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Seasonal changes in phytoplankton community structure in a bioluminescent lagoon, St. Croix, US Virgin Islands

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ABSTRACT: Many persistent bioluminescent bays (biobays) worldwide are the result of dense accumulations of bioluminescent dinoflagellates. One such biobay, Mangrove Lagoon, is a man-made lagoon in Salt River Bay, St. Croix, US Virgin Islands, that exhibits year-round bioluminescence. The causes of variations in the abundances of dinoflagellates as well as other phytoplankton in the lagoon are unknown. The purpose of this research was to quantify the seasonal changes in phytoplankton community structure in Mangrove Lagoon, with emphasis on dinoflagellates. A secondary goal was to relate phytoplankton abundances to corresponding changes in nutrient availability, salinity, and water residence time. Weekly to bi-weekly water samples were collected in 2013 (February–December) and 2015–2016 (June–August). Phytoplankton community composition was determined using a combination of microscopy, high-performance liquid chromatography photopigment analysis, and ChemTax. Nutrient and salinity concentrations were also measured, while water residence times were calculated based on predicted tidal elevations. Dinoflagellates were a consistent and major component of the phytoplankton community. Blooms (defined as a significant increase in biomass as chl *a*) of diatoms, cryptophytes, and chlorophytes coincided with dinoflagellate blooms. Cyanobacterial blooms occurred mostly during the summer months under high salinity conditions. There were no correlations between phytoplankton blooms and nutrient concentrations, salinity, or nitrogen:phosphorus ratio ($n = 78$, $p > 0.05$). However, dinoflagellate blooms occasionally occurred during periodic, tidally driven long water residence times in the lagoon. Phytoplankton and dinoflagellate abundance dynamics reported in this study provide insights into the potential role of physical processes driving the variability in bioluminescence in Mangrove Lagoon.

KEY WORDS: ChemTax · Caribbean · Biobay · Mangrove · Dinoflagellate · Residence time · Spatiotemporal distribution

INTRODUCTION

Many bioluminescent bays (biobays) are the result of dense accumulations of bioluminescent dinoflagellates that produce spectacular light displays during the evening hours. There are only 14 known persistent biobays worldwide, and 11 have been reported in the Caribbean region (Pinckney et al. 2014). These biobays have common characteristics including prolonged water retention times, a narrow inlet, shallow basin bathymetry, limited tidal range, and mangroves surrounding the perimeter of the lagoon

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(Seliger et al. 1970, 1971, Sastre et al. 2013). One of these persistent biobays is Mangrove Lagoon, a man-made embayment, located in Salt River Bay on the island of St. Croix, US Virgin Islands (USVI). Bioluminescence in the waters of Mangrove Lagoon occurs year-round, but the intensity varies seasonally, with the highest intensity usually occurring in the wet winter months (L. Dunton pers. comm.). This feature of Mangrove Lagoon has become an important ecotourism attraction for St. Croix. Over the last 15 yr, nightly kayak tours have been conducted to observe the natural bioluminescence in Mangrove Lagoon. Initially, tours were once per month, increasing to twice per week 10 yr ago and most recently up to 5 times per week, providing a bioluminescence experience for up to 20 to 30 kayaks per tour (L. Dunton pers. comm.).

Most biobays in the Caribbean region are surrounded by the red mangrove *Rhizophora mangle*, which may provide nutrients and dissolved organic matter that appear to be essential for the growth of the dinoflagellates (Prakash & Rashid 1968, Seliger et al. 1971, Phlips et al. 2006, O'Connell et al. 2007, Trainer 2007, Zimberlin 2013, Soler-Figueroa & Otero 2016). However, the nutrients or compounds responsible for stimulating and supporting growth are unknown. One hypothesis is that mangroves may supply vitamin B_{12} (cyanocobalamin), which is essential for dinoflagellate growth (Burkholder & Burkholder 1958, Koch et al. 2011).

A variety of biotic and abiotic processes, which vary over spatial and temporal scales, can regulate phytoplankton community structure. The horizontal microscale (tens of meters) variability in community composition is overlooked in most sampling programs. Since biobays are usually enclosed embayments with limited exchange with larger water bodies, they offer a unique opportunity to observe phytoplankton community compositional dynamics in a natural microcosm. We know little about the spatial and temporal extent of blooms (defined as a significant increase in biomass as chl *a*) in these small systems. In particular, we do not know if the blooms of dinoflagellates correspond with blooms of other phytoplankton groups such as diatoms, cyanobacteria, and cryptophytes. Dinoflagellates usually have slower growth rates than other phytoplankton species (Reynolds 2006), which promotes questions about how the dinoflagellates are able to out-compete other algal groups for limiting resources in these biobays. Furthermore, these tropical systems exhibit typical wet–dry seasons that result in marked differences in environmental conditions over an annual cycle.

Most previous studies on biobays have emphasized the autecology of dinoflagellates (e.g. Seliger et al. 1970, Phlips et al. 2006, Usup et al. 2012, Soler-Figueroa & Otero 2016). However, dinoflagellates are likely only a fraction of the entire phytoplankton community in these systems. Few studies have examined the relative contribution of dinoflagellates to the overall phytoplankton community and how this relationship changes over time in these small (<50 ha) lagoons. The purpose of this research was to quantify the seasonal changes in phytoplankton community structure in a Caribbean biobay, with particular emphasis on dinoflagellates. A secondary goal was to relate phytoplankton abundances to corresponding changes in nutrient availability, salinity, and water residence times.

MATERIALS AND METHODS

The biobay considered here, Mangrove Lagoon (17° 46′ 43″ N, 64° 45′ 4″ W), is located on the eastern side of Salt River Bay, south of Hemer's Peninsula on St. Croix, USVI. The oval-shaped Mangrove Lagoon is a small (250 × 130 m, 3.8 ha), shallow (average depth = 2.3 m) man-made embayment that resulted from a mangrove wetlands dredging project to create a marina basin (1960–1970s); the project was abandoned in the 1970s and the area left fallow, allowing mangroves to recruit along the lagoon shoreline (Kendall et al. 2005, Reidhaar et al. 2016) (Fig. 1). Approximately 15 yr ago, the bay began to exhibit bioluminescence. Salinity in the lagoon ranges from 30 to 40, water temperature varies seasonally between 20 and 35°C, and water residence time ranges between 5 and 75 d, depending on the tidal range and wind conditions (Pinckney et al. 2014).

Weekly to fortnightly water samples were collected by hand between 10:00 and 12:00 h local time from a kayak using a 1 m integrated water column sampler (surface waters) and a horizontal Niskin bottle (0.5 m from the bottom) from 5 fixed locations within Mangrove Lagoon in 2013 (February–December) and from 4 locations in 2015 (June–December) and 2016 (January–August) (Fig. 1). A single replicate for phytoplankton, nutrients, and salinity was obtained for each location and sample depth. Sample water was transported in opaque containers stored on ice in an insulated cooler. The collected water (ca. 100–500 ml) was filtered onto glass fiber filters (25 mm diameter Whatman GF/F) for phytoplankton photopigment analysis and stored in opaque microcentrifuge tubes. Water chemistry

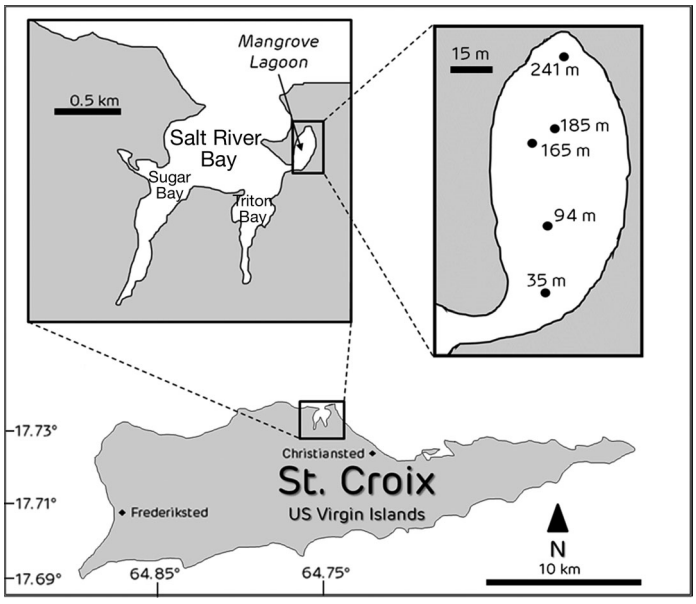

Fig. 1. Study site and sampling locations in Mangrove Lagoon, Salt River Bay, St. Croix, US Virgin Islands. Distances of sampling stations from the mouth of the lagoon are shown in the map inset

samples were filtered through sterile 0.45 µm pore size cellulose acetate membrane syringe filters and immediately frozen for later nutrient analyses. Filtered water samples (ca. 50 ml each) and phytoplankton samples were stored frozen at −80°C and shipped quarterly to the University of South Carolina (USC) for nutrient and photopigment analyses. For 2013, salinity was measured at hourly intervals *in situ* in the center of the lagoon at a depth of 1 m using a YSI 6920 sonde. For 2015 and 2016, salinity was measured with a refractometer at all locations at 1 m depth and 0.5 m from the bottom and averaged for analysis.

Daily rainfall amounts at Mangrove Lagoon were recorded at 15 min intervals using a HOBO data logging weather station, managed and maintained by the US National Park Service (NPS). Cumulative daily rainfall amounts were summed for the 7 d prior to water quality and phytoplankton sampling for correlation analyses.

High-performance liquid chromatography (HPLC) was used to separate, identify, and quantify phytoplankton photosynthetic pigments. First, filters were lyophilized (−50°C, vacuum of 0.50 atm [51 kPa]) for 20 to 24 h, followed by extraction in 90% aqueous acetone (600–750 µl at −20°C for 18–22 h). The internal standard was the synthetic carotenoid pigment β-apo-8′-carotenal (Sigma). Filtered extracts (250 µl) were injected into a Shimadzu HPLC (LC-10AT) equipped with reverse-phase C18 columns (Rainin Microsorb, 0.46 × 1.5 cm, 3 µm packing and Vydac 201TP54, 0.46 × 25 cm, 5 µm packing) in series as the solid phase. Gradients and flow conditions are described in Pinckney et al. (2001). A Shimadzu SPD-M10av photodiode array detector was used to obtain absorption spectra and chromatograms (440 ± 4 nm). Pure standards (DHI) were used to confirm peak identities and retention times. The concentrations of dissolved inorganic nitrogen (DIN), i.e. nitrite + nitrate (NO_2^- + NO_3^-) and ammonium (NH_4^+), were determined using a Lachat Quickchem 8000T autoanalyzer according to well-established wet chemical methods (Johnson & Petty 1983, Grasshoff et al. 1999). Samples for dissolved inorganic phosphorus (DIP as orthophosphate, PO_4^{3-}) were measured using the method of Monaghan & Ruttenberg (1999).

ChemTax (v. 1.95) was used to determine the relative abundances of major phytoplankton groups based on photopigment concentrations (Pinckney et al. 2001, Higgins et al. 2011). The major phytoplankton groups used for ChemTax categories were based on qualitative microscopic examinations of water samples. The initial pigment ratio matrix was derived from Schlüter et al. (2000), and the convergence procedure outlined by Latasa (2007) was used iteratively to correct for inaccuracies in pigment ratio seed values. Homogeneous groups for separate ChemTax analyses were determined using a 2-step cluster analysis procedure (IBM SPSS v. 24) based on log-likelihood distance measures of 19 photopigment variables (see Supplement 1 at www.int-res.com/articles/suppl/a081p109_supp.pdf). The cluster analysis grouped the samples into 2 clusters consisting of 595 (87%) and 88 (13%) samples. These 2 clusters were used to define analysis bins in ChemTax. Final RMSEs of 0.1795 and 0.2917, respectively, were achieved for the 2 bins after 10 separate runs (Supplement 2 at www.int-res.com/articles/suppl/a081p109_supp.pdf). Total chl *a* was used as a proxy for phytoplankton biomass (i.e. the sum of all phytoplankton groups). The relative abundances of the different algal groups were expressed as absolute concentrations of chl *a* for each group.

A sampling program for phytoplankton species composition was undertaken in March 2013 only in Mangrove Lagoon to confirm dinoflagellate species present at this single time point. Samples consisted of whole water samples taken within the upper 0.5 m at each sampling station (Fig. 1). In addition to whole water samples, benthic screen samplers (Tester et al.

2014) were deployed at the same stations. These screen samplers were retrieved after 2 d, and trapped phytoplankton harvested were returned to the laboratory for processing. Aliquots of the whole water and screen samples were placed in labeled bottles, fixed with Lugol's solution, and archived for later study. Live samples were first examined with an Olympus CK40 inverted microscope; initial rough selection was then made using pulled Pasteur pipettes, and cells were placed in labeled liquid scintillation vials. Since Mangrove Lagoon was noted for its bioluminescence, 1 nighttime sampling was also undertaken on 6 March, where surface and bottom samples were taken at 4 stations roughly in the main axis of the lagoon. These samples were taken for later examination at the University of the Virgin Islands (UVI) laboratory. Again, aliquots of sample water from each of the stations (surface and bottom) were fixed with Lugol's for later study. Upon return to the University of North Carolina Wilmington (UNCW) laboratory, enumeration of cell density from fixed samples was accomplished by the Uttermöhl settling method and inverted microscopes. Cells were further processed for examination using electron microscopy as described in Karafas & Tomas (2015).

Residence times for waters in Mangrove Lagoon were calculated based on estimates of lagoon surface area, lagoon volume, and tidal prism volumes. The calculations were as follows:

$$TV = (A_L \times TR)/V_L \quad (1)$$

where TV = percent tidal volume of Mangrove Lagoon, A_L = surface area of Mangrove Lagoon = 38 083 m^2, TR = tidal range determined from predicted tide tables (m), and V_L = volume of water in Mangrove Lagoon = 89 263 m^3.

Thus,

$$\text{Residence time (d)} = 1/TV \quad (2)$$

Tidal data were obtained from the primary NOAA tidal gauge station (9751364) at nearby Christiansted Harbor, St. Croix, USVI. Daily values of residence time were calculated using a 30 d running average of predicted residence times to smooth the data and reflect conditions within Mangrove Lagoon.

All statistical analyses (paired *t*-tests, cluster analysis, non-parametric Kendall's tau correlation analysis) were accomplished using the statistical package IBM SPSS (v. 24). Assumptions of all tests were verified prior to conducting the respective analyses. Contour plots were constructed using the kriging algorithm in Surfer (Golden Software v. 12.8).

RESULTS

The single time point sampling in Mangrove Lagoon in March 2013 revealed that the phytoplankton community consisted primarily of dinoflagellates and diatoms based on microscopic examination of the samples. The major bioluminescent component was, as suspected, *Pyrodinium bahamense*, which was found throughout the lagoon (Fig. 2A–C). Nighttime samples indicated that the highest densities (10^5 cells l^{-1}) were in the upper meter of the lagoon. During the day, cells were found throughout the water column at 10^4 cells l^{-1}, with a slight increase toward the bottom of the lagoon. In addition to *P. bahamense*, other benthic dinoflagellates were prevalent. The lagoon area had populations of *Gambierdiscus* sp. (Fig. 2D,E). Few single cells were found in the water column of the lagoon. Normally, populations were below 10^3 cells l^{-1}. Other species present included several unknown species of *Ostreopsis* (Fig. 2F) and the newly described species *Coolia santacroce* (Fig. 2G–I) (Karafas et al. 2015). Another prominent species observed throughout was *Bysmatrum subsalsum* (Fig. 2J,K). Additional dinoflagellate species were also found along with the dominants consisting of several *Amphidinium* species. These are presently under study in a larger review of *Amphidinium* species from tropical waters. Few pelagic diatom species were present, but a few benthic species (*Navicula*, *Pleurosigma*, and *Stephanopyxis*) were found at low concentrations.

Phytoplankton chl *a* averaged 3.75 ± 3.32 µg l^{-1} (mean ± SD) and ranged from 0.22 to 34.68 over the duration of the study (Table 1). ChemTax analysis identified 6 algal groups that contributed >98% of total phytoplankton abundance. Further analysis was limited to dinoflagellates, *Karenia*-like dinoflagellates, diatoms, chlorophytes, cyanobacteria, and cryptophytes. Dinoflagellates were separated into 2 groups based on the presence of accessory photopigments peridinin (dinoflagellates) and fucoxanthin/gyroxanthin (*Karenia*-like dinoflagellates). Summary statistics for group abundances show that dinoflagellates were the most abundant group (1.32 µg chl *a* l^{-1}) over the study period, followed by cyanobacteria (0.87) and diatoms (0.69) (Table 1). Dinoflagellate abundances ranged from 0.00 to 22.57 µg chl *a* l^{-1}, while the *Karenia*-like dinoflagellates had a range of 0.00 to 6.53. On average, dinoflagellates (including *Karenia*-like dinoflagellates) composed nearly a third (32.7%) of the phytoplankton community. However, during bloom periods, dinoflagellates made up nearly 97% of the phytoplankton chl *a* in Mangrove Lagoon.

Nutrient concentrations were generally low, with the exception of NH_4^+, which had an average of 7.22 ± 9.66 µmol l^{-1} and a range of 1.03 to 65.63 (Table 1). Total DIN was calculated as the sum of NO_3^-, NO_2^-, and NH_4^+ concentrations. DIP was the concentration of PO_4^{3-}. The molar DIN:DIP ratio, an indicator of nutrient status, averaged 11.09 ± 11.56 and suggests that the system was nitrogen (N) limited relative to the Redfield ratio of 16:1.

The spatiotemporal distributions of phytoplankton groups were plotted to identify patterns of bloom dynamics (Fig. 3A–H). Blooms occurred in both the 2013 and 2015–2016 periods. Highest accumulations of algal biomass were associated with the central

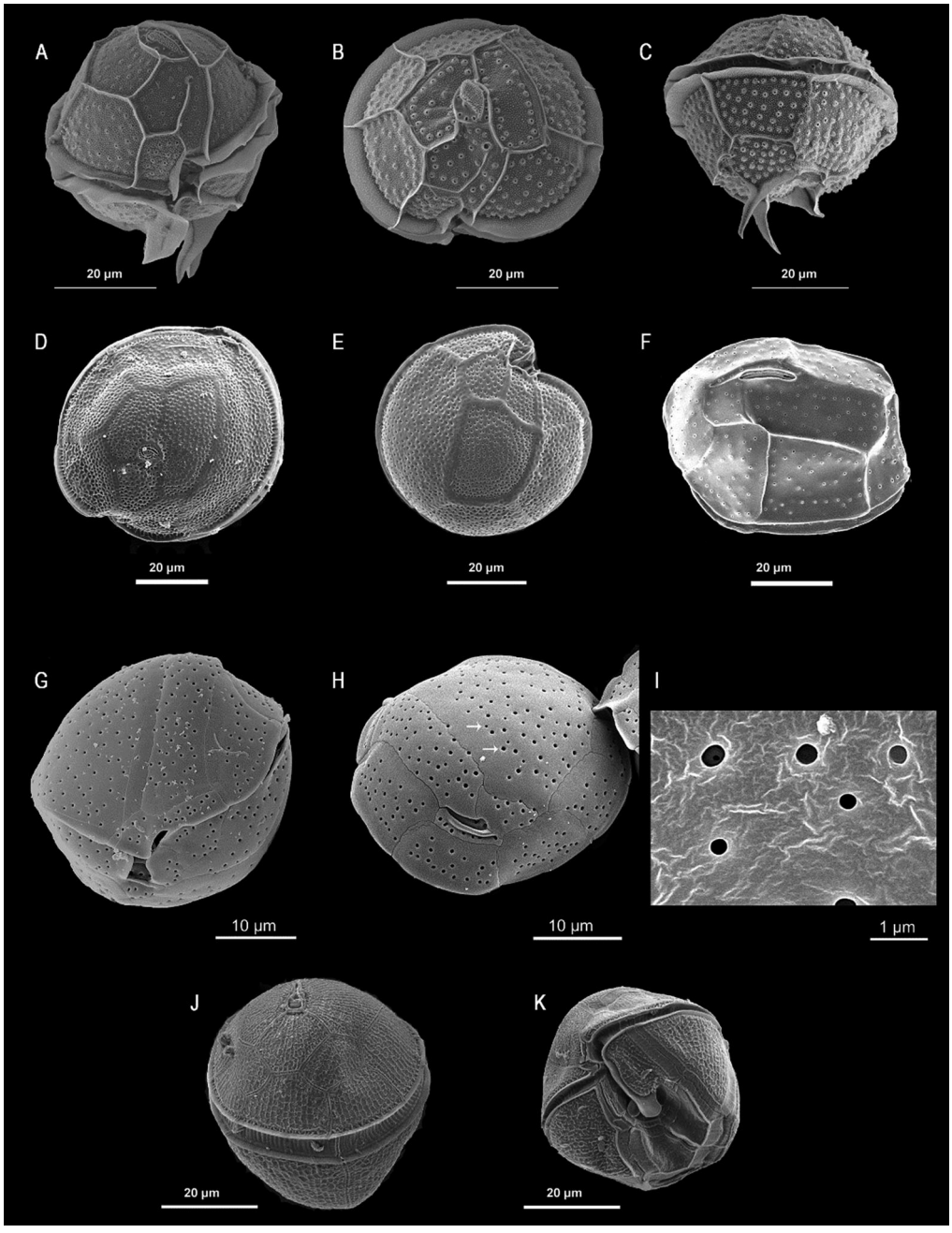

Fig. 2. Selected species found in Mangrove Lagoon, St. Croix, US Virgin Islands. *Pyrodinium bahamense* (A) ventral view, (B) apical view, and (C) dorsal view; *Gambierdiscus* sp. (D) apical view and (E) antapical view; *Ostreopsis* sp. (F) apical view showing apical pore complex; *Coolia santacroce* (G) antapical view, (H) apical view showing apical pore complex and different-sized pores (arrows), and (I) details of pores including imbedded pores and size differences; and *Bysmatra subsalsum* (J) dorsal view showing apical pore complex and (K) ventral view

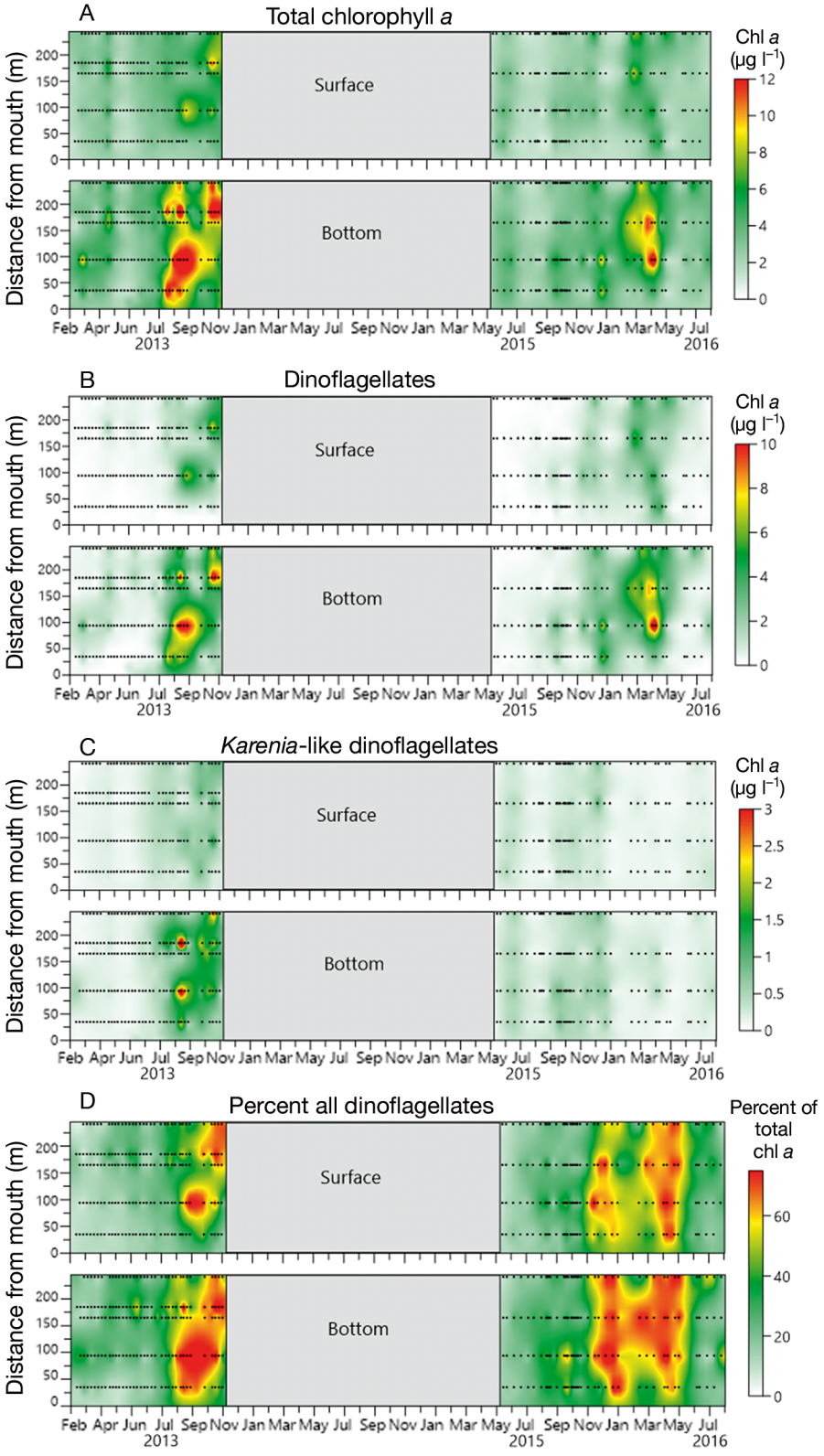

Fig. 3. (Above and following page) Spatio-temporal contour maps of surface and bottom water abundances of (A) total chl *a*, (B) dinoflagellates, (C) *Karenia*-like dinoflagellates, (D) percent of all dinoflagellates, (E) diatoms, (F) chlorophytes, (G) cyanobacteria, and (H) cryptophytes. Distance is measured as a transect line from the mouth of the lagoon. Dots represent sampling locations and dates. Gray area represents the interval between sampling periods where no data were collected

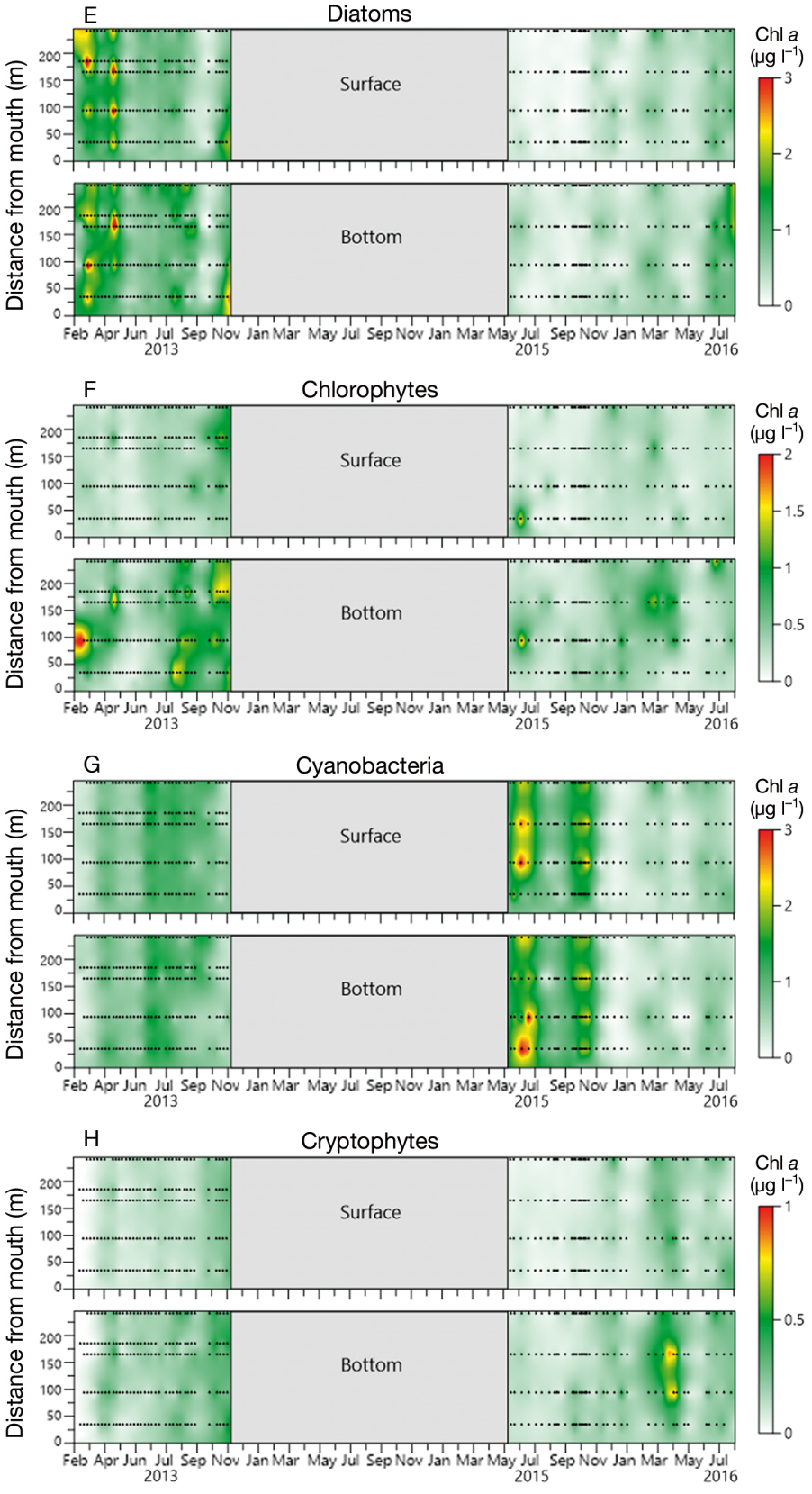

Fig. 3 (continued)

area of the lagoon, which is also the deepest area of the lagoon. In general, blooms were limited to different regions as patches of high abundance. There were no cases where the bloom encompassed the entire lagoon. Paired *t*-test comparisons of surface vs. bottom abundances for each group indicated that bottom water concentrations were significantly ($p < 0.001$) higher than surface water concentrations for total chl *a* as well as each of the phytoplankton groups except the cyanobacteria ($p = 0.91$).

Phytoplankton abundances were averaged for each sampling date across all sampling locations (Fig. 4). The total phytoplankton community (total chl *a*) exhibited blooms in May 2013, August and September 2013, November 2013, December 2015, and March to May 2016. The April 2013 bloom could be attributed primarily to diatoms. A combination of diatoms and dinoflagellates was the primary source for the late 2013 blooms, while dinoflagellates were responsible for the blooms in 2015–2016. Cyanobac-

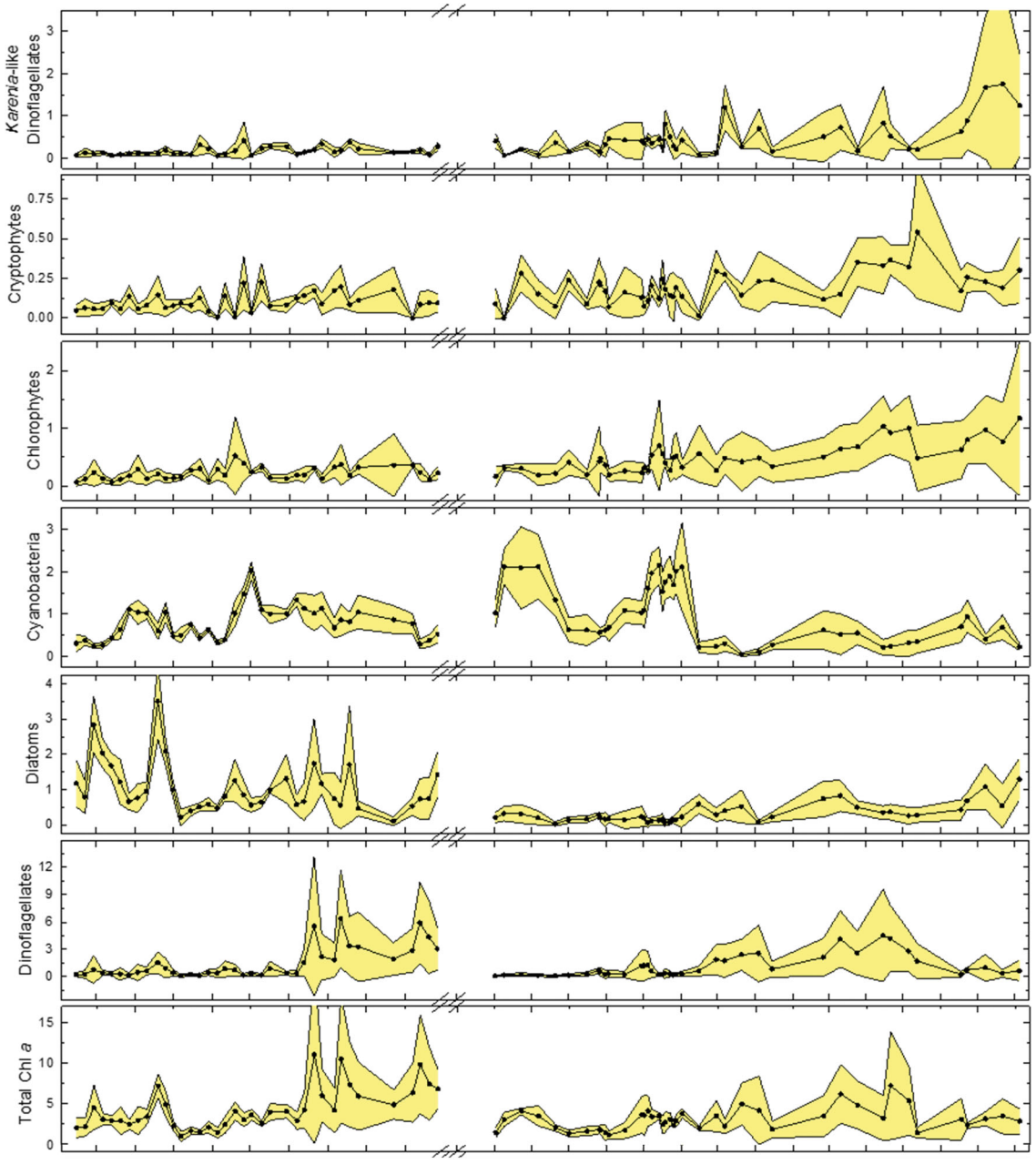

Fig. 4. Averaged phytoplankton abundances for sampling dates in Mangrove Lagoon. Shaded area denotes ±1 SD. Units are µg chl *a* l^{-1}

Table 1. Summary statistics for phytoplankton and nutrient measurements in Mangrove Lagoon. Percent dinoflagellates was calculated from the ratio of all dinoflagellates to all phytoplankton. DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphorus (as orthophosphate)

Parameter	n	Mean	±1 SD	Min.	Max.
Phytoplankton (µg chl *a* l^{-1})					
All phytoplankton (total chl *a*)	683	3.75	3.32	0.22	34.68
Chlorophytes		0.37	0.41	0.00	4.39
Cryptophytes		0.15	0.14	0.00	1.42
Cyanobacteria		0.87	0.64	0.00	3.64
Diatoms		0.69	0.77	0.00	5.40
Dinoflagellates		1.32	2.42	0.00	22.57
Karenia-like dinoflagellates		0.34	0.56	0.01	6.53
Percent dinoflagellates (%)		32.7	22.3	2.2	96.5
Nutrients (µmol l^{-1})					
Nitrate + nitrite	234	1.72	0.93	0.34	4.38
Ammonium		7.22	9.66	1.03	65.63
Orthophosphate (DIP)		0.78	0.15	0.49	1.19
Total DIN		8.97	9.97	1.50	68.96
DIN:DIP ratio (unitless)		11.09	11.56	2.41	81.67

terial abundances peaked in July and November 2015, while chlorophytes, cryptophytes, and *Karenia*-like dinoflagellates increased in April to June 2016. Overall, concentrations of these groups were low relative to the dinoflagellates and diatoms. A notable exception was the absence of high diatom concentrations during 2015–2016, while the blooms of other groups (chlorophytes and cryptophytes) corresponded with the dinoflagellate increases.

The contribution of dinoflagellates (including *Karenia*-like dinoflagellates) to total phytoplankton abundance (total chl *a*) was calculated to illustrate the dominance of dinoflagellates in Mangrove Lagoon during extended periods (Fig. 5). More than half of the phytoplankton community in both the bottom water and surface water was composed of dinoflagellates in September to November 2013 and December 2015 to April 2016. At times, as much as 97% of the phytoplankton biomass was composed of dinoflagellates (Table 1). In addition, dinoflagellates were a significant component of the phytoplankton community year-round.

The relationships between all phytoplankton, dinoflagellates, salinity, and nutrients were plotted to identify potential causal factors for the variations in phytoplankton abundance (Fig. 6). Salinity in Mangrove Lagoon averaged 36.9 ± 2.0 in 2013 and 39.5 ± 2.4 in 2015–2016. Salinity was homogeneous from the surface to the bottom, indicating a well-mixed water column (data not shown). A *t*-test of means indicated that salinity was significantly higher ($p < 0.001$) in 2015–2016, likely due to an extended drought during

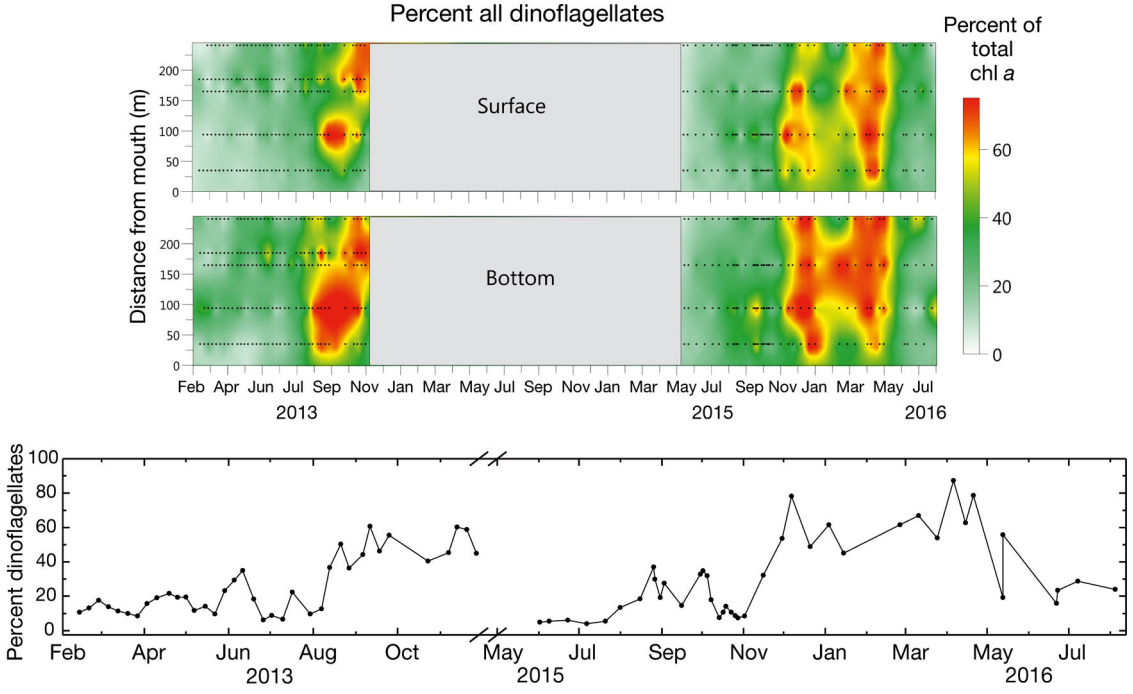

Fig. 5. Spatio-temporal contour plot of the dinoflagellate percentage of total phytoplankton community in units of chl *a* (µg l^{-1}) and plot of the average percent contribution of dinoflagellates for the entire Mangrove Lagoon

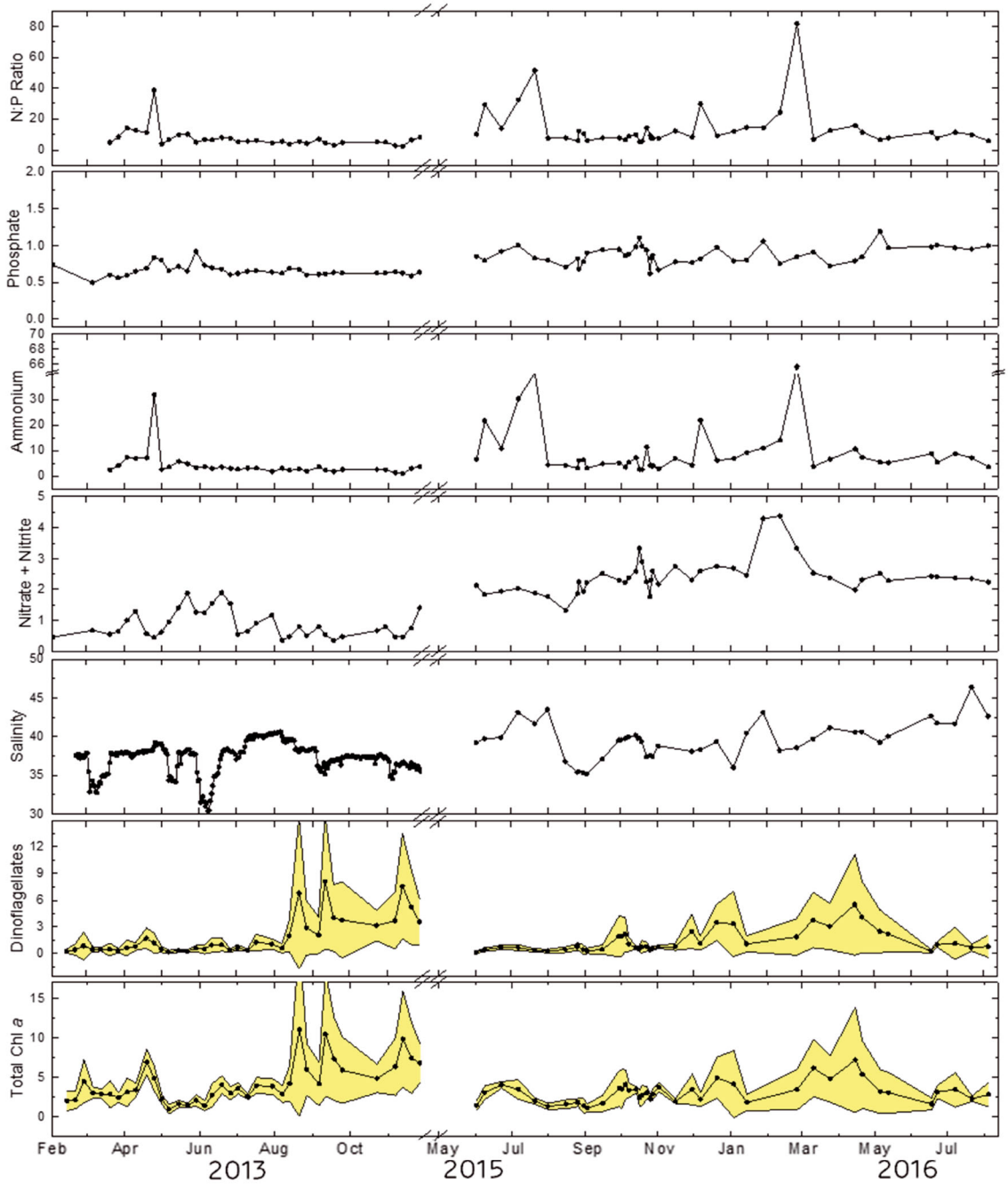

Fig. 6. Averaged phytoplankton abundances, salinity, nutrients, and dissolved inorganic nitrogen:dissolved inorganic phosphorus (N:P) ratio for sampling dates in Mangrove Lagoon. Shaded area denotes ±1 SD. Units are µg chl *a* l^{-1} for phytoplankton, ppt for salinity, and µmol l^{-1} for nutrients

those years. Similarly, $NO_2^- + NO_3^-$, NH_4^+, and PO_4^{3-} concentrations were higher in 2015–2016 (*t*-test, $p < 0.01$). The DIN:DIP ratio roughly mirrored the variations in NH_4^+ and was relatively constant except for peaks in April 2013, July 2015, and February 2016. There were no obvious persistent trends among the variables, and a non-parametric Kendall's tau correlation analysis did not reveal any significant (linear) correlations between phytoplankton and salinity, nutrients, or DIN:DIP ratios ($p > 0.05$).

Total phytoplankton and dinoflagellate concentrations were compared with daily rainfall totals and the estimated water residence time in Mangrove Lagoon (Fig. 7). A visual comparison between phytoplankton and rainfall amounts does not show any evident correlation. Dinoflagellate abundance was compared to

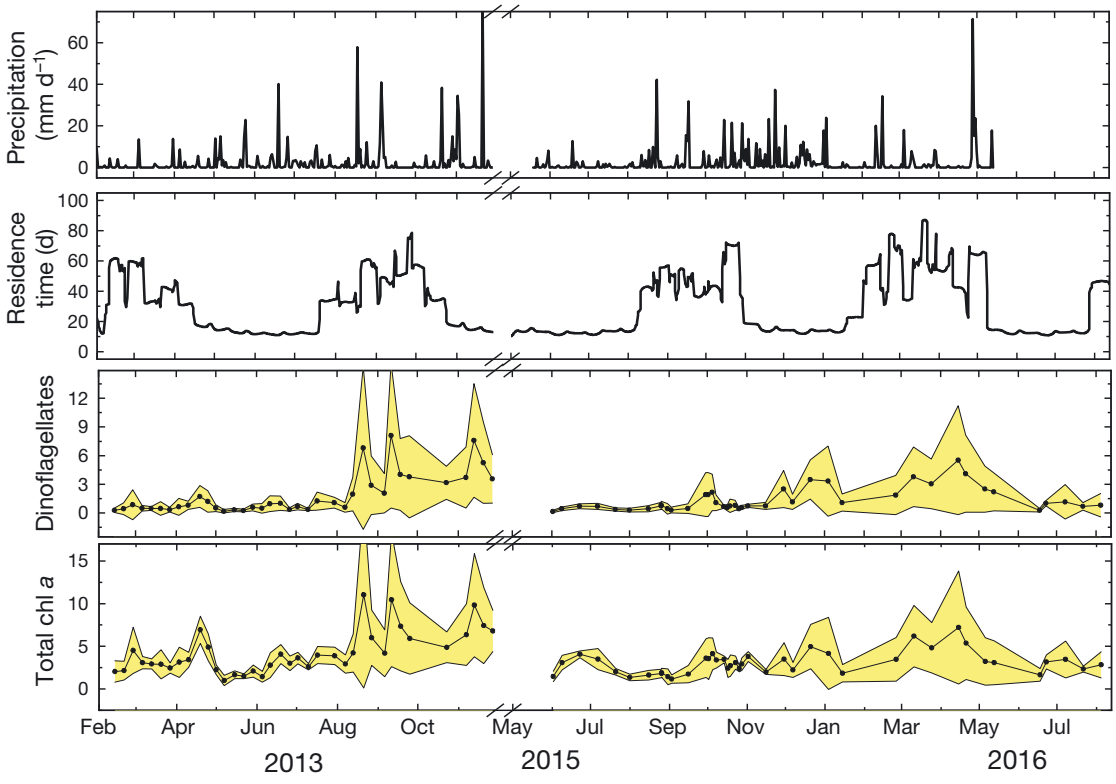

Fig. 7. Averaged total phytoplankton and dinoflagellate abundances, daily precipitation, and calculated water residence time in Mangrove Lagoon based on predicted tide data. Shaded area denotes ±1 SD. Units are µg chl *a* l^{-1} for phytoplankton

the 7 d antecedent cumulative rainfall totals to determine potential stimulatory effects of rainfall on bloom events (Fig. 8). Correlation analysis revealed no significant correlation between the 2 variables (Spearman's rho = 0.14, p = 0.282). Bloom events in the lagoon appeared to correspond with long residence times, especially the peaks in phytoplankton concentrations in late 2013 and March to May 2016 (Fig. 7). However, long water residence times did not always result in large phytoplankton blooms (e.g. February–March 2013, August–October 2015). The variation in calculated residence times was due to local tidal harmonics (variations in solar and lunar cycles) that resulted in reduced tidal ranges and, consequently, lower flushing of the lagoon.

For St. Croix, the wet season usually occurs in May to December and the dry season extends from December to April. Phytoplankton abundance data were divided into 2 groups based on the season (wet–dry) in which the samples were collected. Mean abundances for each group were compared to determine if there were significant differences in abundances for wet and dry seasons (Table 2). The combined groups (all phytoplankton), cryptophytes, and dinoflagellates did not exhibit significant differences in abundance between seasons ($p > 0.05$). However, diatom and chlorophyte abundances were higher in the dry season, while cyanobacterial and *Karenia*-like dinoflagellate abundances were higher in the wet season.

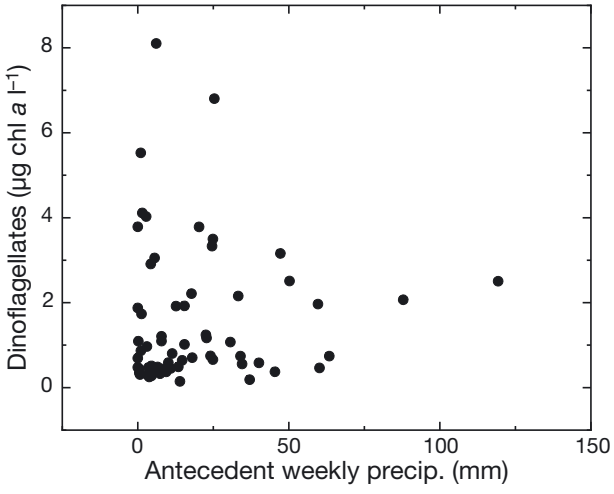

Fig. 8. Comparison of dinoflagellate abundance and cumulative rainfall amounts from the week prior to phytoplankton sampling collection. Antecedent weekly precip. is the total precipitaion in the 7 days prior to sampling

Table 2. Non-parametric Kruskal-Wallis test comparing wet season and dry season means of phytoplankton group abundances. Sample size was 20 for the dry season and 58 for the wet season collection dates

Algal group	Wet season		Dry season		Kruskal-Wallis	
	Mean	±1 SD	Mean	±1 SD	χ^2	p-value
All phytoplankton	3.56	2.21	3.94	1.66	1.917	0.166
Chlorophytes	0.34	0.26	0.43	0.18	7.924	<0.01
Cryptophytes	0.14	0.08	0.18	0.14	1.389	0.239
Cyanobacteria	1.00	0.59	0.43	0.18	13.16	<0.01
Diatoms	0.51	0.43	1.08	0.91	8.78	<0.01
Dinoflagellates	1.17	1.50	1.57	1.43	2.946	0.086
Karenia-like dinoflagellates	0.37	0.36	0.19	0.15	5.186	<0.05

DISCUSSION

The single phytoplankton species collection and identifications performed in March 2013 do not permit a detailed distributional analysis, and further studies are warranted to discern the seasonal variation as well as the greater detailed species composition of phytoplankton of the lagoon. However, these collections allowed the confirmation of a variety of dinoflagellate species in the lagoon.

Although the dinoflagellate blooms were likely the result of several different species, the one known bioluminescent species reported for Mangrove Lagoon is *Pyrodinium bahamense* var. *bahamense.* This species is a bioluminescent dinoflagellate common to all known biobays in the Caribbean (Steidinger et al. 1980, Steidinger & Tangen 1997, Gasparich 2007, Soler-Figueroa & Otero 2016). Bioluminescence intensity has been significantly correlated with *P. bahamense* abundance (Soler-Figueroa & Otero 2016). This species is found in warm tropical waters (>20°C) and ranges in size from 30 to 60 µm (Gasparich 2007). The generation time is reported to be as long as 3 to 3.5 d (Seliger et al. 1970), while Usup et al. (2012) found an average growth rate of 0.4 d^{-1}. Blooms of this species may occur at temperatures greater than 20°C (Phlips et al. 2006). *Pyrodinium* blooms may occur at salinities <25, though most blooms have been reported at salinities >25 (Phlips et al. 2004, 2006, Sastre et al. 2013, Soler-Figueroa & Otero 2015, 2016, Alkawri et al. 2016), as this genus has a wide salinity tolerance ranging from 14 to 46 (Phlips et al. 2004). Salinities in the nearby biobay Bahía Fosforescente, Puerto Rico, range from 35.6 to 37.7 in the wet and dry seasons, respectively (Soler-Figueroa & Otero 2016). Average salinities in the current study ranged from 36.9 to 39.5. In the current study as well as others, the highest concentrations of *P. bahamense* cells were usually found furthest from the mouth of enclosed lagoons due to a more stable environment and lower flushing rates (Gasparich 2007, Algeo 2008).

ChemTax was used to estimate the relative contribution of individual algal groups to the total phytoplankton biomass (in units of µg chl *a* l^{-1}) (Higgins et al. 2011). The advantages and disadvantages of this approach have been well discussed in the literature (Higgins et al. 2011). In this study, the major contributors to phyto plankton biomass were dinoflagellates, which have the unambiguous photopigment biomarkers peridinin and gyroxanthin. The dominance of dinoflagellates was confirmed through qualitative light microscopy examination of samples collected in Mangrove Lagoon to validate ChemTax results (e.g. Reed et al. 2016). Although not perfect, ChemTax and photopigment-based approaches provide a reasonable big-picture view of phytoplankton community dynamics (Roy et al. 2011). We chose this method because microscopic cell counts are inherently selective and biased toward easily identifiable species and cells that remain intact after preservation (Lund et al. 1958, Rott 1981, Wilhelm et al. 1991, Schlüter et al. 2000). Our approach allowed the timely analysis of 683 individual samples (a monumental task for direct microscopy).

The HPLC photopigment data and subsequent ChemTax analysis showed that the phytoplankton community in Mangrove Lagoon consisted of a mixed assemblage of algal groups including dinoflagellates, diatoms, cryptophytes, chlorophytes, and cyanobacteria. Of these groups, dinoflagellates were consistently the most abundant, averaging 33% of total phytoplankton abundance and reaching values as high as 97%. The dinoflagellate group was likely composed of several species, including the bioluminescent species *P. bahamense*. The *Karenia*-like dinoflagellates, which were differentiated from other dinoflagellates by the presence of the accessory photopigments gyroxanthin and fucoxanthin rather than peridinin, were also abundant in 2016 and comprised 10 to 15% of phytoplankton biomass. Large blooms of diatoms were rare, and cyanobacteria showed increases in relative abundance during the warm summer months which corresponded with drought conditions and high salinities in Mangrove Lagoon. Cryptophytes and chlorophytes were minor contributors to overall phytoplankton biomass. Bloom concentrations of the major groups were generally lim-

ited to ca. 50–100 m diameter patches and persisted for periods of 2 to 4 wk per event.

Surface and bottom water sampling was conducted primarily during daylight hours (ca. 10:00–12:00 h). Thus, the observed spatial patterns do not reflect distributional changes due to dinoflagellate vertical migration at night. Seliger et al. (1971) note that *P. bahamense* in tropical sunlight is positively phototaxic and migrates to depths at dusk, allowing for nutrient uptake from the depths at night. However, others have reported that *P. bahamense* migrates to the surface at dusk, but individuals occur throughout the water column (Soli 1966). Measurements of light attenuation in the lagoon revealed as much as 10 to 20% of surface irradiance reached the bottom sediments (Pinckney et al. 2014) and suggested there was sufficient sunlight to support photosynthesis in bottom waters. Previous studies have shown that the waters of the lagoon appear to be homogeneous from top to bottom with respect to temperature, salinity, pH, and dissolved oxygen (Pinckney et al. 2014). Vertical profiles of chl *a* fluorescence during day and night indicated that as much as 25% of the phytoplankton community migrated from bottom to surface waters during nighttime (Pinckney et al. 2014). Our results show a higher abundance of phytoplankton groups (except cyanobacteria) in bottom waters during the daytime, and evidence from Pinckney et al. (2014) suggests vertical migration of a portion of the community to surface waters at night.

Phytoplankton growth in estuarine and coastal systems is usually limited by sunlight or, more commonly, nutrients (Hecky & Kilham 1988, Cloern 1999). In Mangrove Lagoon, the ratio for DIN:DIP averaged 11.1, suggesting N limitation for phytoplankton growth based on nutrient stoichiometry. Nutrient addition bioassays by Pinckney et al. (2014) in Mangrove Lagoon showed that the total phytoplankton community (as chl *a*) and the chlorophytes, cryptophytes, cyanobacteria, and diatoms were co-limited for N and phosphorus (P). In contrast, dinoflagellates did not respond to nutrient additions, which suggested that neither N nor P nor N + P were limiting for growth (Zimberlin 2013, Pinckney et al. 2014). These authors' results were in contrast to other studies (Sellner et al. 2001, Fan et al. 2003, Badylak et al. 2004, Phlips et al. 2004, 2011), which showed that nutrient enrichment stimulated growth of dinoflagellates. However, the Pinckney et al. (2014) bioassay results were consistent with the suggestion by Usup et al. (2012) that *P. bahamense* is not competitive and will not become dominant under high N and P conditions. In the Indian River Lagoon, Florida, Phlips et al. (2006) reported that concentrations of *Pyrodinium* were not correlated with N or P concentrations. Phlips et al. (2006) also reported that there was no relationship between *Pyrodinium* abundance and N:P ratios. Our findings that there was no correlation between phytoplankton abundances and N concentrations, P concentrations, or N:P ratios are consistent with those of Phlips et al. (2006). Although the concentrations of NH_4^+ were relatively high in the lagoon, combined N is likely the limiting nutrient. We speculate that the high NH_4^+ concentrations were the result of active benthic infauna, coupled with a shallow water column and relatively long water residence times for the lagoon.

Reidhaar et al. (2016) undertook a study of the distribution and abundance of *P. bahamense* cysts preserved in the sediments of Mangrove Lagoon. According to their study, *P. bahamense* blooms have been occurring in the lagoon since the 1960s, when the lagoon was dredged and connected to Salt River Bay. However, downcore profiles suggested that the size of *P. bahamense* blooms has been declining in recent years. They hypothesize that a gradual restriction of water exchange with Salt River Bay due to a shallowing sill at the mouth of the lagoon was responsible for the *P. bahamense* decline. The cyst data showed highest abundances (up to 466 cysts g^{-1} dry sediment) on the eastern quadrant of the lagoon. The measured *P. bahamense* cyst concentrations in Mangrove Lagoon were lower than in other bioluminescent lagoons in the region. For example, Puerto Mosquito Bay and Laguna Grande in Puerto Rico have average cyst concentrations that exceed 4500 cysts g^{-1} wet sediment (Hereid 2007, Lane et al. 2013). Reidhaar et al. (2016) proposed that cyclonic circulation in the lagoon due to prevailing easterly winds coupled with nutrients supplied from mangroves and inflowing waters fostered blooms in this area of the lagoon.

Working in Mangrove Lagoon, Zimberlin (2013) conducted a series of phytoplankton bioassays which included additions of mangrove leaves as well as nutrients (N and P). After incubations of 72 h, the results showed a negative response of most phytoplankton groups except the dinoflagellates, which were not negatively impacted. Zimberlin (2013) suggested that mangrove organic matter supplied as leachate from the leaves may suppress the growth of phytoplankton and allow the dinoflagellates to outcompete the other groups. This is one hypothesis that could explain the close association between *P. bahamense* abundance and mangroves.

In the nearby tropical bioluminescent bay Bahía Fosforescente in Puerto Rico, Soler-Figueroa & Otero (2016) concluded that the seasonality in dinoflagellate bioluminescence and composition in their system was most closely related to precipitation. In our study, we did not observe any correlation with individual rainfall events or variations in salinity, but there does seem to be a weak pattern of higher dinoflagellate abundances in fall and winter months.

Slow growth rates and high nutrient loading rates may preclude the growth of dinoflagellates (Tang 1996, Phlips et al. 2006, Usup et al. 2012). However, the large size and motility of *P. bahamense* and other dinoflagellates, combined with the restricted or episodic supply of new inorganic nutrients, may provide the ability to search for and store nutrients in more stable water columns (Phlips et al. 2006, Reynolds 2006, Usup et al. 2012). We suspect that long water residence time and calm conditions in Mangrove Lagoon further contribute to a favorable environment for dinoflagellate growth (Seliger et al. 1970, 1971, Margalef 1978, Sullivan & Swift 2003, Greeney 2007, Phlips et al. 2011). However, these conditions do not always result in dinoflagellate/phytoplankton blooms. Clearly, other factors are necessary to promote and sustain blooms in Mangrove Lagoon. Stable water columns and high residence times have been shown to promote dinoflagellate abundances, often relative to other phytoplankton taxa (Hallegraeff et al. 1995, Vila et al. 2001, Yamamoto et al. 2013). Thus, our finding that residence time is associated with greater biomass of dinoflagellates in Mangrove Lagoon is consistent with prior research.

For the overall phytoplankton community, there was no difference in total biomass (as chl *a*) between wet and dry seasons. However, cyanobacteria and *Karenia*-like dinoflagellate abundances were significantly higher (by a factor of 2) during the wet season. In contrast, diatom and chlorophyte abundances were higher (by a factor of 2 for the diatoms) during the dry season. Although not significant, dinoflagellate biomass tended to be higher during the dry season. Although our time series is too short, one intriguing hypothesis is that the combination of residence time, wet vs. dry season, and rainfall events may all act to support dinoflagellate and other phytoplankton bloom events in Mangrove Lagoon.

The current study provides an important contrast to other bioluminescent bays (biobays) in the Caribbean region. Mangrove Lagoon (3.8 ha) is much smaller than other biobays in Puerto Rico, the Bahamas, Jamaica, and Florida that range in size from 100 to more than 1000 ha in area (Sastre et al. 2013). Another contrast is that other similar studies have demonstrated a positive correlation between rainfall and dinoflagellate abundances (Phlips et al. 2006, Sastre et al. 2013, Soler-Figueroa & Otero 2015, 2016). Rainfall events did not result in increases in dinoflagellate abundances in the current study. However, other characteristics such as long water residence times (>10 d) of the lagoons, close proximity of mangroves, and high salinities (>25) are consistent with other biobays in the region. Thus, Mangrove Lagoon shares many common features with other biobays but is unique in its small size and absence of dinoflagellate blooms triggered by rainfall inputs.

CONCLUSIONS

The primary finding of this study was that dinoflagellates are a consistent and major component of the phytoplankton community in Mangrove Lagoon. Blooms of other phytoplankton groups such as diatoms, cryptophytes, and chlorophytes coincided with dinoflagellate blooms. Cyanobacterial blooms occurred mostly during the summer months under high salinity conditions. Phytoplankton blooms of several weeks duration rarely encompassed the entire lagoon, instead forming patches within the lagoon. There was no correlation between phytoplankton blooms and nutrient concentrations, salinity, or N:P ratio. However, dinoflagellate blooms occasionally occurred during periodic, tidally driven long water residence times in the lagoon. The results of this study offer insights into the potential role of physical conditions on phytoplankton and dinoflagellate abundance dynamics and variability in bioluminescence in Mangrove Lagoon.

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