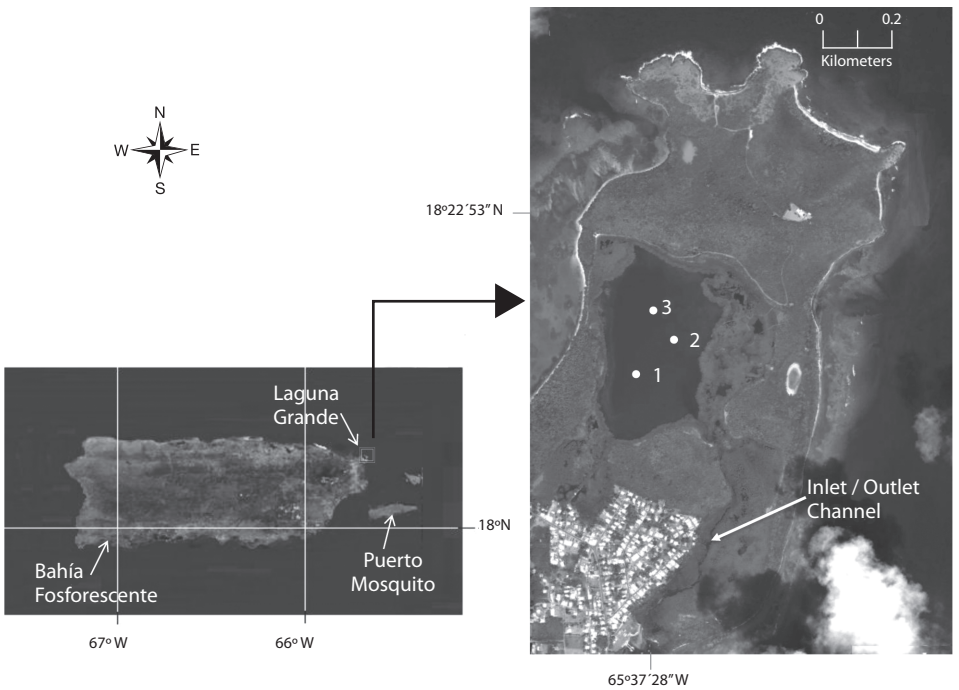

Fig. 1. Satellite image of Puerto Rico (left) showing the location of Laguna Grande, Puerto Mosquito and Bahía Fosforescente; and a detailed image of Laguna Grande (right), showing the position of the study sites and the inlet/outlet channel. Image from the US Geological Survey 2011.

and 1m deep (Zayas, 1979; Weaver, Ramírez & Coll, 1998; Soler-López & Santos, 2010).

Zayas (1979) reported salinity concentrations ranging from 20.5 to 36.2psu (mean=33.6psu), oxygen concentrations ranging from 2.0 to 7.2mg/L (mean=5.3mg/L), and phosphate concentrations ranging from 0.01 to 0.10µg-at /L (mean=0.03µg-at /L), for stations located in the center of the lagoon. The average flushing rate of Laguna Grande is 7.7d (Soler-López & Santos, 2010).

Field methods: Three sampling stations were established in the central portion of Laguna Grande, using a Garmin® GPS model 73 (Station 1: 18°22'31.69" N - 65°37'28.98" W; Station 2: 18°22'34.93" N - 65°37'22.24" W; Station 3: 18°22'40.32" N - 65°37'26.31" W; Fig. 1). At each station, water samples were collected in triplicate using 500mL bottles, at the surface and at 2m depth (the maximum depth at mean low tide in one of the stations is approximately 2.4m). Samples were collected once or twice every month during a period of approximately three years, from May 3, 2003 to May 25, 2006. The Surface samples were collected at approximately 10cm below the surface by grab sampling. A manual diaphragm water pump connected to a 2.5cm diameter flexible tube, attached to a 3.5m-long pole, was used to collect the 2m depth water samples. The water pump was flushed with water from each station before collecting each sample. After sample collection, bottles were covered with aluminum paper and immediately placed under ice in a cooler.

The water temperature in each sample was determined immediately after collection using an alcohol laboratory thermometer. Water transparency was determined at each station using a 20cm diameter black/white quadrant limnological Secchi disk. All samples were collected during the daytime. The field sampling procedures lasted approximately two hours.

Laboratory methods: Water samples were taken to the laboratory within one hour after performing the field sampling procedures. Sample bottles were mixed by inversion in order to distribute the sample homogeneously. One 250mL sub-sample was taken from each sampling bottle for each physico-chemical parameter to be analyzed.

Chlorophyll-*a* was analyzed using a Turner Designs Model TD-700 fluorometer, using the *In-Vivo* method (Turner Designs, 1999). The fluorometer was configured with a 340-500nm excitation filter, a 680nm emission filter and a Blue Mercury Vapor lamp. Borosilicate Test Tubes (13x100mm) were used to perform the analyses (Turner Designs, 1999). All blanks and samples were read at 25°C. Relative fluorescence measurements were reported as Relative Fluorescence Units (RFU). The fluorometer was calibrated prior to each use by means of a blank and a solid secondary standard (Turner Designs, 1999).

Nitrates and phosphates were analyzed using a calibrated and certified (Calitek, Inc., Bayamón, Puerto Rico) Hach spectrophotometer model DR-2000 (Hach Company, 1996a). Nitrates were analyzed according to Method 8192 (cadmium reduction method), and orthophosphates according to Method 8048 (ascorbic acid method; Hach Company, 1996b).

Salinity was determined using a Fisher model 13-946-27 refractometer, calibrated with distilled water prior to analysis. Daily rainfall data was obtained from NOAA's Paraíso station, located approximately 13km Southwest of the study area. Rainfall data was recorded using a Fischer Porter Rain Gauge. Cumulative precipitation was calculated for 3, 6, 9, 12, 15 and 18 day intervals before each sampling date.

In order to preserve samples, each sampling bottle was mixed several times by gentle inversion. Immediately afterwards, a 248.5mL sub-sample was poured into a modified sedimentation chamber and preserved in 1% Lugol's solution, making a final volume of 250mL. The preserved subsamples were allowed to settle by gravity for three days (Wetzel & Likens, 2000). Each subsample was concentrated to 100mL by carefully removing the supernatant with a pipette. The supernatant was gravity filtered through a 20µ Nitex®

Nylon Bolt Cloth, and the cloth inspected for the presence of phytoplankton. If organisms were observed in the cloth, these were returned to the sedimentation chambers.

After mixing each 100mL concentrated plankton sample, one mL subsample was obtained, with an automatic volumetric pipet, and transferred to a Sedwick-Rafter (S-R) counting cell (Wetzel & Likens, 2000). The S-R cell was covered with a coverglass and all *P. bahamense* var. *bahamense* and *C. furca* were counted under a Nikon Eclipse E-600, or a Leica CME microscope, at 100 or 200X magnification. Each subsample was counted once.

A main effects three-way ANOVA was used to evaluate differences between months, sites and depths, for population densities of *P. bahamense* var. *bahamense* and *C. furca,* and for all physical-chemical parameters; except Secchi depth, which was evaluated using a two-way ANOVA (Secchi depth was measured vertically in the water column, therefore depths effects are not applicable). Data were transformed with log e, log 10 and square root to try to conform to assumptions of ANOVA. Levene's tests were used to assess homoscedasticity in transformed and non-transformed data. All ANOVA tests were carried out only using homoscedastic data. Kruskal-Wallis non-parametric tests were applied to variables that showed significant heteroscedasticity, even after been transformed. We assume independence among observations in the dataset.

A multiple correlation was used to determine possible relationships among all variables (Sokal & Rohlf, 1994). Statistical analyses were performed using IBM® SPSS® Statistics 19 software (http://www.spss.com). Any correlation equal to, or less than 0.5 was considered significant.

RESULTS

Temperature: The average water temperature during the sampling period was 29.1°C (SD=1.8, n=40; Table 1). The highest temperature (34.0°C) was recorded during August 16, 2003, and the lowest one, 24.3°C, during January 31, 2006. Kruskal-Wallis non-parametric tests did not detect significant differences between depths nor between sites ($p>0.05$). However, significant differences were detected between months ($p<0.001$).

Salinity: Salinity ranged from 18 to 42 practical salinity units (psu, Fig. 2). During strong rainfall events decreases in salinity were observed both in surface and 2m depth waters. During less severe events, decreases in salinity were observed only in surface waters. ANOVA detected significant differences between months ($p<0.001$) and depths ($p=0.001$) but not between sites ($p>0.05$, Table 2).

Water transparency: Average Secchi depth was 1.68m (SD=0.43, n=35; Table 1). It was positively correlated with salinity ($r=0.385$, $df=33$, $0.02<p<0.05$) and negatively correlated with fluorescence ($r=-0.634$, $df=34$, $p<0.001$). The lowest readings in Secchi depth were recorded during November 21, 2003 (0.76m) and September 21, 2004 (0.61m), which corresponded with high fluorescence concentrations. ANOVA detected significant differences between months ($p<0.001$) but not between sites ($p>0.05$, Table 2).

Rainfall: Daily rainfall at Paraíso Station ranged from 0 to 10.4cm (Table 1). Cumulative 9-d, 12-d, 15-d and 18-d rainfall data (cumulative amount of rain recorded prior to each sampling occasion) was negatively correlated with salinity. Also, cumulative 6-d, 9-d, 12-d, 15-d and 18-d rainfall data was negatively correlated with fluorescence. Cumulative 9-d rainfall was negatively correlated with Secchi depth; and cumulative 3-d rainfall was correlated with phosphates (Table 3).

The lowest salinity concentrations (18psu, Table 1), recorded during September 21, 2004, corresponded to the passage of Hurricane Jeanne on September 14 (22.9cm of rain in 24hr at the Paraíso station). The second lowest salinities, recorded during November 21, 2003, were caused by the heavy rains received during the occurrence of a tropical wave in November

TABLE 1

Minimum, maximum and mean values of surface and bottom samples; and minimum, maximum and grand mean of the resulting mean values of surface and bottom samples, of biological and physico-chemical parameters

	Surface			Bottom			Surface and bottom mean			n
	Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Grand mean±SD	
Biological Parameters:										
- *Pyrodinium bahamense* densities (cells/L)	0.48	103 511.1	20 286.8 ±22 486.9	0.48	78 444.4	19 690.3 ±21 263.6	0.48	90 977.8	19 988.5 ±21 390.0	39
- *Ceratium furca* densities (cells/L)	1.0	13 800.0	2 741.0 ±3238.8	1.0	8 600.0	2 462.8 ±2 726.0	1.0	11 200.0	2 601.9 ±2 843.1	40
Physico-chemical Parameters:										
- Temperature (°C)	25.7	34.0	29.1±1.8	24.3	34.0	29.1±1.95	25.0	34.0	29.1±1.85	40
- Salinity (psu)	17.8	42.2	35.5±4.7	25.7	42.0	36.1±3.7	22.3	42.1	35.8±4.1	42
- Soluble Reactive Phosphorus (mg/L)	0.01	0.61	0.08±0.10	0.01	0.65	0.09±0.12	0.01	0.63	0.08±0.11	41
- Nitrates (mg/L)	0.04	0.56	0.12±0.10	0.05	0.26	0.11±0.06	0.05	0.41	0.12±0.08	32
- Fluorescence (RFU)	6.4	698.5	71.5±112.7	8.0	374.1	66.7±65.6	7.7	536.3	69.1±87.5	43
	Min	Max	Mean±SD	n						
- Secchi depth (m)	0.61	2.49	1.68±0.43	35						
- Daily rainfall at Paraíso Station (cm)	0	10.54	0.85±1.88	43						

Min=minimum, Max=maximum, SD=standard deviation, n=number of sampling periods, orgs.=organisms, RFU=relative fluorescence units.

Secchi depth was measured vertically in the water column, therefore surface and bottom values are not applicable.

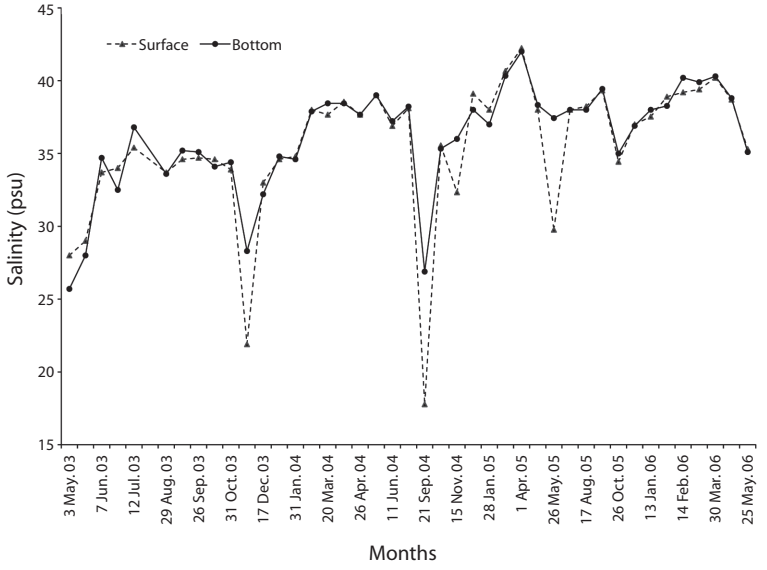

Fig. 2. Monthly variations of surface and 2-meter depth salinity concentrations in the water at all sampling stations (mean of all sampling stations). psu: practical salinity units.

TABLE 2
Main effects ANOVA of physical-chemical parameters and population densities

Parameter	Factor	F	df	p
Pyrodinium bahamense	Months	10.472	36; 187	< .001
	Sites	2.575	2; 187	0.079
	Depths	0.101	1; 187	0.750
Ceratium furca	Months	8.578	36; 188	< .001
	Sites	0.531	2; 188	0.589
	Depths	1.128	1; 188	0.290
Temperature*	Months	32.074	35; 179	< .001
	Sites	1.539	2; 179	0.217
	Depths	2.325	1; 179	0.129
Salinity	Months	32.195	36; 188	< .001
	Sites	2.117	2; 188	1.123
	Depths	12.267	1; 188	0.001
Soluble Reactive Phosphorus* (log 10 transformed)	Months	335.360	36; 187	< .001
	Sites	1.475	2; 187	0.231
	Depths	0.029	1; 187	0.865
Nitrates	Months	7.589	30; 158	< .001
	Sites	0.521	2; 158	0.595
	Depths	0.764	1; 158	0.383
Fluorescence (log 10 transformed)	Months	22.795	37; 191	< .001
	Sites	5.879	2; 191	0.003
	Depths	1.315	1; 191	0.253
Secchi depth	Months	126.72	35;68	< .001
	Sites	22.40	2;68	< .001

*, Levene's test p<0.05.
F=F statistic, df=degrees of freedom, p=probability.
All parameters were tested using three-way ANOVA, except water transparency which was tested using a two-way ANOVA.

TABLE 3
Significant correlations between cumulative rainfall and water quality parameters

Comparison	r	df	p
9-d vs. salinity	-0.6392	40	< .001
12-d vs. salinity	-0.6610	40	< .001
15-d vs. salinity	-0.6446	40	< .001
18-d vs. salinity	-0.4308	40	0.02 < p < 0.05
6- d vs. fluorescence	0.4923	40	0.001 < p < 0.01
9-d vs. fluorescence	0.7892	40	< .001
12-d vs. fluorescence	0.7667	40	< .001
15-d vs. fluorescence	0.6766	40	< .001
18-d vs. fluorescence	0.4904	40	0.001 < p < 0.01
9-d vs. Secchi depth	-0.4238	31	.01 < p < .05
3-d vs. soluble reactive phosphorus	0.4876	37	.001 < p < .01

r=correlation coefficient, df=degrees of freedom, p=probability, 3-d=3-day cumulative rainfall, 6-d=6-day cumulative rainfall, 9-d=9-day cumulative rainfall, 12-d=12-day cumulative rainfall, 15-d=15-day cumulative rainfall, 18-d=18-day cumulative rainfall.

13, 2003 (20.8cm of rain in 24hr at the Paraíso station). The highest salinity (42psu), recorded during April 1, 2005, corresponded with a period of very low rainfall previous to the sampling date (2.6cm of rain in 23d at the Paraíso station, Figure 2). Even though periods of 7 and 8d had passed between the passage of Hurricane Jeanne, the tropical wave; and the sampling dates after those events, low salinity readings were still recorded at Laguna Grande.

Phosphates: Soluble reactive phosphorus (SRP) levels up to 0.63mg/L were observed (Fig. 3). Average SRP concentration was 0.08 mg/L (SD=0.10, n=41; Table 1). Kruskal-Wallis non-parametric tests detected significant differences between months (p<0.001) but not between depths or sites (p>0.05).

Nitrates: Nitrate concentrations up to 0.41mg/L were observed (Fig. 3). The lowest concentration, 0.05mg/L, was observed during January 31, 2006. Average nitrate concentration was 0.12mg/L (SD=0.08, n=32, Table 1). ANOVA detected significant differences between months (p<0.001) but not between depths or sites (p>0.05, Table 2).

Fluorescence: The highest peaks in fluorescence (Table 1) were observed during the low salinity dates of November 21, 2003; September 21, 2004; and May 26, 2005 (Fig. 4). During the first two peaks fluorescence was higher in the surface; however, during the third peak, higher readings were observed in the 2m depth samples. Fluorescence was negatively correlated with salinity (r=-0.802, df=40, p<.0001). ANOVA detected significant differences between months (p<0.001) but not between depths or sites (p>0.05, Table 2).

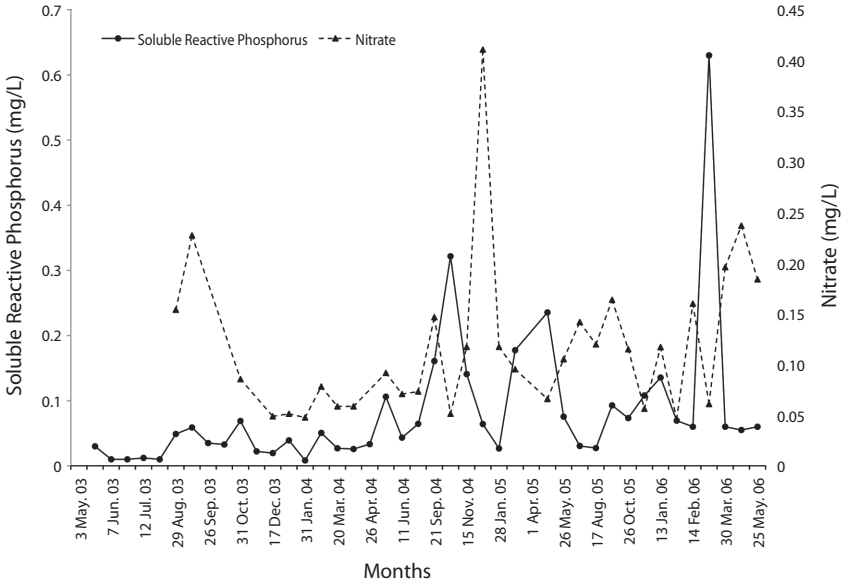

Fig. 3. Monthly variations of soluble reactive phosphorus and nitrate concentrations in the water at all sampling stations (mean of all sampling stations and depths).

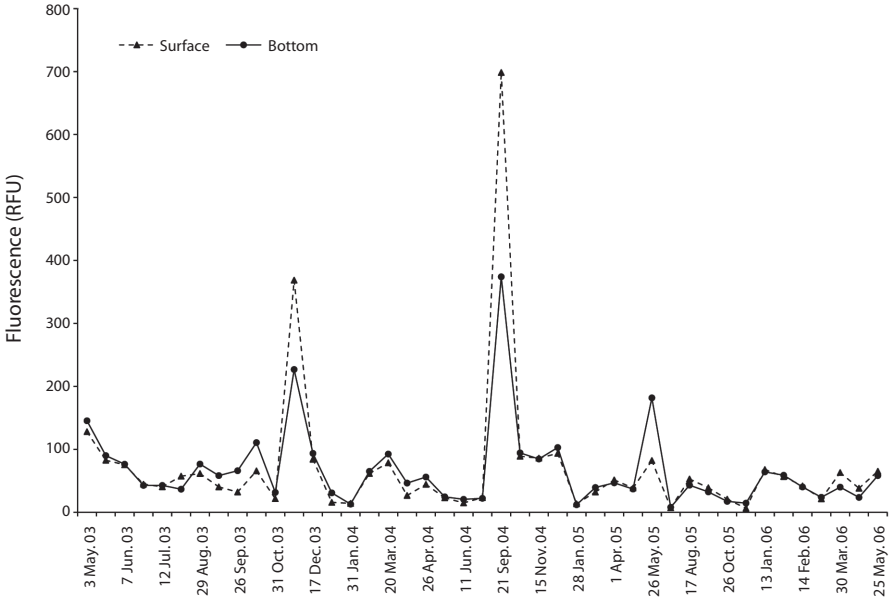

Fig. 4. Monthly variations of surface and 2-meter depth fluorescence concentrations in the water at all sampling stations (mean of all sampling stations). RFU: relative fluorescence units.

Densities of *P. bahamense* var. *bahamense* and *C. furca*: ANOVA detected significant differences between months but not between stations or depths, for densities of *P. bahamense* var. *bahamense* and *C. furca* (Table 2).

Densities of *P. bahamense* var. *bahamense* ranged from 0.48 to 90 978 cells/L (Figs. 5 and 6). Even though no seasonality was observed for *P. bahamense* var. *bahamense* density fluctuations, higher densities were observed from April to September and lower densities from October to February.

C. furca densities ranged from 0 to 11 200 cells/L (Figs. 5 and 6). Population densities

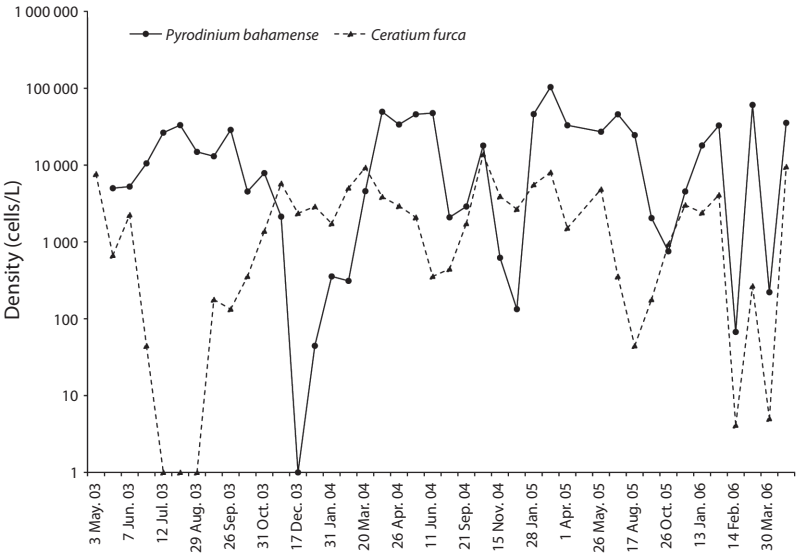

Fig. 5. Monthly variations of *Pyrodinium bahamense* and *Ceratium furca* densities in the water at all sampling stations (mean of all sampling stations and depths).

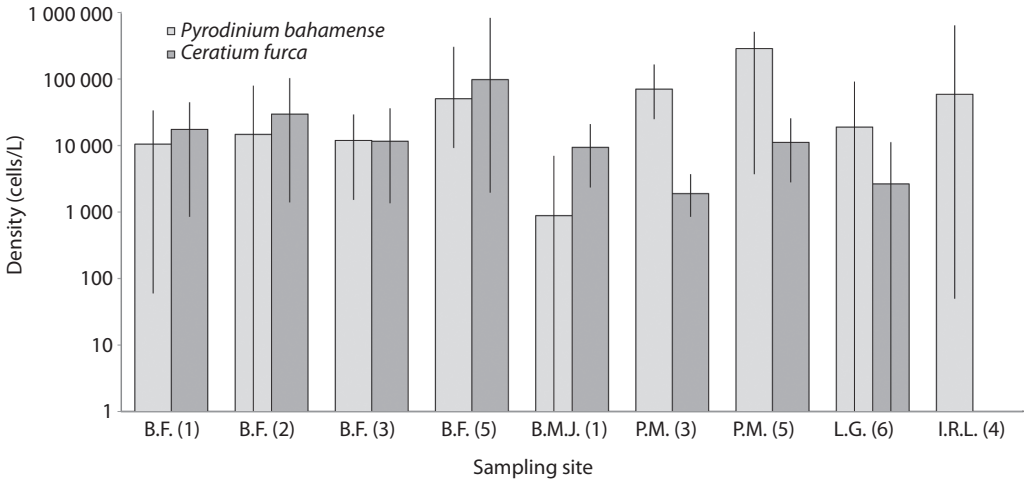

Fig. 6. Mean (grand mean) population densities of *Pyrodinium bahamense* and *Ceratium furca* in Puerto Rico and Florida. Vertical line indicates range. B. F., Bahía Fosforescente; B. M. J., Bahía Monsio José; P. M., Puerto Mosquito; L. G., Laguna Grande; I. R. L., Indian River Lagoon (no data for *C. furca*). (1), Seixas, 1983; (2), Seixas, 1988; (3), Walker, 1997; (4), Phlips et al., 2004; (5), Soler, 2006; (6), this study.

of *P. bahamense* var. *bahamense* were negatively correlated with *C. furca* densities during the first year of sampling ($r=-0.576$, $df=13$, $.01<p<.05$). During the third year of sampling they were positively correlated to each other ($r=0.759$, $df=13$, $.001<p<.005$). No significant correlation was detected (including lagged correlations) between population densities of *P. bahamense* var. *bahamense* or *C. furca,* and any of the physical-chemical parameters.

DISCUSSION

Laguna Grande has less water interchange with the ocean than the bioluminescent bays Puerto Mosquito, Bahía Fosforescente and Bahía Monsio José; because of the presence of the shallow (average depth approximately 1m) and narrow 1.5km long tidal inlet/outlet channel, that limits water interchange between the lagoon and the ocean (Fig. 1). The restricted water flow into the lagoon causes a 3.5hr delay between the tidal peaks in the ocean and the corresponding tidal peaks inside the lagoon (Soler-López & Santos, 2010). It is visibly evident that the currents in the channel can cause strong turbulence and mixing in the water column. Therefore, the "negative" estuary (or hypersaline estuary) type of circulation, described by Margalef (1961) and further discussed by Seliger et al. (1971), does not occur in Laguna Grande. The average residence (or flushing) time of Laguna Grande is 7.7d (Soler-López & Santos, 2010). Most other bioluminescent lagoons or bays in Puerto Rico should have shorter water residence times because of their wider and less restrictive entrances. Therefore, the retention of nutrients, humic substances and microalgae should be longer in Laguna Grande. Also, a broader range in physical-chemical parameters is expected. Bays and/or lagoon habitats where *P. bahamense* var. *bahamense* dominate are characterized by relatively long residence times, both in Florida (Phlips et al., 2006) and Puerto Rico (Margalef, 1961).

In this study *P. bahamense* var. *bahamense* was observed at temperatures up to 34°C (range: 24-34°C), higher than what has been reported for other populations in Puerto Rico (combined range: 22-32°C; Seixas, 1983, 1988; Walker, 1997; Soler-Figueroa, 2006) and Florida (combined range: 12-31°C; Phlips et al., 2006). Puerto Rico's tropical *P. bahamense* var. *bahamense* populations and Florida Bay's tropical/subtropical populations (range 15-31°C) seem to be more constant throughout the year than the subtropical warm/temperate populations of Indian River Lagoon and Tampa Bay, Northern Florida (combined range 12-31°C), where vegetative cells are generally restricted to the warm season, when temperatures exceed 20°C (Phlips et al., 2006). The temperature range observed for the *C. furca* population in Laguna Grande, and in other Puerto Rico sites (Seixas, 1983, 1988; Walker, 1997; Soler-Figueroa, 2006) is within the combined temperature range (18-34°C) for sites in the United States, Norway, Mexico, Thailand and Japan (Baek, Shimode & Kikuchi, 2008).

A broader salinity range was observed in Laguna Grande (18-42psu) than in other bioluminescent water bodies in Puerto Rico (combined range: 32-39psu; Seixas, 1983 1988; Walker, 1997; Soler-Figueroa, 2006). The salinity range observed in Laguna Grande is within the range reported by Phlips et al. (2006, 10-45psu) for various populations in Florida. *P. bahamense* var. *bahamense* blooms in the Indian River Lagoon, Florida, and *P. bahamense* var. *compressa* blooms in the Indo-Pacific, seem to be associated with periods of elevated rainfall (and lower salinities); and lower densities, or the absence of planktonic forms, are associated with low rainfall (and higher salinity) periods (Azanza & Taylor, 2001; Phlips et al., 2006). In this study, *C. furca* was observed in salinities up to 42psu, higher than previously reported salinities for this species in the United States (16-22psu), Norway (20-25psu), Mexico (13-35psu), Thailand (21-25psu) and Japan (30-32psu; Baek et al., 2008).

Since the geomorphological and hydrological characteristics of Laguna Grande can lead to water accumulation in the lagoon, we calculated cumulative rainfall (3, 6, 9, 12, 15 and 18 day intervals before each sampling date,

using NOAA Paraíso station data) and correlated it with the physical-chemical parameters observed in this study. The resulting correlation coefficients measured the degree of association between cumulative rainfall and each of the other parameters. The various significant correlations between cumulative rainfall; salinity and fluorescence could be due, in part, because of the relatively low water interchange between Laguna Grande and the ocean. The effects of cumulative rainfall on salinity and fluorescence could be significantly detected for up to 18d. The strong negative correlation between salinity and fluorescence can be explained by the washout of algae and leaf particles, from the channels and other areas of the Laguna Grande basin, to the sampling stations located in the central portion of the lagoon. Rainfall events can increase the nitrogen: phosphorus ratio and push the system towards phosphorus limitation (Smalley & Coats, 2002), changing the planktonic species composition. In the Arabian Sea, an increase in the nitrogen: phosphorus ratio, following monsoon rains, favors the diatom *Biddulphia sinensis* relative to the dinoflagellate *C. furca* (Qasim, Bhattathiri & Devassy, 1973). In Kuwait's waters (Arabian Sea), lower salinity water, associated with monsoon rains, is correlated with a high prevalence of diatoms whereas higher salinity water is correlated with a higher dominance of dinoflagellates (Polikarpov, Al-Yamani & Saburova, 2009).

The non-significant correlations between the fluorescence values; and the densities of *P. bahamense* var. *bahamense,* and *C. furca* were possibly due to the fact that all the other photosynthetic planktonic microalgae in the lagoon were not considered in these analyses. The densities of *P. bahamense* var. *bahamense* and *C. furca* are only a portion of the total density of all the photosynthetic planktonic microalgae. Therefore, the omission of most microalgae species could explain these non-significant correlations.

The non-significant differences detected in Laguna Grande, between surface and 2m depth samples, for temperature, salinity, phosphates, nitrates, fluorescence, and densities of *P. bahamense* var. *bahamense* and *C. furca,* suggest a vertically mixed water column. Relatively strong trade winds, combined with an average depth of only approximately three meters (Soler-López & Santos, 2010) facilitate the mixing process. However, vertical stratification in salinity and fluorescence, affecting the entire water column, was observed after the most significant rain events, recorded on November 21, 2003 and September 21, 2004. Less severe rain events affected only the salinity in the surface (e.g. May 26, 2005). Soler-López & Santos (2010) also observed some vertical stratification in Laguna Grande.

The correlations between the density of *P. bahamense* var. *bahamense* and *C. furca* were performed to show numerical relationships between these species but not to infer predator-prey or competition relationships. However, significant negative correlations might indicate potentially competing species. The correlation was significantly positive during the first year of our study, significantly negative during the third year, but not significant over the entire three-year period. Similarly, Seixas (1983, 1988), Walker (1997) and Soler-Figueroa (2006) did not observe significant relationships between these species in other bioluminescent bays/lagoons in Puerto Rico.

Heterotrophic dinoflagellates of the genus *Protoperidinium* (Hansen & Calado, 1999), observed at low densities in Laguna Grande, might prey on *C. furca* and/or *P. bahamense* and may affect their densities. However, we have not detected this interaction in live phytoplankton samples from Laguna Grande.

During this study, the highest monthly mean density of *P. bahamense* var. *bahamense* was 90 978 cells/L (SD=39 431, n=6); and for *C. furca* was 11 200 cells/L (SD=6 601, n=6). Monthly mean densities of *P. bahamense* var. *bahamense*, up to 511 882 cells/L, and of *C. furca,* up to 25 613 cells/L, have been reported for Puerto Mosquito; and monthly mean densities of *P. bahamense* var. *bahamense*, up to 303 053 cells/L, and of *C. furca*, up to 830 199 cells/L, have been reported for Bahía Fosforescente (Soler-Figueroa, 2006). In coastal waters

of Florida, *P. bahamense* var. *bahamense* bloom densities of 776 000 cells/L have been reported in the Indian River Lagoon, and of 380 000 cells/L in Tampa Bay (Phlips et al., 2006). In Laguna Grande, we observed patches of *P. bahamense* var. *bahamense*, having mean cell densities of 481 000 cells/L (SD=143 846, n=25), on October 14, 2002. Typical peak cell densities in a *P. bahamense* var. *compressum* (Pacific variety) red tide patch is in the order of 10^6 cells/L (Usup & Azanza, 1998). The highest reported monthly mean density of *C. furca* in Bahía Fosforescente, Puerto Rico (830 199 cells/L; Soler-Figueroa, 2006), is higher than the density of a bloom in Chesapeake Bay (up to 324 000 cells/L; Smalley & Coats, 2002) and the density in the Bay of Bengal (0-20 cells/L; Naik, Hegde & Anil, 2011). However, much lower than bloom peak densities reported for Puerto Escondido, Baja California, Mexico (10^7 cells/L; Orellana-Cepeda, Granados-Machuca & Serrano-Esquer, 2004).

An attempt was made to compare the range and the mean population densities of *P. bahamense* var. *bahamense* and *C. furca* observed in this study (Table 1), with those reported by Seixas (1983, 1988), Walker (1997), Soler-Figueroa (2006) and Phlips, Badylak, Youn & Kelley (2004, no data for *C. furca*). The mean population density of *P. bahamense* var. *bahamense* and *C. furca* in Laguna Grande is within the range of these other studies. The density of *P. bahamense* var. *bahamense* is higher than that of *C. furca* in Laguna Grande and Puerto Mosquito, but mostly lower in Bahía Fosforescente and Bahía Monsio José. The mean population density of *P. bahamense* var. *bahamense* in the Indian River Lagoon, Florida (58 934 cells/L; Phlips et al., 2004) is within the range of the mean densities reported in the Puerto Rico sites.

In order to conserve the continuous *P. bahamense* var. *bahamense* populations in Laguna Grande, as well as most of its bioluminescence, it is important to maintain the existing water flow levels in the tidal inlet/outlet channel, connecting Laguna Grande to Las Croabas Bay. This will help to keep the existing water exchange rates with the ocean, maintaining the actual nutrient and salinity levels of the lagoon. Even though *P. bahamense* var. *bahamense* is considered a euryhaline sub-species, having tolerance salinity limits in Florida of approximately form 10 to 45psu (Phlips et al., 2006), and very likely, similar tolerance limits in Puerto Rico; should the channel close, either by natural or artificial means, the salinity and nutrient levels in Laguna Grande will be altered. This should lead to changes in the planktonic species composition and to the eventual elimination, or population crash, of *P. bahamense* var. *bahamense*, as well as many other species non-tolerant to very low or very high salinities. It is also important to maintain unpolluted water quality parameters within the bay, hydrographical basin and adjacent waters; preserve mangrove communities within the basin and adjacent areas; and establish sound management plans. Light pollution should be minimized to increase human perception of bioluminescence. Bioluminescence in the bay is a touristic attraction and the intensity of the biochemical reaction impact the natural experience which influences the local economy.

In order to have more accurate measurements of density variations through time in Laguna Grande it is important to sample at more frequent intervals since the replication rate of *P. bahamense* var. *bahamense* and *C. furca* is much faster than the sampling interval used in this study.

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RESUMEN

Este estudio describe parámetros de calidad de agua y fluctuaciones en la densidad poblacional de *Pyrodinium bahamense* Plate 1906 y *Ceratium furca* (Ehrenberg) Claparède & Lachmann 1859, los dos dinoflagelados más abundantes en las bahías y lagunas bioluminiscentes de Puerto Rico, durante un periodo de tres años, en Laguna Grande, Puerto Rico. En *P. bahamense* se observó un patrón de densidad poblacional, donde se observaron las densidades más altas mayormente desde abril hasta septiembre y las más bajas desde octubre hasta febrero. En *C. furca* las fluctuaciones en densidad fueron más erráticas y no se observó un patrón repetitivo. La densidad poblacional promedio de *P. bahamense* (media=18 958.5 células/L) fue significantemente mayor que la de *C. furca* (media=2 601.9 células/L). No se encontraron diferencias significantes entre las muestras de superficie y las de 2m de profundidad para temperatura, fosfatos, nitratos, salinidad, fluorescencia, y las densidades de *P. bahamense* y *C. furca*, lo que sugiere que la columna de agua está mezclada verticalmente. La densidad poblacional media de *P. bahamense* y *C. furca* en Laguna Grande está dentro del rango de las densidades que han sido reportadas para otras lagunas bioluminiscentes en Puerto Rico y Florida, EE.UU.

Palabras clave: *Pyrodinium bahamense, Ceratium furca,* Laguna Grande, Puerto Rico, bioluminiscencia, lagunas bioluminiscentes.

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