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Factors associated with moderate blooms of *Pyrodinium bahamense* in shallow and restricted subtropical lagoons in the Gulf of California

Abstract: We examined the environmental and biological factors related to blooms of the toxic dinoflagellate *Pyrodinium bahamense* in three shallow, restricted subtropical lagoons in the Gulf of California during the rainy summer. In the San José, Yavaros, and El Colorado lagoons, the vegetative stage peaked at 63, 108, and 151 ($\times 10^3$ cells l⁻¹), respectively. At San José, production of cysts peaked at 9.7×10^3 g⁻¹ of dry sediment mass as the bloom declined. Large diatoms predominated, with *P. bahamense* the most common dinoflagellate during the blooms. Abundance of *P. bahamense* at San José was positively correlated with salinity ($r=0.50$, $p=0.0003$), seawater temperature ($r=0.44$, $p=0.005$), silicates ($r=0.45$, $p=0.003$), and ammonium ($r=0.32$, $p=0.005$), and negatively correlated with dissolved oxygen ($r=-0.34$, $p<0.0001$). No such correlations were found at El Colorado and Yavaros. The environmental window that favors development of blooms is restricted to the summer and is influenced by seawater temperature, salinity, and relative concentrations of ammonium and phosphates that, in turn, depend on rainfall and runoff, which is greater on the eastern side of the Gulf where seawater quality is degraded.

Keywords: bloom dynamics; cysts; ecology; Gulf of California; Mexico; *Pyrodinium bahamense*; subtropical lagoons.

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Introduction

The distribution pattern and factors related to bloom formation of the bioluminescent, toxic dinoflagellate *Pyrodinium bahamense* plate have been extensively described for coastal areas of the tropical Indo-Pacific (MacLean 1989, Azanza 1997, Azanza and Taylor 2001) and tropical-subtropical North Atlantic (Phlips et al. 2004, 2006, Soler-Figueroa 2006). In the northeastern Pacific, descriptions are less comprehensive and ecological information is limited.

In the central Indo-Pacific region, *P. bahamense* blooms in shallow coastal environments during the warm period (April through October), peaking mainly during the onset of the rainy season toward the end of the summer, which, in combination with tidal currents, promotes resuspension of cysts (Azanza 1997, Villanoy et al. 2006). Increased thermal stratification with vertical stability of the water column favors initiation of the bloom and its prolongation (Villanoy et al. 2006). The dinoflagellate caused 877 cases of paralytic shellfish poisoning (PSP) and 44 deaths in the Philippines during the period 1988–1998. Just as the blooms unexpectedly appeared in 1988, they abruptly disappeared in 1999 and seem not to have occurred since; nevertheless, specimens of the species were still found during the months when blooms are expected to occur (Jacinto et al. 2006).

Along the Florida coastline, there is a seasonal fluctuation in the abundance of *P. bahamense*; the dinoflagellate is found nearly year-round in the Indian River Lagoon and Tampa Bay, but is generally restricted to summer (Phlips et al. 2006). Twenty degrees Celsius appears to be the lower limit for the occurrence of highest population abundance. Salinity tolerance ranges from 10 to 45. The bloom potential of *P. bahamense* is most closely associated with shallow-water ecosystems with long water residence times; peak levels of biomass are correlated with nutrient concentrations (Phlips et al. 2006). In the Indian River Lagoon, *P. bahamense* increases sporadically, suggesting that rainstorms after

a regional drought flush bioavailable phosphorus and nitrogen into the lagoon, which, in turn, stimulates bloom formation (Phlips et al. 2004). *Pyrodinium bahamense* is considered the putative source of saxitoxin puffer fish poisoning in the Indian River Lagoon; several isolates tested positive for saxitoxins (Landsberg et al. 2006).

Blooms also occur in the southern Gulf of Mexico and Caribbean Sea (Gómez-Aguirre 1998, Licea et al. 2004); however, these have not been linked to outbreaks of PSP and there is no evidence of toxicity in strains from these areas. The first report of *Pyrodinium bahamense* in the northeastern Pacific came from the coast of Mexico near the border with Guatemala (Osorio-Tafall 1942). Blooms occurred between 1985 and 2004 and were linked to outbreaks of PSP, with 393 human poisonings and 20 fatalities (Cortés-Altamirano et al. 1993, Orellana-Cepeda et al. 1998, INP-SAGARPA 2004). Very low abundances of *P. bahamense* in the Gulf of California have been confirmed recently (Martínez-López et al. 2007a, Morquecho 2008, Gárate-Lizárraga and González-Armas 2011), but blooms have not been previously reported in this region. Interest in the distribution of this dinoflagellate and the factors that regulate its seasonality and bloom dynamics in this region has increased.

The ecophysiology and bloom dynamics of *P. bahamense* are still not well understood, and comparative studies between populations are needed to better appreciate the bloom dynamics of this species (Usup et al. 2012). This study examined, for the first time, the factors related to the occurrence and bloom development of *P. bahamense* in three shallow and restricted subtropical lagoons in the Gulf of California, where the impact of urban, agricultural, and aquacultural activities varies from none to intense.

Materials and methods

Study areas

On the western side of the Gulf of California, at the southern end of Isla San José (24° 52' 32.63" N, 110° 33' 30.23" W), there is a small lagoon of ~86 ha (Figure 1), which is bordered by a mangrove forest and a narrow sand bar. The lagoon is connected to the sea by a long, narrow channel to the north-northwest and a small outlet to the southwest. The lagoon is part of a natural protected area; with the exception of local fishing, it is not impacted by human activity.

On the eastern side of the Gulf of California are Laguna Yavaros (~10,000 ha; 26° 42' 15.56" N, 109° 29' 39.90" W; Figure 1) and Laguna El Colorado (~16,400 ha; 25° 42' 15.16" N, 109° 21' 31.29" W; Figure 1). Laguna Yavaros is bordered by a mangrove forest and two sand bars, and is about 2 m deep. The lagoon is influenced by the Río Mayo and annually receives ~70 t of nitrogen and 8 t of phosphorus from the surrounding agricultural and urban activities (Arreola-Lizárraga et al. 2009). The Río Fuerte, which discharges into the northern end of Laguna El Colorado, runs through sparsely populated territory; its drainage basin is large and the water from it collects in Río Fuerte Viejo and Río Fuerte Nuevo. On the northeast and southeast coasts of the lagoon, there are mangrove forests. The lagoon is part of a natural protected area; however, there is a significant impact from aquacultural activity and fishing, as well as waste water from the surrounding population (Páez-Osuna et al. 2007).

Sampling and measurements

At Isla San José, nine sampling stations were selected, 13 at Laguna Yavaros, and 12 at Laguna El Colorado (Figure 1). At Isla San José, samples were taken monthly from July through October 2008; at Laguna El Colorado, samples were taken in June 2006; at Laguna Yavaros, samples were taken in September 2008. Measurements of temperature, salinity, and dissolved oxygen were made with probes (600XLM and 556MPS, YSI, Yellow Springs, OH, USA, and DS5X Hydrolab, Hach, Loveland, CO, USA). Monthly mean air temperature and monthly accumulated precipitation were assembled from daily data obtained in 2006 and 2008 from meteorological stations relatively close to the study areas. The influence of storms was recorded, based on the bulletins of the meteorological service.

Seawater samples were collected for nutrient analysis (n=105; 250 ml each) and phytoplankton analysis (n=105; 250-ml vials) with a standard water sampler at 1 m depth and near the sea bottom at San José and Laguna El Colorado, and near the surface only at Laguna Yavaros. Samples of phytoplankton for taxonomic analysis (n=49; 50-ml vials) were also collected by vertical hauls with a 20- μ m mesh net. Both types of phytoplankton samples were fixed with 5% acid Lugol's solution (Thronsdon 1978). At San José, sediment samples (n=32) for cyst analysis were collected in 50-ml plastic tubes from the first centimeter of the sea floor by a scuba diver. The sediment samples were wrapped in aluminum foil and stored in the dark at 4°C until analysis.

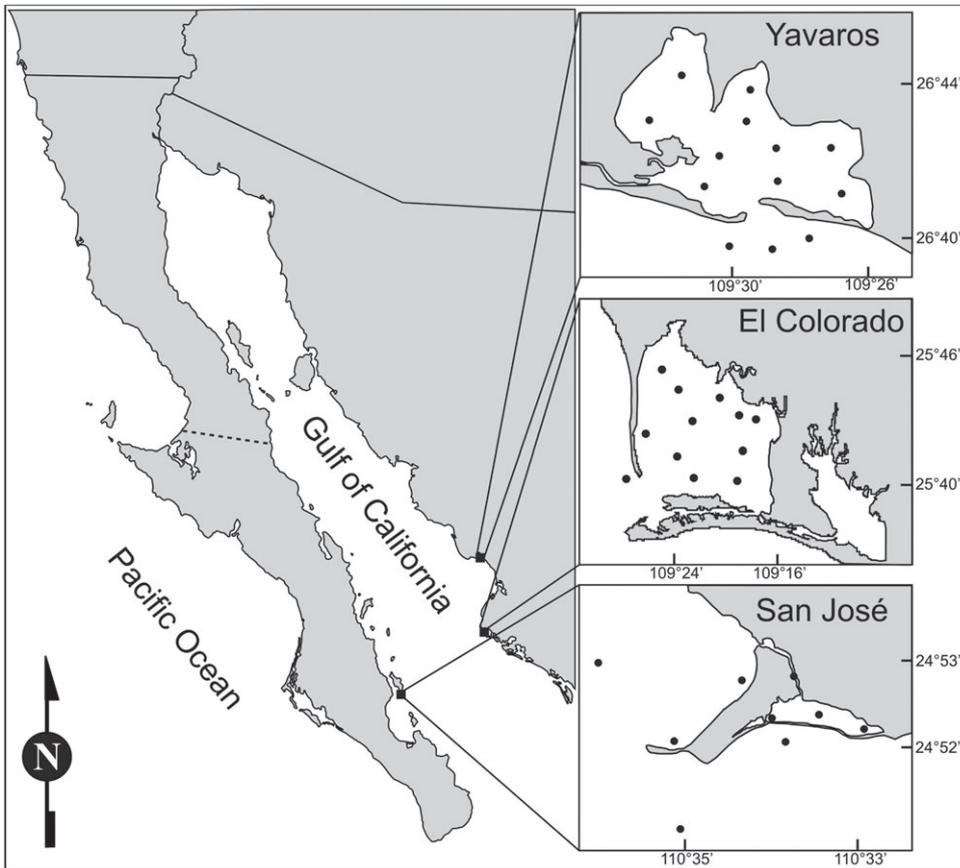


Figure 1 *Pyrodinium bahamense*: study areas in the Gulf of California: ■, locations of the lagoons; ●, locations of sampling stations.

Nutrient analysis

Seawater samples were analyzed for nitrite, nitrate, ammonium, soluble phosphorus, and silicates (Armstrong 1951; Strickland and Parsons 1972; Valderrama 1981). Absorption values were measured by spectrophotometry (Spectronic 21D, Milton Roy, Ivyland, PA, USA, and Thermo Spectronic AquaMate UV, ThermoFisher Scientific, Waltham, MA, USA).

Phytoplankton and dinoflagellate cyst analysis

Counts of *Pyrodinium bahamense* and other common phytoplankton species at San José and Yavaros were determined in 50-ml Utermöhl counting chambers under an inverted microscope (Axiovert 100, Carl Zeiss, Göttingen, Germany); phytoplankton samples from El Colorado were counted with a 1-ml Sedgewick-Rafter chamber and a compound microscope (Pysers-SGI Ltd.,

Edenbridge, Kent, UK). A phase-contrast microscope (BX41TF, Olympus, Tokyo, Japan) was used to confirm the taxonomic identification of common species. For the identification and enumeration of *P. bahamense* cysts, the sediment was treated as described by Matsuoka and Fukuyo (2000). Abundance of living and empty *P. bahamense* cysts is expressed here as cysts per gram of dried sediment.

Statistical data analysis

We used multivariate, multidimensional, non-parametric scaling on data transformed ($\log x+1$) and standardized to determine whether the lagoons were similar in terms of water quality during *Pyrodinium bahamense* peaks; the PRIMER 6 statistical software (Primer-E, Ivybridge, UK) was used to perform the analyses. The Arid Zone Coastal Water Quality Index (AZCI), based on the pooled effect of NO_2^- , NO_3^- , NH_4^+ , and PO_4^{3-} , was used to assess water quality (Mendoza-Salgado et al. 2005). AZCI values were

analyzed by comparison of means with one-way analysis of variance. Because of the non-normality in the data, the Spearman's rho correlation coefficient was used to determine the interrelationships between abundance of *P. bahamense* vegetative cells and hydrologic variables. For the latter, significance was recognized at $\alpha < 0.05$.

Results

Meteorological conditions

At the Yavaros and El Colorado lagoons, average maximum air temperature (35°C–39°C) and rain occurred from July through October. During summer 2006 at El Colorado, total monthly rain fluctuated between 52 mm and 82 mm, whereas in 2008 at Yavaros, the peak monthly rainfall was 373 mm in September (Figure 2). At San José, peak air temperatures (39°C and 42°C) and the rainy season occurred from June to October; peak total monthly precipitation was below 50 mm (Figure 2).

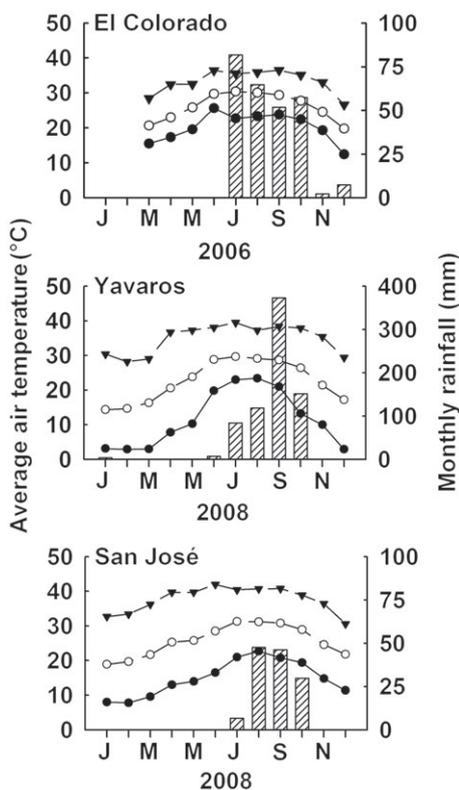


Figure 2 *Pyrodinium bahamense*: average monthly variation in temperature (O, average; ●, minimum; and ▼, maximum) and monthly rainfall (bars) in 2006 and 2008 in the southern Gulf of California. Data came from weather stations closest to the study areas.

During 2006, the Pacific coast of Mexico was impacted by 21 tropical storms, but they did not provide rain to this part of the southern Gulf of California. During 2008, the rainy season was influenced by two tropical storms (August and September) and one hurricane at the beginning of October. At Isla San José and Yavaros, the maximum rainfall produced by these storms was 20–50 mm, whereas the 24-h accumulated rainfalls produced by the hurricane were 100–150 mm near Isla San José and 40–60 mm near Yavaros.

Seawater physical and chemical characteristics

Inside the lagoons, water temperature ranged between 25.8°C and 34.5°C (Table 1). In 2008, temperatures were highest in August and September at San José and Yavaros. At El Colorado, the highest water temperature was 34.5°C in July 2006. Temperatures outside the lagoons were usually slightly lower. At San José, water temperatures were markedly different inside and outside the lagoon; temperature differences were less extreme by late summer.

At San José, salinity from July through October 2008 was similar inside and outside the lagoon, ranging from 34.5 to 36.2 (Table 1). At El Colorado, salinity was generally similar inside and outside the lagoon; however, in very shallow areas, it reached 46.1. At Yavaros, salinity inside the lagoon decreased during the rainy season. At San José, dissolved oxygen ranged from 4.3 to 11.6 mg l⁻¹ but tended toward lower concentrations inside the lagoon as the summer advanced. At Yavaros and El Colorado, dissolved oxygen ranged from 6.1 to 6.3 mg l⁻¹ and from 3.8 to 7.5 mg l⁻¹, respectively (Table 1).

Among the three forms of nitrogen, NH₄⁺ was the most common in the three areas; the highest concentrations occurred in Laguna El Colorado. At San José, the three nitrogen components were frequently below levels of detection; however, in October 2008, NH₄⁺ increased from 0.57 to 4 μM inside and outside the lagoon. The concentration of PO₄³⁻ was similar in the three areas, ranging from 0.38 to 2.87 μM. Concentrations of SiO₃²⁻ at San José ranged from 1 to 11.7 μM and were usually higher in the inner part of the lagoon, mainly in October. Concentrations of SiO₃²⁻ were much higher at El Colorado in June 2006, where the mean and maximum values were 20.32 and 43.6 μM, respectively (Table 1).

Phytoplankton assemblages

The phytoplankton community was composed mainly of diatoms and dinoflagellates. Diatoms were more common

	Statistics	El Colorado		San José						Yavaros			
		2006		2008						2008			
		June		July		August		September		October		September	
	IL	OL	IL	OL	IL	OL	IL	OL	IL	OL	IL	ML	
Temperature (°C)	Mean	32.9	32.3	26.8	23.7	30.9	29.5	30.8	29.6	27.3	27.5	31.3	30.3
	Minimum	32.1	32.3	25.8	22.5	30.2	29.1	30.3	28.7	26.9	27.3	30.8	30.2
	Maximum	34.5	34.2	27.4	24.9	31.7	29.9	31.2	30.0	27.6	27.6	31.7	30.4
Salinity	Mean	41.7	37.3	35.2	34.8	36.1	35.8	35.6	35.6	35.9	35.6	31.9	34.8
	Minimum	37.5	37.3	35.0	34.5	35.9	35.8	35.3	35.5	35.7	35.5	31.1	33.9
	Maximum	46.1	37.4	35.2	34.9	36.2	35.8	35.8	35.7	36.1	35.7	32.5	35.3
Dissolved oxygen (mg l ⁻¹)	Mean	5.3	5.8	10.0	10.6	6.9	7.1	5.2	5.8	5.7	6.0	7.5	6.2
	Minimum	3.8	5.8	7.6	9.5	5.2	6.3	4.3	5.7	4.9	5.2	6.1	6.1
	Maximum	7.5	5.8	11.6	11.6	9.4	8.9	6.1	6.0	6.2	6.3	8.8	6.3
NO ₂ ⁻ (μM)	Mean	0.22	0.25	0.06	0.04	0.05	0.03	0.03	0.02	0.02	0.00	0.19	0.14
	Minimum	0.04	0.17	0.00	0.00	0.01	0.07	0.00	0.00	0.00	0.00	0.02	0.09
	Maximum	1.02	0.32	0.14	0.10	0.08	0.00	0.06	0.06	0.05	0.03	1.07	0.23
NO ₃ ⁻ (μM)	Mean	1.20	0.99	0.05	0.46	0.19	0.04	0.06	0.14	0.04	0.01	0.42	0.27
	Minimum	0.24	0.61	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.04	0.10
	Maximum	4.66	1.37	0.34	2.08	0.85	0.26	0.39	0.67	0.26	0.07	3.68	0.59
NH ₄ ⁺ (μM)	Mean	8.14	8.53	0.45	0.26	0.84	0.72	0.53	0.52	2.07	2.10	1.79	1.45
	Minimum	0.00	5.63	0.17	0.13	0.24	0.35	0.19	0.27	0.57	0.73	0.54	1.28
	Maximum	33.04	11.44	0.92	0.37	1.86	1.85	1.10	0.71	3.90	3.98	6.88	1.76
DIN (μM)	Mean	9.56	9.77	0.55	0.76	1.08	0.79	0.62	0.68	2.13	2.11	2.40	1.86
	Minimum	1.48	7.32	0.22	0.21	0.35	0.35	0.22	0.29	0.61	0.73	0.61	1.47
	Maximum	33.6	12.22	1.40	2.47	2.79	1.92	1.55	1.18	3.90	3.98	11.63	2.58
PO ₄ ³⁻ (μM)	Mean	1.96	1.18	1.20	1.40	1.12	1.12	1.14	1.09	0.60	0.58	1.07	0.83
	Minimum	0.38	0.77	1.05	0.82	0.94	1.05	0.97	0.84	0.47	0.51	0.71	0.66
	Maximum	2.87	1.59	1.49	1.67	1.39	1.26	1.31	1.17	0.75	0.68	2.79	1.04
SiO ₃ ²⁻ (μM)	Mean	20.32	1.34	4.99	1.99	4.22	2.19	3.35	2.57	8.03	5.88	NR	NR
	Minimum	0.27	0.76	3.68	1.00	2.59	1.00	1.64	1.05	5.55	4.22	NR	NR
	Maximum	43.56	1.92	8.44	3.77	6.47	5.63	5.81	5.16	11.70	7.63	NR	NR
Nitrogen/phosphorus ratio	Mean	7.37	8.62	0.44	0.51	0.97	0.70	0.54	0.62	3.44	3.62	1.83	2.22
	Minimum	0.79	7.71	0.20	0.15	2.40	0.33	0.23	0.26	1.08	1.28	0.74	1.97
	Maximum	37.91	9.53	0.94	1.48	0.25	1.69	1.44	1.01	5.96	7.26	4.17	2.48

Table 1 *Pyrodinium bahamense*: average values and ranges of physical variables and nutrients in three lagoons in the Gulf of California from which samples were collected.

IL, inside the lagoon; OL, outside the lagoon; ML, mouth of the lagoon; DIN, dissolved inorganic nitrogen; NR, not recorded.

than dinoflagellates inside and outside the lagoons. Chlorophytes, Cyanobacteria, euglenophytes, raphidophytes, and silicoflagellates were also found, but at very low abundance (<6% of the total). Assemblages were dominated by specific abundant taxa (Table 2). At El Colorado, *Thalassiosionema* ($\leq 0.29 \times 10^6$ cells l⁻¹) was the most common genus among diatoms. At Yavaros, it was *Skeletonema costatum* ($\leq 3.1 \times 10^6$ cells l⁻¹) and *Nitzschia longissima* ($\leq 0.24 \times 10^6$ cells l⁻¹). At San José, the major diatoms were *Pseudonitzschia* cf. *subcurvata* ($\leq 2 \times 10^6$ cells l⁻¹) and *Corethron pennatum* ($\leq 0.25 \times 10^6$ cells l⁻¹), the first occurring inside and outside the lagoon from July through October 2008 and the latter blooming inside the lagoon in September 2008.

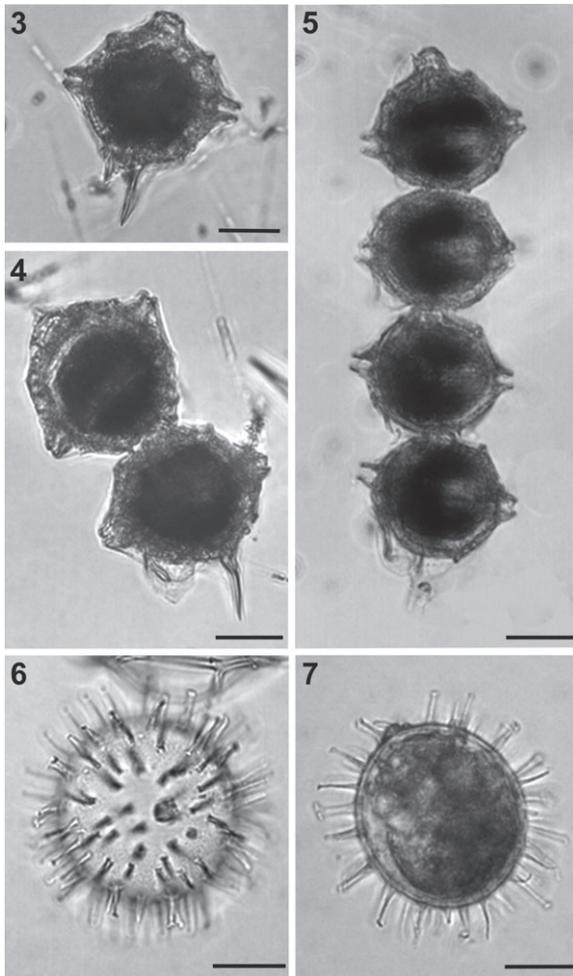
Pyrodinium bahamense was one of the most abundant dinoflagellates in the three lagoons. The vegetative stage was found mainly as single cells; cell pairs and four-cell chains were rarely observed (Figures 3–5). At El Colorado, a moderate bloom occurred in late June 2006; at San José and Yavaros, moderate blooms occurred in September 2008 (Table 3). At San José, the vegetative stage peaked at 63×10^3 cells l⁻¹. At El Colorado and Yavaros, the maxima were 151×10^3 and 108×10^3 cells l⁻¹, respectively. All blooms occurred in the lagoons and, particularly at Yavaros and San José, occurred in the warmest months and during the peak of the rainy season (Figure 2 and Table 3).

Protoperdinium spp. were also abundant at El Colorado and Yavaros, *Prorocentrum minimum* at Yavaros, and

Group	Genus or species	El Colorado		San José		Yavaros	
		06/2006	07/2008	08/2008	09/2008	10/2008	09/2008
Diatoms	<i>Chaetoceros</i> spp.	■		■			
	<i>Corethron pennatum</i> (Grunow) Ostenfeld*			■			
	<i>Leptocylindrus</i> spp.	■					
	<i>Nitzschia longissima</i> (Brébisson) Ralfs*		■		■		■
	<i>Nitzschia</i> spp.	■					
	<i>Pseudo-nitzschia</i> cf. <i>subcurvata</i> (Hasle) Fryxell**		■	■	■	■	■
	<i>Skeletonema costatum</i> (Greville) Cleve**						
	<i>Thalassionema</i> spp.*	■					
	<i>Thalassiosira</i> spp.*		■	■			
	<i>Akashiwo sanguinea</i> (Hirasaka) Hansen et Moestrup		■	■	■		
	<i>Alexandrium</i> spp.						
	cf. <i>Heterocapsa</i> sp.*	■	■	■	■		
	<i>Neoceratium furca</i> (Ehrenberg) Gómez, Moreira et López-García						
	<i>Cochlodinium</i> cf. <i>fulvescens</i> Iwataki, Kawami et Matsuoka			■	■		
	<i>Gonyaulax verior</i> Sourmia			■	■		
	<i>Katodinium glaucum</i> (Lebour) Loeblich III			■	■		
	<i>Pyrodinium bahamense</i> Plate*	■			■	■	■
<i>Prorocentrum gracile</i> Schütt							
<i>Prorocentrum micans</i> Ehrenberg			■	■			
<i>Prorocentrum minimum</i> (Pavillard) Schiller*							
<i>Protoperidinium</i> spp.*	■					■	
<i>Protoperidinium bipes</i> (Paulsen) Balech						■	
Chlorophytes							
Cyanobacteria	■						
Euglenophytes			■			■	
Raphidophytes		■					
Silicoflagellates			■			■	

Table 2 *Pyrodinium bahamense*: most abundant associated phytoplankton taxa in the southern Gulf of California.

Genera or species that developed moderate blooms (50×10^3 up to $\sim 1 \times 10^6$ cells l^{-1}) and massive blooms ($> 1 \times 10^6$ cells l^{-1}) are marked with an asterisk or a double asterisk, respectively.



Figures 3–7 *Pyrodinium bahamense*: Vegetative and resting stages from the Gulf of California.

(3) Single vegetative cell. (4) Pair of vegetative cells. (5) Chain of four vegetative cells. (6) Empty cyst. (7) Living cyst. Scale bars=20 μm .

a small, armored dinoflagellate, cf. *Heterocapsa* sp., at San José (Table 2). Dinoflagellates occurring commonly in the assemblage with *P. bahamense* differed among lagoons (Table 2). The monthly sampling at San José showed that dinoflagellates were primarily located in the lagoon. In September, the bloom predominantly comprised *P. bahamense*, but also included *Corethron pennatum*, *Nitzschia longissima*, *Pseudo-nitzschia* cf. *subcurvata*, *Skeletonema costatum*, *Akashiwo sanguinea*, cf. *Heterocapsa* sp., and *Neoceratium furca*. Prior to the bloom, the phytoplankton community mainly included *Chaetoceros* spp., *P.* cf. *subcurvata*, *Thalassiosira* spp., *A. sanguinea*, cf. *Heterocapsa* sp., *Cochlodinium* cf. *fulvescens*, *Gonyaulax verior*, *Katodinium glaucum*, *Prorocentrum gracile*, and *Prorocentrum micans*.

Pyrodinium bahamense cysts at Isla San José

Dinoflagellate cysts occurred mainly inside the lagoon; outside the lagoon, cysts were uncommon. The cyst assemblage included (in order of abundance) the Gonyaulacales, calcareous Peridinales, Gymnodinales, and organic Peridinales. Among the Gonyaulacales, the cysts of *P. bahamense* were the most abundant and representative morphotypes. Most of these cysts were empty; however, a few living cysts were found at very low concentrations in July and August (Table 3, Figures 6 and 7). During October 2008, the mass of cysts in the most protected parts of the lagoon peaked at $9.7 \times 10^3 \text{ g}^{-1}$ (sediment dry mass), coinciding with the decline of the vegetative form (Table 3).

Year	Lagoon	Month	Location	Vegetative stage (cells l ⁻¹)			Empty cysts g ⁻¹ (dried weight)			Living cysts g ⁻¹ (dried weight)		
				Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
2006	El Colorado	June	IL	64,145	500	151,500	ND	ND	ND	ND	ND	ND
			OL	500	500	500	ND	ND	ND	ND	ND	ND
2008	San José	July	IL	NR	NR	NR	4967	69	10,769	12	35	74
			OL	NR	NR	NR	93	62	124	10	31	31
		August	IL	471	40	2800	4214	23	13,667	17	204	204
			OL	NR	NR	NR	79	49	269	NR	NR	NR
	September	IL	23,139	5180	62,700	1961	31	5492	10	31	61	
		OL	8	80	80	7	13	13	7	13	13	
	October	IL	4910	480	22,400	7803	84	26,464	2433	139	9712	
		OL	48	120	220	51	16	186	6	16	37	
Yavaros	September	IL	30,600	800	107,600	ND	ND	ND	ND	ND	ND	
		ML	1333	400	2400	ND	ND	ND	ND	ND	ND	

Table 3 *Pyrodinium bahamense*: abundance of vegetative and resting stages in the El Colorado, San José, and Yavaros lagoons. IL, inside the lagoon; OL, outside the lagoon; ML, mouth of the lagoon; NR, not recorded; ND, no data.

Seawater quality and hydrological factors related to *Pyrodinium bahamense*

Multivariate non-parametric, multidimensional scaling (nMDS) clearly indicated a differentiation in water quality among the three lagoons during peaks of *P. bahamense* abundance. The parameters at El Colorado were most strongly differentiated (Figure 8). The AZCI showed that water quality was good at San José and Yavaros, but poor at El Colorado (Figure 9).

Spearman's rho correlation coefficient, a statistical test for correlation between two rank-ordered scales, yields a statement of the degree of interdependence of the scores of the two scales. It showed that *P. bahamense* abundance at Isla San José was moderately correlated with salinity ($r=0.50$, $p=0.0003$), temperature ($r=0.44$, $p=0.005$), silicates ($r=0.45$, $p=0.003$), and ammonium ($r=0.32$, $p=0.005$), and was moderately negatively correlated with dissolved oxygen ($r=-0.34$, $p<0.0001$). At El Colorado and Yavaros, no correlations with any hydrological variable were found.

Discussion

Seasonality and habitat characteristics of *Pyrodinium bahamense*

In the Gulf of California, cysts of *P. bahamense* were reported for the first time by Martínez-Hernández and Hernández-Campos (1991). The motile stage was reported in spring

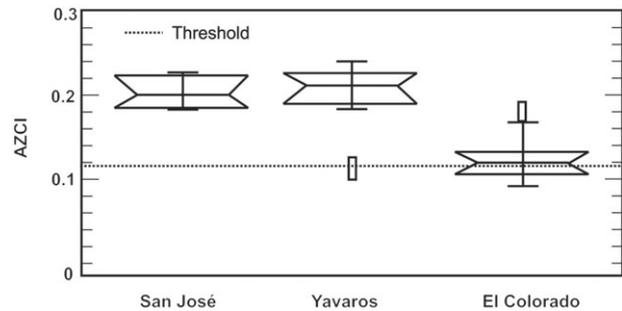


Figure 9 *Pyrodinium bahamense*: Box and whisker plot of the Arid Zone Coastal Water Quality Index (AZCI) for the lagoons during peak events. Median, quartiles, ranges, and outliers of data are shown for each event. Dotted line shows the threshold of water quality (<0.12). El Colorado had some significant differences, compared to the San José and Yavaros lagoons.

through autumn, first at low abundances (Martínez-López et al. 2007a, Morquecho 2008, Gárate-Lizárraga and González-Armas 2011) and now in bloom proportions in our study. In the upper reaches of Tampa Bay, Florida, a similar scenario was described by Badylak et al. (2007). From spring to fall in 2002, *P. bahamense* was observed in bloom proportions, but previously did not appear in accounts of this bay, which suggests that this species is controlled by long-term trends or that there may be a lack of comprehensive long-term data that represent real population changes. It is likely that blooms in the Gulf of California went unnoticed because they developed in restricted and shallow lagoons with mangrove swamps for short periods during the summer rainy season.

In dry zone mangroves on the eastern side of the Gulf of California, litter fall is greatest during summer

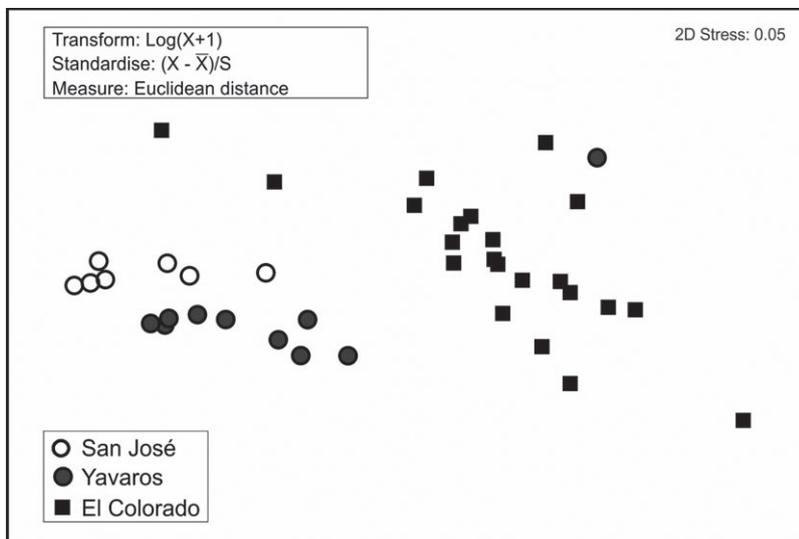


Figure 8 *Pyrodinium bahamense*: nMDS ordination of water quality parameters for the lagoons when peaks occurred. All water quality parameters were included in the multivariate analysis.

rains (Arreola-Lizárraga et al. 2004, Sánchez-Andrés et al. 2010), and recycling of organic phosphorus is probably more efficient, contributing to long-term phosphorus storage through improved binding to fulvic and humic acids (Sánchez-Carrillo et al. 2009, Sánchez-Andrés et al. 2010). *Pyrodinium bahamense* blooms during the summer rainy season, suggesting that organic matter from the mangrove contributes to its population dynamics and that lagoons and mangroves are the most suitable habitat for this dinoflagellate. In Gulf of Mexico lagoons, blooms of the dinoflagellates *Peridinium* aff. *quinquecorne* Abé and *Prorocentrum cordatum* (Ostenfeld) Dodge are associated with high concentrations of dissolved active phenolic substances (tannins and lignins), which are present when maximum litter fall occurs (Aké-Castillo and Vázquez 2008).

Rainfall and seawater quality

In the Gulf of California, moderate abundances of *Pyrodinium bahamense* were found after the onset of the hurricane season and between La Niña and El Niño events (NOAA data at http://www.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ensoyears.shtml). Indeed, peak abundance at Yavaros matched the peak rainfall in September 2008. Rainy season conditions promote *P. bahamense* blooms in the western Pacific; blooms occur during either the northeast or the southwest monsoon periods (MacLean 1989, Usup and Lung 1991). In coastal Florida, the abundance of *P. bahamense* increases during rainfall and decreases during prolonged droughts, responding to periodic climatic cycles (El Niño and La Niña) and stochastic rainfall events (Phlips et al. 2006).

The distribution range of the vegetative stage correlates with the distribution of the resting stage described by Martínez-Hernández and Hernández-Campos in 1991. *Pyrodinium bahamense* blooms were stronger on the eastern side of the Gulf of California, mainly at Laguna El Colorado, where seawater quality was severely degraded, as indicated by AZCI values. Several rivers discharge into the lagoons, mainly during the rainy season, discharging considerable loads of nutrients that favor phytoplankton blooms (Páez-Osuna et al. 2007, Martínez-López et al. 2007b). This is consistent with evidence from our nMDS analysis, where El Colorado lagoon was different from Yavaros and San José, which is attributable both to higher nutrient concentrations and to higher water quality variability. El Colorado has several sites where agricultural water runoff is discharged into the lagoon, resulting in a concentration gradient, whereas in Yavaros there is only

one discharge channel and San José has none. On the western side of the Gulf, creeks discharge water to the gulf mainly under the influence of tropical storms or hurricanes, and this coastal area is also affected by seasonal upwelling. In this area, cysts of *P. bahamense* have very restricted distribution, occurring only in the sediments containing phosphorite, where it is the dominant morphotype (relative frequency of 34%). This suggests that hypersaline and warmer seawater masses affect abundance (Martínez-Hernández and Hernández-Campos 1991). Cysts of *P. bahamense* are common in marine sediments of the Gulf of Tehuantepec (Vásquez-Bedoya et al. 2008, Limoges et al. 2010), in which there are also phosphorite sediments (Morales de la Garza and Carranza-Edwards 1995) and an upwelling zone, which has led to massive toxic blooms (Cortés-Altamirano et al. 1993). In Puerto Rico, *P. bahamense* inhabits several lagoons; the intensity of blooms varies and tends to be higher in lagoons that are reservoirs of bioavailable phosphorus (Pain 2008).

In the Gulf of California, *P. bahamense* was mainly found inside the lagoons, where seawater temperature was highest. Summer temperatures are similar in distant areas with blooms of *P. bahamense*. Laboratory studies of cyst germination and establishment of cultures of isolates from Isla San José (L. Morquecho, R. Alonso-Rodríguez, G. Martínez-Tepacuacho, A. Reyes-Salinas, unpublished data) demonstrate a temperature range of 20°C–35°C for germination and 25°C–30°C for optimal growth of vegetative cells, which is similar to the range of temperature at San José.

Salinity in the Gulf of California fluctuates slightly, with an average of 35 (Lavín et al. 1997). It seems to be an important factor in *P. bahamense* blooms. Our data suggest that the species prefers water with moderate salinity (31.1–46.1), and this is the case for populations in the Central Indo-Pacific (Usup et al. 1989, Corrales et al. 1995) and Caribbean Sea (Seliger et al. 1971, Faust et al. 2005, Soler-Figueroa 2006). Populations from the coast of Florida tolerate a salinity range from 10 to 45 (Phlips et al. 2006). An *in vitro* assay of cysts from San José also showed the euryhaline character of this dinoflagellate, which is able to germinate at salinities from 15 to 35 (L. Morquecho, R. Alonso-Rodríguez, G. Martínez-Tepacuacho, A. Reyes-Salinas, unpublished data).

In our study, peak abundance of *P. bahamense* seemed to be associated with high phosphate and ammonium concentrations, mainly in populations in the eastern lagoons after heavy rains. The lagoons at El Colorado and Yavaros are affected by rainfall, as well as by agriculture, aquaculture, and urban wastewater, and are highly susceptible to eutrophication. The role of phosphorus in

P. bahamense blooms is well supported by the results of Philips et al. (2006) in the Indian River Lagoon after an 8-year study. Most major blooms coincide with the summer rainy season, when phosphate levels increase. Rainfall and phosphorus during blooms are discussed by others (Seliger 1989, Azanza and Miranda 2001, Badylak et al. 2007). Therefore, the bloom potential of *P. bahamense* in the Gulf of California appears to be controlled by limiting nutrients of terrestrial origin and mangrove litter fall, which, in turn, depends on the amount of rainfall and runoff. We postulate that, along the Gulf of California, the habitat of *P. bahamense* is restricted to areas near mangroves and where there is an accumulation of phosphorite sediments and upwelling events occur.

Structure of phytoplankton communities

During our surveys, diatoms predominated among the phytoplankton in the lagoons, particularly when blooms were present, and they occurred with *P. bahamense* at Isla San José. This is very similar to the structure and species composition in Manila Bay (Azanza and Miranda 2001), Indian River Lagoon (Badylak and Philips 2004), and Tampa Bay (Badylak et al. 2007). The diatom assemblage at San José was composed of large diatoms, particularly *Corethron pennatum*, *Skeletonema costatum*, *Pseudonitzschia* cf. *subcurvata*, and *Nitzschia longissima*. In Japan, *S. costatum* only blooms in summer, when water temperature is high enough for growth, and dissolved inorganic phosphorus is plentiful (Shikata et al. 2008). In China, *S. costatum* blooms occur after strong rains at the end of spring (Huo et al. 2001). Most of these factors were also found at San José and Yavaros, supporting the postulate that *S. costatum* may be linked to a succession that favors *P. bahamense*. In laboratory experiments, Lorenzo et al. (2007) found that exudates of *S. costatum* during the exponential and stationary growth phases dissolve organic matter that promotes rapid production of humic material.

Dinoflagellates occurring with *P. bahamense* at San José were also similar to those in coastal areas of the Philippines and the USA, including *Neoceratium furca*, *Akashiwo sanguinea*, *Prorocentrum micans*, *Prorocentrum gracile*, and *Protoperidinium* spp. The mixotrophic and auxotrophic growth capacities of most of these species (Gaines and Elbrächter 1987; Burkholder et al. 2008) suggest that dinoflagellate assemblages in lagoons of the Gulf of California use this advantage to exploit different sources of nutrients and bloom during the summer. Because *P. bahamense* was the dominant species in the

three lagoons, it suggests that its success is related to its ability to absorb phosphate groups more efficiently from many types of molecules; endogenous alkaline phosphatase activity has been detected in this dinoflagellate (González-Gil et al. 1998).

Role of cysts in the dynamics of *Pyrodinium bahamense* blooms

The relative importance of resting cysts of *P. bahamense* in initiating blooms may vary with location (Usup et al. 2012). In our study, cysts of *P. bahamense* were dominant in a moderate-to-low diversity of dinocysts from July through October (data not shown). This pattern is characteristic of unstable coastal environments, suggesting that this species is an opportunistic bloom-forming, r-selected phytoplankton (De Verteuil and Norris 1996). *Pyrodinium bahamense* cyst production at Isla San José seems to be related to the decline of the bloom assemblage, which occurs as temperature falls at the end of summer. From July through October, empty cysts were always abundant and *in situ* cyst production occurred in the most protected areas of the lagoon, being most evident after October. Living cysts were in moderate abundances in superficial sediments at that time, although they were also present, but in very low abundances, from July to September (<204 cysts g⁻¹ sediment). Living cysts were found in the water column, but at very low densities (<200 cysts l⁻¹). This pattern suggests that living cysts decreased in abundance when there were no blooms because they were germinating; however, vegetative cells were unable to develop and spread because adverse conditions prevailed (low temperatures, limited nutrients, and competition with diatoms). This postulate is supported by *in vitro* tests of living cysts collected during October 2008 at Isla San José, where cysts germinate at temperatures from 20°C to 35°C (L. Morquecho, R. Alonso-Rodríguez, G. Martínez-Tepacuacho, A. Reyes-Salinas, unpublished data).

In summary, in the Gulf of California, *P. bahamense* occurs in restricted shallow lagoons that are partly surrounded by mangroves and where environmental factors favor the development of short-term blooms (August–September). Bloom potential and intensity are influenced by the summer rainy season, relatively high seawater temperature (25°C–32°C), typical salinity (31–36), intense sunlight, and relatively high concentrations of ammonium and phosphates, the last one, in turn, depending on rainfall and runoff and seems stronger on the eastern side of the gulf. Living cysts appeared to be more prevalent when blooms declined at the end of summer.

Future investigations should study the morphological, toxicological, and genetic characteristics of *P. bahamense* in populations in the Gulf of California and Gulf of Tehuantepec. To identify the mechanisms underlying *P. bahamense* population dynamics and toxicity, a comparative study of ecosystems needs to be done in these areas.

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