The distribution of *Pyrodinium bahamense* cysts in Old Tampa Bay sediments.

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Introduction

Extensive blooms of the potentially toxic dinoflagellate *Pyrodinium bahamense* have been reoccurring in Old Tampa Bay periodically since 2000 and with increasing frequency and intensity since 2008. This species forms resting cysts which remain dormant in the sediments until water conditions are favorable for a bloom to occur. This study is a follow up to a previous pilot study evaluating the abundance and distribution of *Pyrodinium bahamense* cysts in Old Tampa Bay sediments conducted in May 2010 (Karlen and Miller 2011). Sediment samples were collected at 21 sites from Old Tampa Bay in 2010, including 19 monthly surface water quality monitoring stations sampled by the Environmental Protection Commission of Hillsborough County (EPCHC). The purpose of that study was to:

1. Map the distribution of *Pyrodinium bahamense* cysts in Old Tampa Bay sediments to provide a baseline for predicting future bloom locations.

2. Evaluate the water quality and sediment characteristics which correspond to the distribution of *Pyrodinium bahamense* cysts in Old Tampa Bay.

Monthly *Pyrodinium bahamense* population trends in Old Tampa Bay were also evaluated for a 32 month period (January 2008 through August 2010) from phytoplankton samples collected at the EPCHC monitoring stations. The phytoplankton data were correlated with corresponding water quality data to evaluate possible relationships between *Pyrodinium bahamense* blooms and surface hydrographic conditions and nutrient concentrations.

Sediment samples for the current study were collected during the late fall of 2011 at the same 21 sites that were sampled in May 2010. The purpose of this study was to evaluate changes in the abundance and distribution of *Pyrodinium bahamense* cysts over time and after the most recent bloom event in August 2011. Four additional sites were also sampled to obtain better coverage within Old Tampa Bay (Figure 1).

Pyrodinium bahamense

The dinoflagellate *Pyrodinium bahamense* was first described by Plate in 1906 from samples collected in the Bahamas (Balech 1985). This species is known for its bioluminescence; and coincident to this phenomenon the establishment of several popular tourist destinations in Puerto Rico has resulted (Hernández-Becerril and Navarro 1996). *Pyrodinium bahamense* has been recorded in both the Atlantic and Indo-Pacific oceans and two geographic variants are currently recognized: *Pyrodinium bahamense* var. *bahamense* in the Caribbean and Gulf of Mexico [and more recently reported in the Gulf of California (Martínez-López et al. 2007, Morquecho 2008)] and *Pyrodinium bahamense* var. *compressa* in the Indo-Pacific region (Steidinger et al. 1980, Balech 1985). Both variants are morphologically similar (Balech 1985); however, *P. bahamense* var. *compressa* cells are more anterio-posteriorly flattened and often form chains of multiple cells. In contrast, the Atlantic form, *P. bahamense* var. *bahamense*, is more rounded in form and is not known to form chains of more than two daughter cells. *Pyrodinium bahamense* var. *compressa* is known to be toxic and has been responsible for outbreaks of paralytic shellfish poisoning (PSP) in the Indo-Pacific (Steidinger et al. 1980, Balech 1985, Azanza and Taylor 2001). *Pyrodinium bahamense* var. *bahamense* was until recently thought to be non-toxic (Steidinger and Tangen 1997). Recent evidence however, has linked an outbreak of saxitoxin poisoning from the consumption of puffer fish to *P. bahamense* populations in the Indian River Lagoon where the fish originated (Landsberg et al. 2006).

The life history cycle of *Pyrodinium bahamense* includes several stages (Figure 2):

1. *Pyrodinium bahamense* exists as a thecate, free-swimming cell during the summer months. This stage is photosynthetic. These cells will reproduce by fission (mitosis), resulting in temporary chains of two joined daughter cells referred to as a couplet stage (Buchanan 1968).
2. When conditions become unfavorable, the cell protoplast will detach from the cell wall and emerge from the theca. The emergence of the protoplasm from the original *Pyrodinium* stage occurs via either the theca splitting along the middle girdle region, or by splitting of individual thecal plates (Buchanan 1968).

3. The protoplasm enters the “Gymnodinioid” stage forming a motile, avalvate cell (Buchanan 1968; Wall and Dale 1969).
4. The cyst stage is formed when the avalvate protoplasm contracts into a sphere and forms a translucent wall. Cysts may also form directly within the theca of the *Pyrodinium* stage, bypassing the avalvate state (Buchanan 1968). The cyst stage is non-motile and accumulates in the bottom sediments. The individual cysts are spheroid with diameters of 43 – 55 μm and covered with irregularly spaced spines that are 6 - 12 μm long and have asymmetrical lobed tips (Figure 2; Wall and Dale 1968). The interior of the cyst contains the cell protoplasm and is characterized by accumulations of starch grains and a conspicuous red pigment spot (“eyespot”) which is possibly photosensitive (Figure 2; Wall and Dale 1969).
5. Excystment occurs when environmental conditions such as temperature and salinity are favorable. The protoplasm detaches and exits the cyst through an opening in the cyst wall referred to as the archeopyle.
6. The excysted protoplasm returns to the avalvate “Gymnodinioid” stage.
7. Thin thecal plates develop within 12 hours after exiting the cyst. This process is referred to as the prevalvate stage (Wall and Dale 1969).
8. The thecal plates thicken as the cell develops back into the free-swimming, thecate stage (1).

Pyrodinium bahamense var. *bahamense* has been known to occur on both coasts of Florida and in the Gulf of Mexico historically (Steidinger et al. 1967; Steidinger and Williams 1970; Licea et al. 2004; Badylak and Phlips 2009) but records of its past occurrence in Tampa Bay have been sparse. Early phytoplankton surveys of Tampa Bay conducted in the 1970’s did not record *P. bahamense* (Turner 1972; Turner and Hopkins 1974). The first recorded occurrences of *P. bahamense* at the EPCHC monitoring sites were in August 1975, primarily in the northwest side of Old Tampa Bay. This was followed by a less extensive bloom in August 1977 and a single record in September 1983, also in the same part of Old Tampa Bay. *Pyrodinium bahamens*e was not recorded in the EPCHC water monitoring database between September 1983 and October 2000 (Boler n.d.). There have been several periodic blooms recorded in Old Tampa Bay since 2000 (Figure 3) (Badylak et al. 2007; Badylak and Phlips

2008, Karlen 2012). This trend appears to be increasing in frequency and intensity in recent years, with blooms reoccurring during the summers of 2008, 2009 and 2011 (Figure 3).

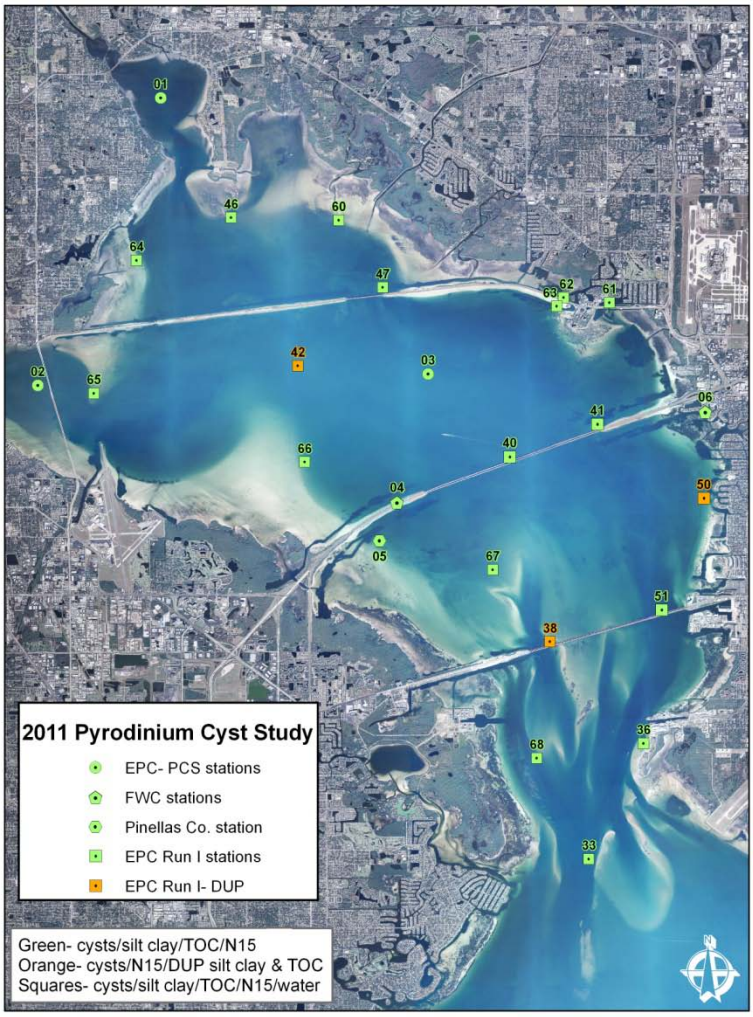

Figure 1 Sediment and water quality sampling stations in Old Tampa Bay. Monthly EPCHC water monitoring stations represented by green squares, additional sampling sites for this study are represented by circles, pentagons (FWC stations) or hexagons (Pinellas Co. stations). Sites represented by orange colored symbols had duplicate sediment samples taken for QA/QC on silt/clay and TOC analysis.

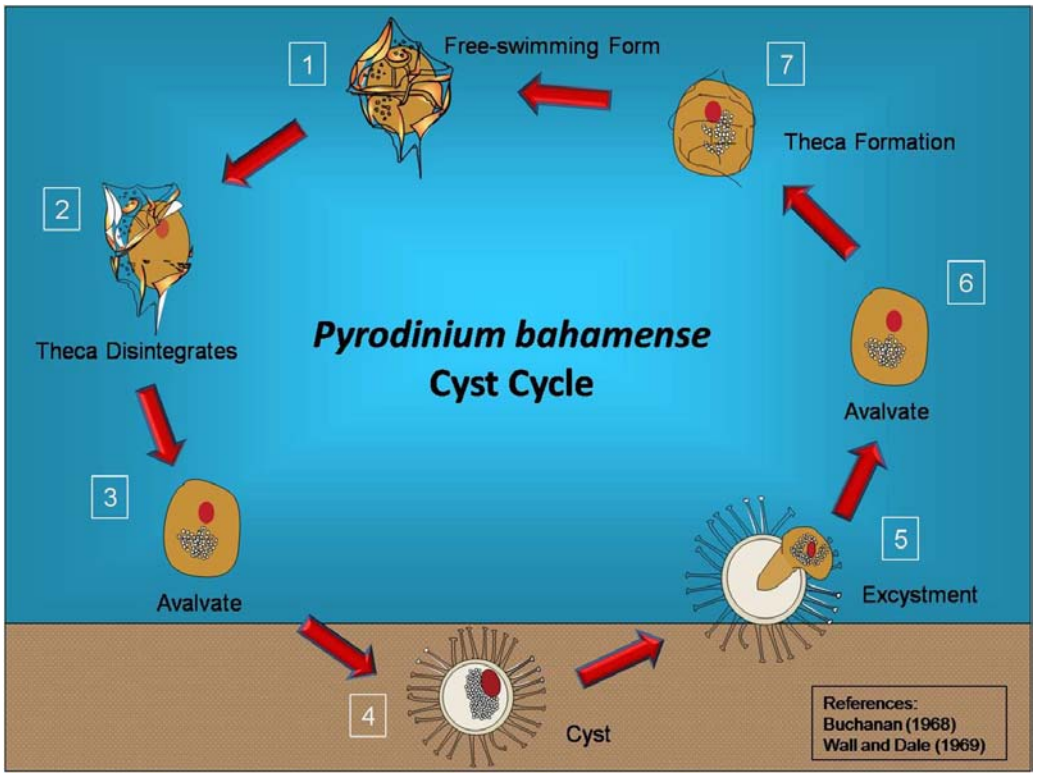

Figure 2 *Pyrodinium bahamense* cyst cycle (From Karlen and Miller 2011).

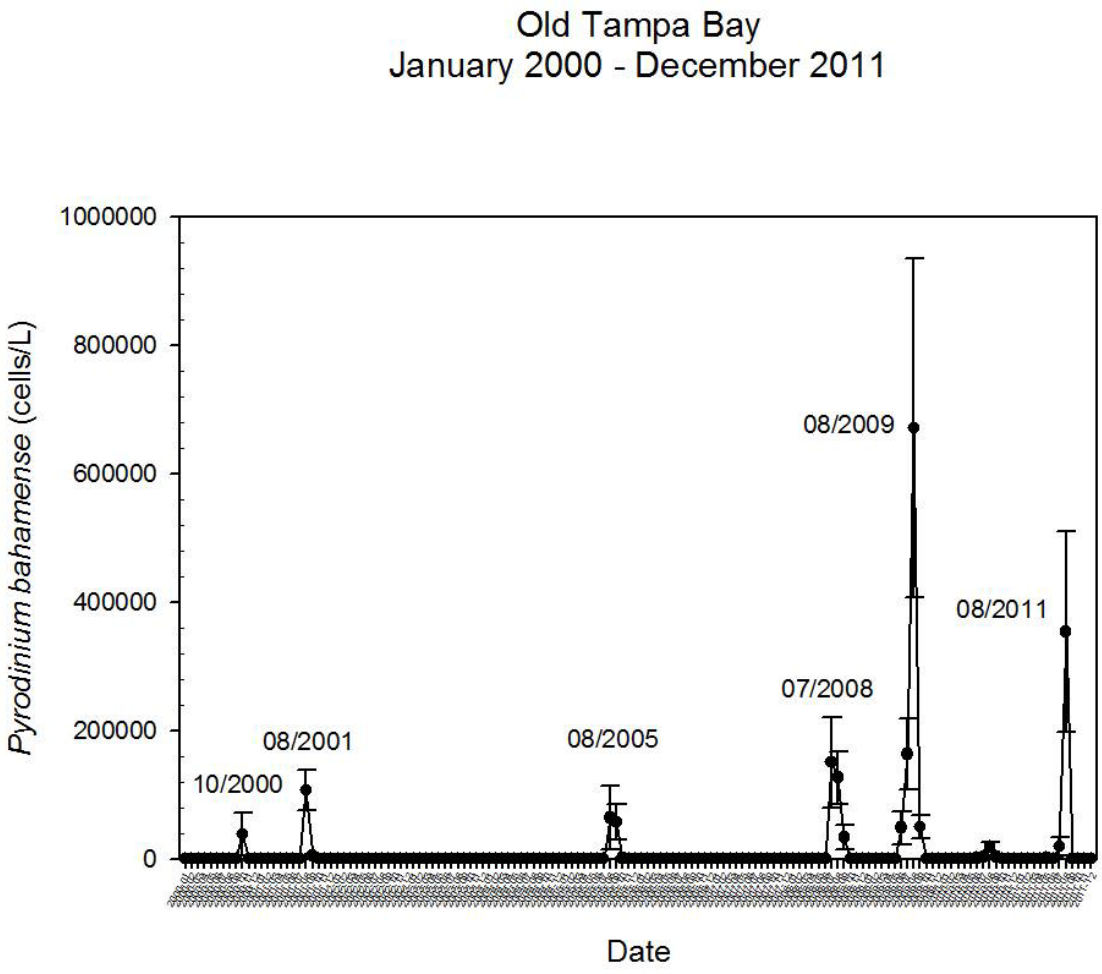

Figure 3 Trends in *Pyrodinium bahamense* cell counts (#/0.1 ml) at EPCHC Old Tampa Bay monitoring sites from January 2000 - December 2011.

Methods

Sample collection:

Sediment samples were collected on four dates during November and December 2011: 14 November (7 sites), 5 December (5 sites), 20 December (9 sites), and 23 December (4 sites)]. The samples were collected at the same 21 sites from the 2010 study which included the 19 EPCHC monthly surface water quality monitoring stations in Old Tampa Bay plus two additional sites; one north of the Courtney Campbell Causeway in the vicinity of Safety Harbor (site PCS01) and one west of the Bayside Bridge in the vicinity of the Clearwater WWTP discharge (site PCS02). Four additional sites were added for the current study including one near the central bay between the Howard Frankland Bridge and Courtney Campbell Causeway (site PCS03), two corresponding to past Florida Fish and Wildlife Conservation Commission sampling locations near the Howard Frankland Bridge (sites PCS04 and PCS06) and one corresponding to a Pinellas County Department of Environmental Management monitoring site (PCS05). Sediments were collected at each site using a Young grab sampler. The top 1cm sediment layer was removed from the grab and homogenized in a stainless steel beaker. The homogenized sediment was split into three subsamples. One subsample was used for the dinoflagellate cyst extraction procedure, one for stable isotope analysis and one for sediment composition analysis (% silt/clay and organic carbon content). Each subsample was placed in a pre-cleaned HDPE bottle (for cyst extraction and sediment composition) or a pre-cleaned glass sample jar (for stable isotope analysis) and refrigerated until laboratory processing.

Cyst extraction and counting:

The procedures used for the extraction of the dinoflagellate cysts from the sediments were adapted from the "Modified Dinoflagellate Cyst Isolation Protocol" provided by the FWC Florida Wildlife Research Institute (Appendix A). The following methods were used in this study:

1. A 100g wet weight sample of sediment for sandy samples or 50g wet weight of sediment for silty samples was used for the cyst extraction.

2. The weighed sediment was placed in a 1000 ml beaker and artificial seawater (23 psu) was added for a final volume of 200 ml. The beaker was agitated at 100 – 150 rpms for 15 minutes to suspend the cysts.
3. The beakers were wrapped in aluminum foil to prevent light exposure and the suspended sediment was allowed to settle overnight.
4. The settled flocculent containing the cysts was removed from the sediment using a wide-mouth pipette and transferred to a 100 ml beaker.
5. The 100 ml beaker containing the flocculent was placed in a larger beaker (250 ml) of cold water and sonicated for 10 minutes using a Bronson 2200 sonicator.
6. The sonicated flocculent was rinsed through a 250 μm sieve with artificial seawater. The filtrate was collected in a 150 ml beaker and the collected sediment > 250 μm was discarded.
7. The 250 μm filtrate was rinsed through a 90 μm sieve. The resulting filtrate was collected in a 150 ml beaker and the collected sediment > 90 μm was discarded.
8. The 90 μm filtrate was rinsed through a 20 μm sieve. The collected sediment was backwashed into a 100 ml beaker and the resulting filtrate was discarded.
9. The collected flocculent from the 20 μm sieve was brought up to a volume of approximately 40 ml with artificial seawater.
10. The flocculent/artificial seawater was mixed thoroughly with a wide-mouth disposable pipette and 40 ml were transferred to 4 clean 15ml conical centrifuge tubes (10 ml per tube).
11. A 4 ml aliquot of Sodium Polytungstate (SPT) was carefully added to the bottom of each centrifuge tube (under the flocculent sample) using a long stemmed Pasture pipette.
12. The samples were centrifuged for 10 minutes at 3250 rpm using an Eppendorf 5810 centrifuge.

13. The top 10 ml of each centrifuged sample was carefully pipetted into a clean centrifuge tube.
14. The samples were then centrifuged for 3 minutes at 2570 rpm.
15. The top 9 ml of each centrifuged sample was removed and discarded and the remaining 1 ml from each tube was placed in a cell culture dish.

The cyst extraction procedure resulted in four 1 ml aliquots for each sample. The four aliquots were combined in a cell culture dish. Culture dishes were wrapped in aluminum foil to prevent light exposure and stored at 4°C. Cysts were counted using an inverted compound microscope at 100x magnification. Final cyst count data was standardized as number of cysts per gram of sediment.

Sediment Analysis:

The percent silt+clay (%SC) was measured following Tampa Bay Benthic Monitoring program protocols (Versar 1993). Sediments categories were estimated from percent silt+clay measurements following the methods outlined in Karlen et al. (2008) and based on the Wentworth size class system. Sediment categories were defined as: Coarse (%SC <1.70); Medium (%SC = 1.70 – 4.51); Fine (%SC = 4.51 – 11.35); Very Fine (%SC = 11.35 – 25.95); and Mud (%SC > 25.95).

The sediment total organic carbon (TOC) content was measured using a Shimadzu TOC-VCPH instrument equipped with a solid sample module, SSM-500A and a non-dispersive infrared detector (NDIR). Stable isotope analysis was conducted by the University of South Florida, Department of Marine Science following the procedures outlined in Peebles et al. (2010).

Data Analysis and Reporting:

Group-average cluster analysis (Bray-Curtis similarity) was done on square root transformed cyst count data using PRIMER v. 6 in order to group the sites based on their *Pyrodinium bahamense* cyst densities. Maps of the cyst abundance and distribution and sediment

characteristics were generated using Arc-GIS and graphing and statistical analysis were done using SigmaPlot 10/SigmaStat 3.5. Basic summary statistics were calculated using SYSTAT 12.

Results

Sediment characteristics:

The sediment percent silt+clay (%SC) and total organic carbon (TOC) results are summarized in Table 1. The %SC content at the 25 sites ranged from 1.5 – 84.5% with a median value of 3.3%. Three sites in particular had extremely high values. Site 11PCS61 located in an enclosed embayment on the northeast side of the Courtney Campbell Causeway near Sweetwater Creek had the highest measured %SC content (84.5 %). Sites 11PCS01 in Safety Harbor and 11PCS02 west of the Bayside Bridge had %SC values of 56.6% and 76.7% respectively. Overall, the sediments were predominantly medium grained sands and the %SC content decreased from north to south in the bay (Figure 4A).

The total organic carbon content at the Old Tampa Bay sites was generally low with a median value of 0.3% and ranging 0.3 – 6.5%. The TOC was strongly correlated with the %SC ($\rho = 0.99$; Pearson correlation on untransformed data) with the highest measurement occurring at 11PCS61. As with the %SC content, the TOC showed a decreasing trend from north to south in Old Tampa Bay (Figure 4B).

Results for the stable isotope analysis were not available at the time of this report and will be presented as a later addendum.

***Pyrodinium bahamense* cyst density and distribution:**

Pyrodinium bahamense cysts were found at all 25 sites. Densities ranged from 0.4 cysts/gram of sediment at site 11PCS38 on the northwest side of the Gandy Bridge to 2,236.3 cysts/gram at site 11PCS03 between the Howard Frankland Bridge and Courtney Campbell Causeway (Table 1; Figure 5). The distribution of *Pyrodinium bahamense* cysts in Old Tampa Bay sediments was highest in the north central area of the bay south of the Courtney Campbell

Causeway (sites 11PCS42 and 11PCS03) and at several sites along the eastern shore of Old Tampa Bay (11PCS06 and 11PCS36). The cluster analysis assembled the sites into four distinct groups based on the Bray Curtis similarity of square root transformed cyst densities (Figure 6). These are designated as groups “a”, “b”, “c”, and “d” in Figures 6 – 9 and Table 2.

The “a” group consisted of the three sites which had the lowest cyst densities (median = 0.7 cysts/gram) and were characterized by sediments with low %SC and TOC (Table 2; Figures 7-9). The “b” group was comprised of six sites with relatively low cyst densities (median = 4.5 cysts/gram; Table 2) (Figure 7). The sediments at the “b” sites had a median %SC content of 2.1% and TOC of 0.3% (Table 2). This group however did also include the site with the highest %SC and TOC values which accounts for the high mean values and standard deviations in Figures 8 and 9. Group “c” was composed of the four sites with the highest cyst densities (median = 1,206 cysts/gram) (Table 2; Figure 7). The group “c” sites had the highest median %SC and TOC values of 10.3% and 0.7% respectively (Table 2) although the average values were lower than for groups “b” and “d” (Figures 8 & 9). Group “d” encompassed the remaining 12 sampling sites with relatively high *Pyrodinium* cyst densities (median = 129 cysts/g; Table 2). The sediments at the group “d” sites had relatively high %SC contents (median = 4.2%) and included two of the three sites with exceedingly high %SC which resulted in the elevated mean values and large standard deviations reflected in Figures 8 and 9.

The cyst density did show a positive correlation ($\rho = 0.32$) with %SC and was highest at sites with very fine grained sediments (Figure 10). This trend however did not hold for the “Mud” sites which exhibited lower cyst densities (Figure 10).

Table 1 Sampling site locations and sediment characteristics

Site	Latitude	Longitude	Group	*P. bahamense* cysts/g	% Silt+clay	% TOC
11PCS01	28.02005	-82.67678	d	183.2	56.6	3.6
11PCS02	27.94743	-82.70850	d	88.8	76.7	5.3
11PCS03	27.95035	-82.60850	c	2236.3	14.1	0.8
11PCS04	27.91750	-82.61620	b	14.7	1.5	0.3
11PCS05	27.90761	-82.62125	d	169.7	2.8	0.3
11PCS06	27.94110	-82.53790	c	929.8	7.4	0.6
11PCS33	27.82610	-82.56750	a	0.7	1.7	0.3
11PCS36	27.85580	-82.55330	c	936.4	6.7	0.6
11PCS38	27.88180	-82.57750	a	0.4	1.8	0.3
11PCS40	27.92910	-82.58730	d	213.2	4.9	0.3
11PCS41	27.93740	-82.56500	d	115.6	2.2	0.3
11PCS42	27.95280	-82.64160	c	1475.7	13.1	0.9
11PCS46	27.99040	-82.65930	d	142.3	10.1	0.6
11PCS47	27.97260	-82.62020	d	72.7	3.3	0.3
11PCS50	27.91850	-82.53790	d	153.1	2.4	0.3
11PCS51	27.89020	-82.54880	b	2.1	2.0	0.3
11PCS60	27.98990	-82.63160	d	70.3	4.4	0.4
11PCS61	27.96870	-82.56210	b	3.4	84.5	6.5
11PCS62	27.97000	-82.57430	b	4.0	3.1	0.3
11PCS63	27.96760	-82.57600	d	251.9	2.3	0.3
11PCS64	27.97940	-82.68330	a	1.1	1.7	0.3
11PCS65	27.94560	-82.69430	b	5.1	2.1	0.3
11PCS66	27.92780	-82.63970	d	53.3	4.0	0.4
11PCS67	27.90020	-82.59200	d	60.9	9.0	0.3
11PCS68	27.85190	-82.58080	b	17.9	2.0	0.3
Minimum				0.4	1.5	0.3
Maximum				2236.3	84.5	6.5
Median				72.7	3.3	0.3
Mean				288.1	12.8	1.0

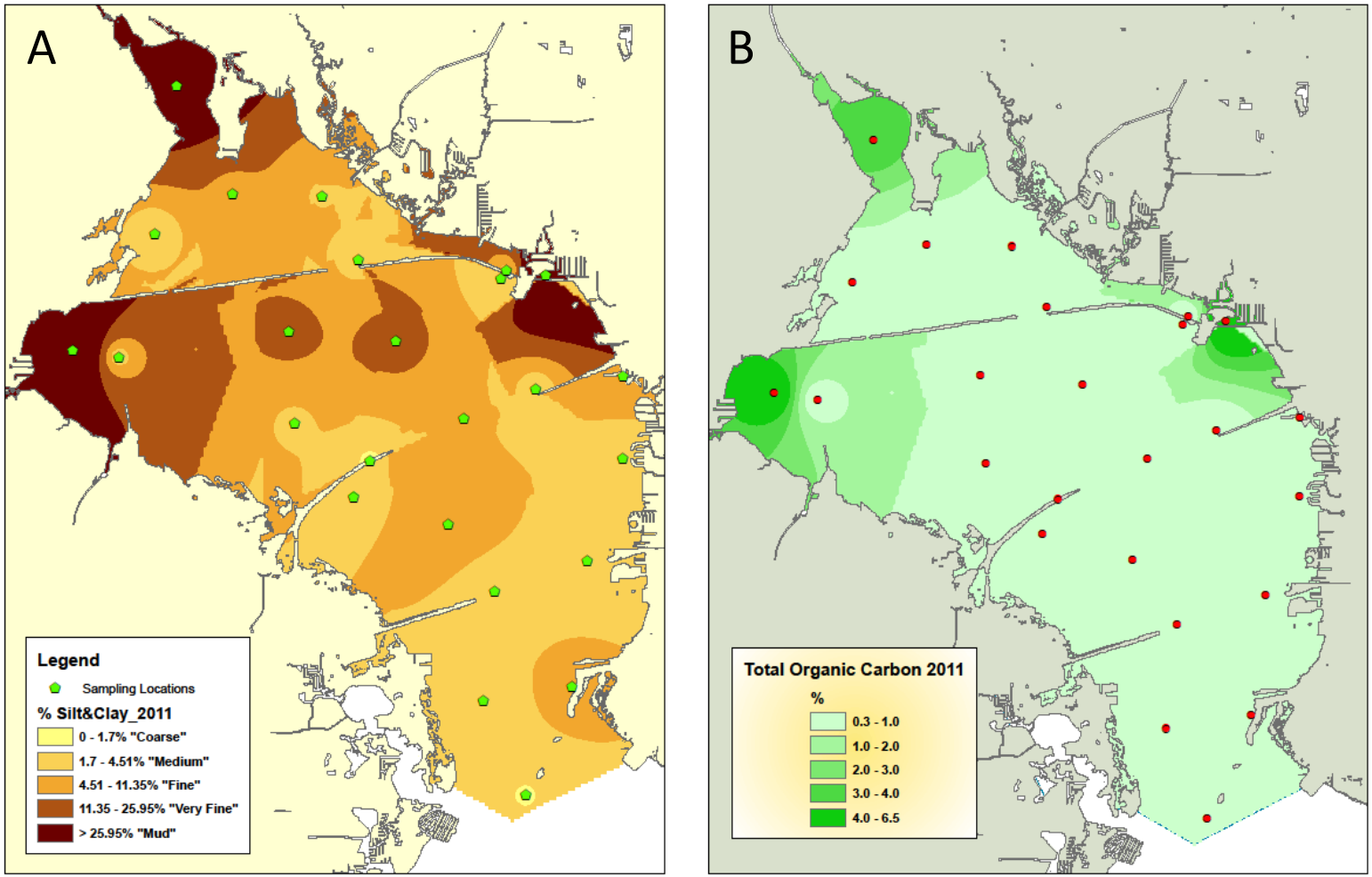

Figure 4 (A) Distribution of sediment type based on % silt/clay content and (B) sediment total organic carbon as % TOC in Old Tampa Bay.

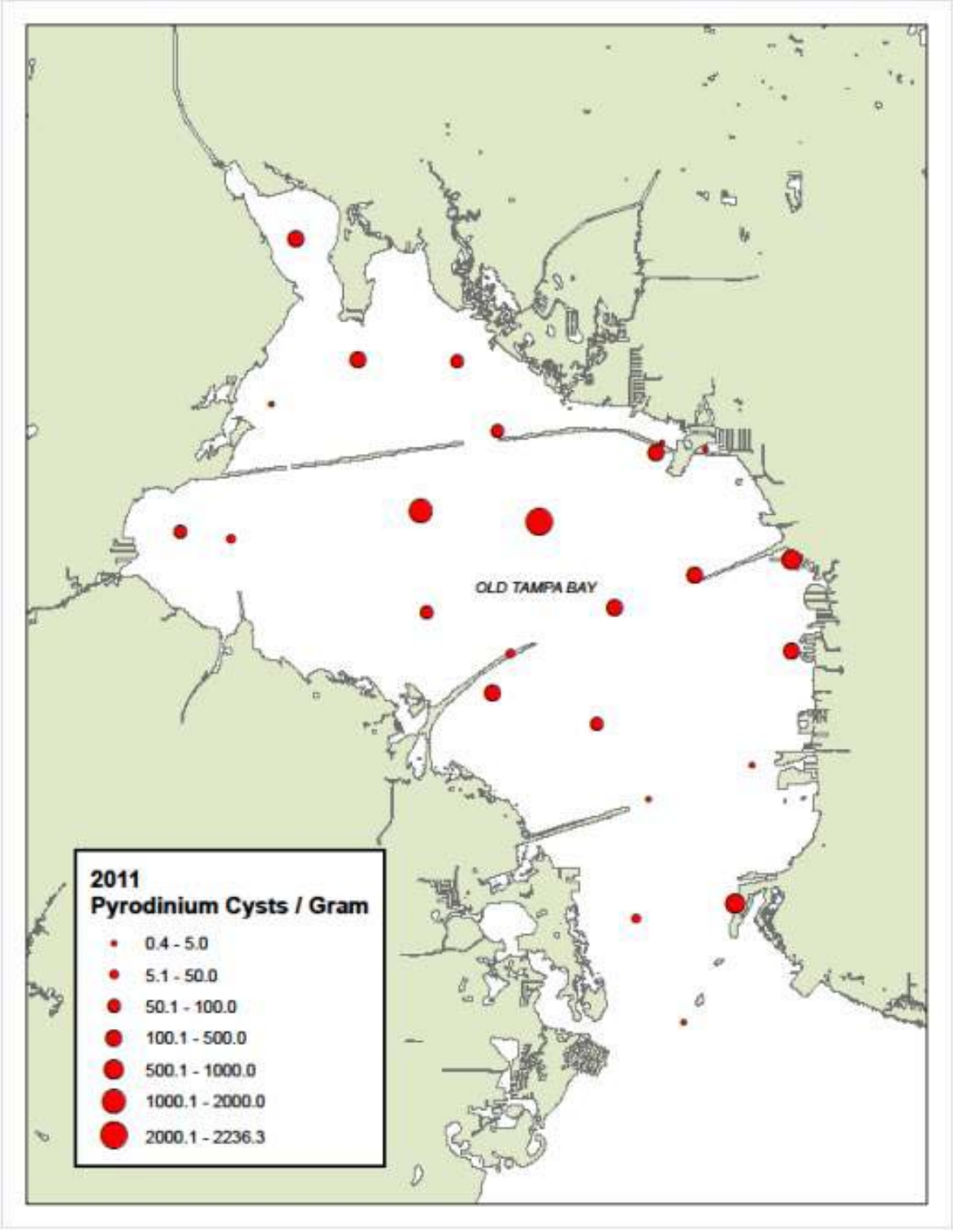

Figure 5: Distribution and density of *Pyrodinium bahamense* cysts in Old Tampa Bay.

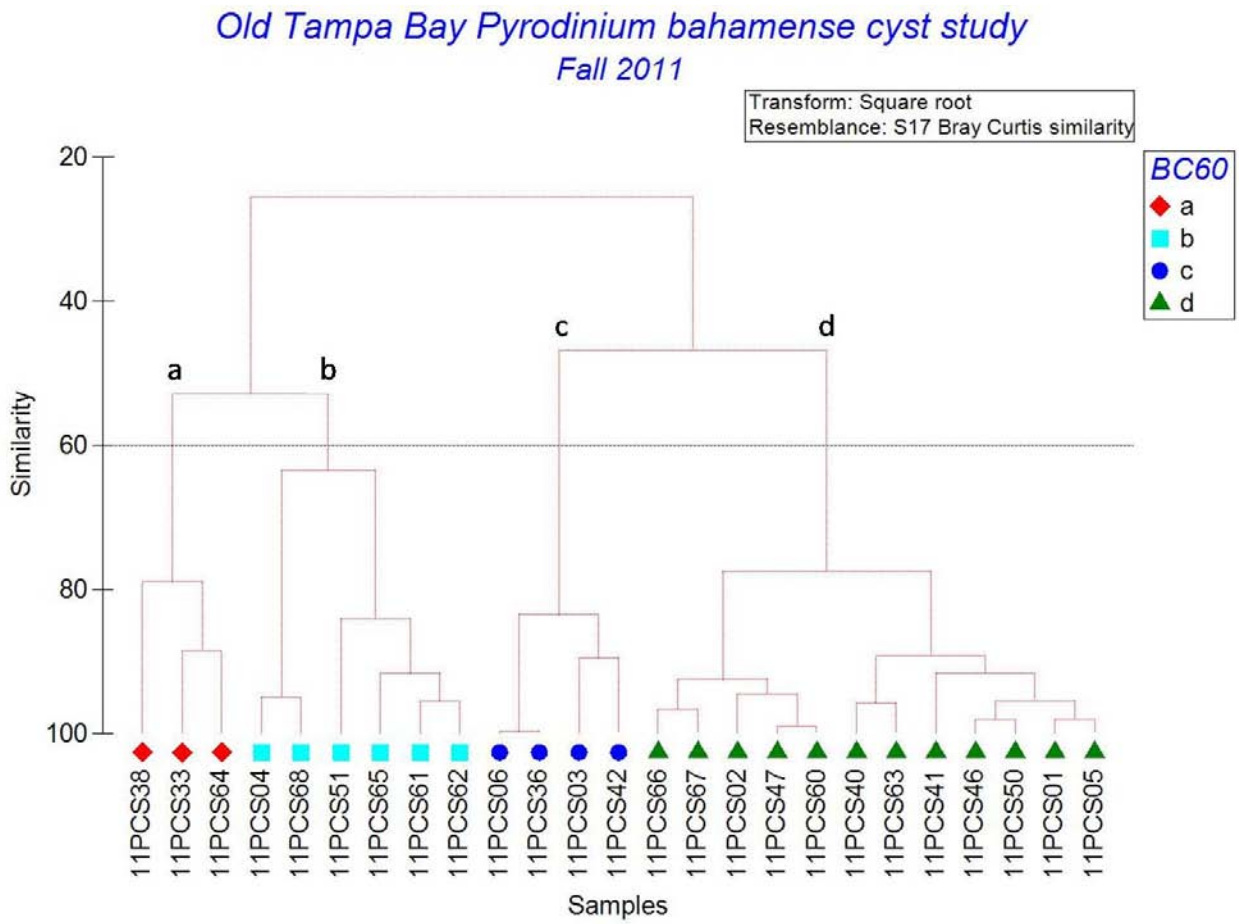

Figure 6 Cluster analysis based on Bray Curtis similarity of square root transformed *Pyrodinium bahamense* cyst counts. Dotted line represents Bray Curtis similarity value of 60.

Table 2 Median (top), minimum (lower left) and maximum (lower right) cyst and sediment values by similarity groups; n = number of sites within group.

	a (n = 3)		b (n = 6)		c (n = 4)		d (n = 12)	
Pyrodinium cysts/gram	0.7		4.5		1206.0		129.0	
	0.4	1.1	2.1	17.9	929.8	2236.3	53.3	251.9
Total cysts/gram	1.7		11.2		1252.0		136.3	
	0.7	1.9	4.6	26.9	929.8	2274.3	56.0	253.0
Silt+clay (%)	1.7		2.1		10.3		4.2	
	1.7	1.8	1.5	84.5	6.7	14.1	2.2	76.7
Total Organic Carbon (%)	0.3		0.3		0.7		0.3	
	0.3	0.3	0.3	6.5	0.6	0.9	0.3	5.3

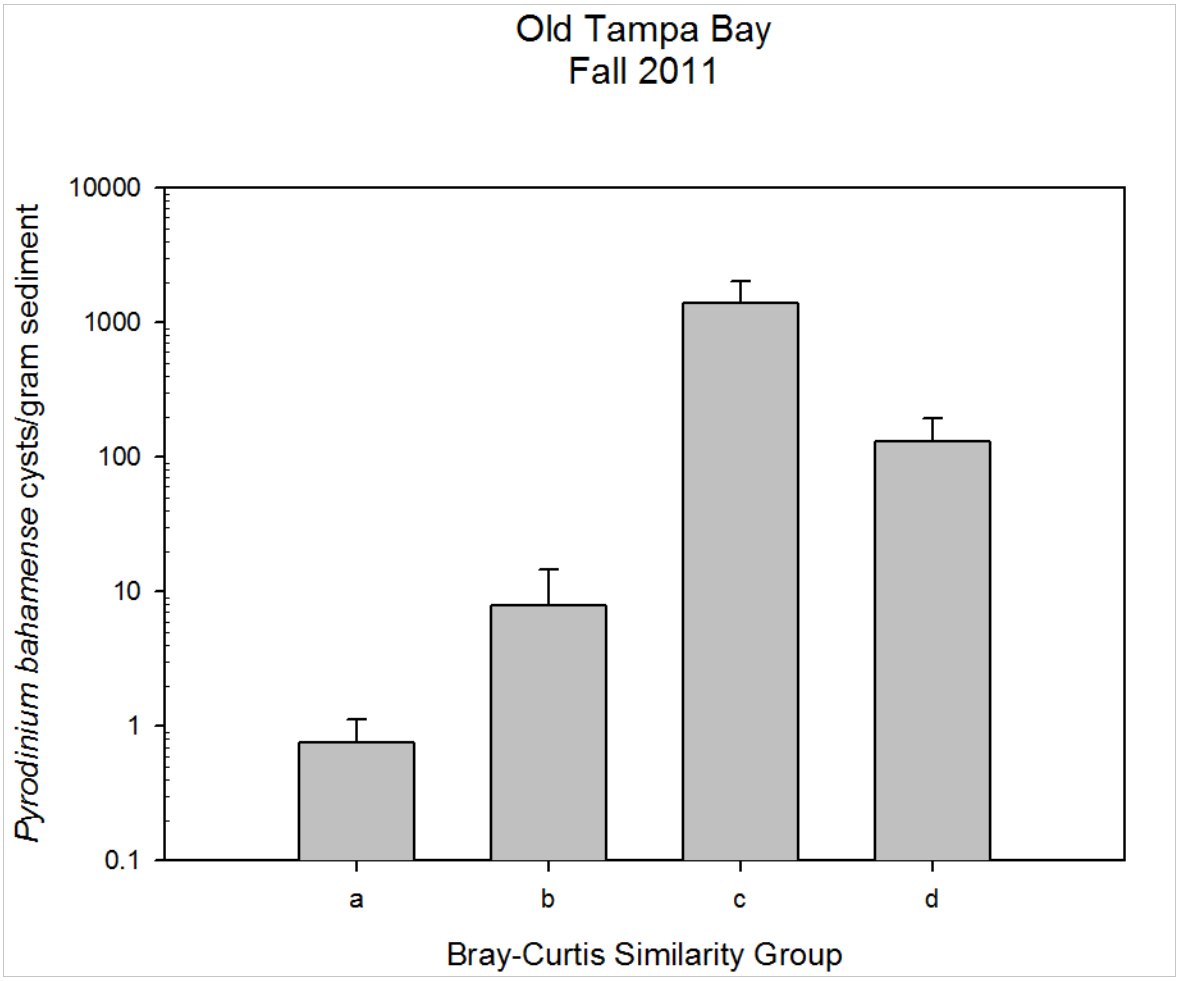

Figure 7. Mean density of *Pyrodinium bahamense* cysts by Bray-Curtis similarity group. Error bars represent 1 standard deviation about the mean.

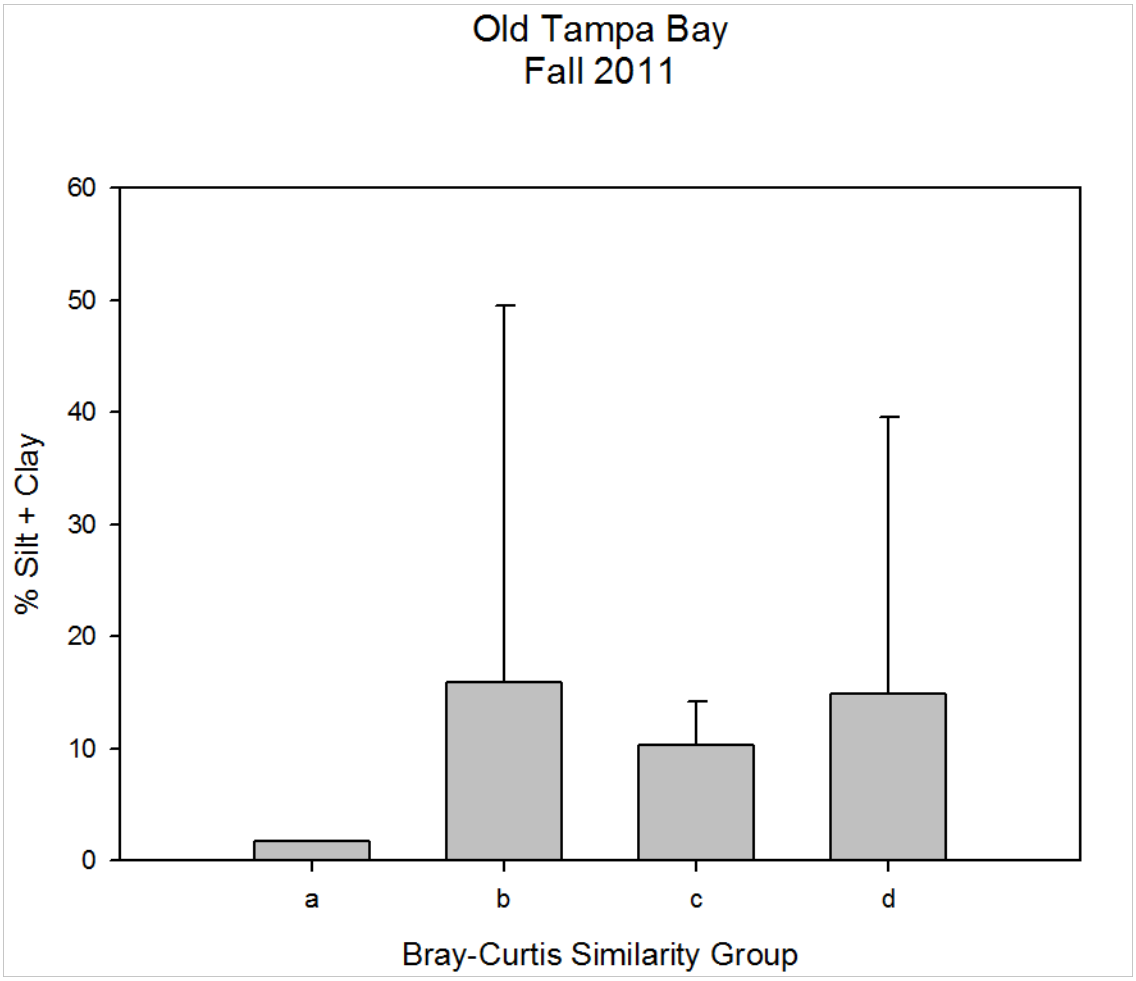

Figure 8. Mean percent silt+clay by Bray-Curtis similarity group. Error bars represent 1 standard deviation about the mean.

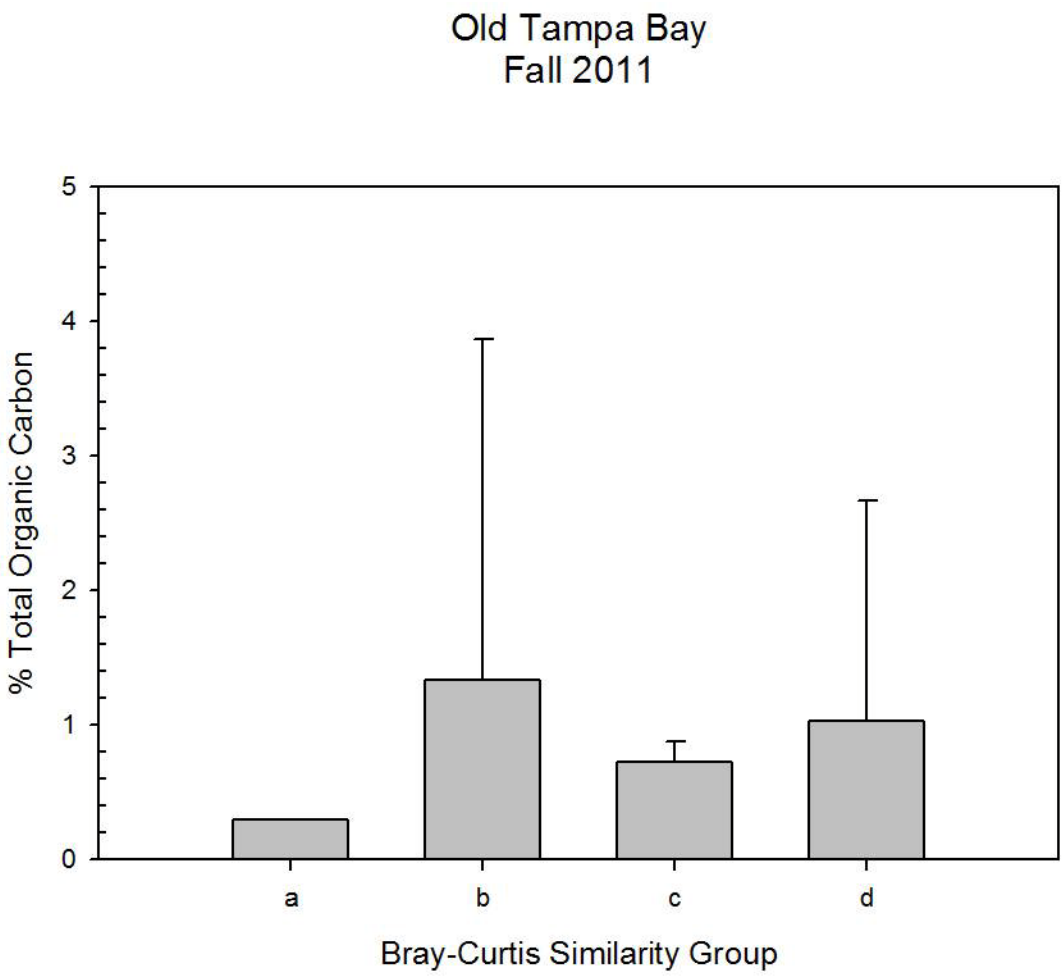

Figure 9. Mean TOC by Bray-Curtis similarity group. Error bars represent 1 standard deviation about the mean.

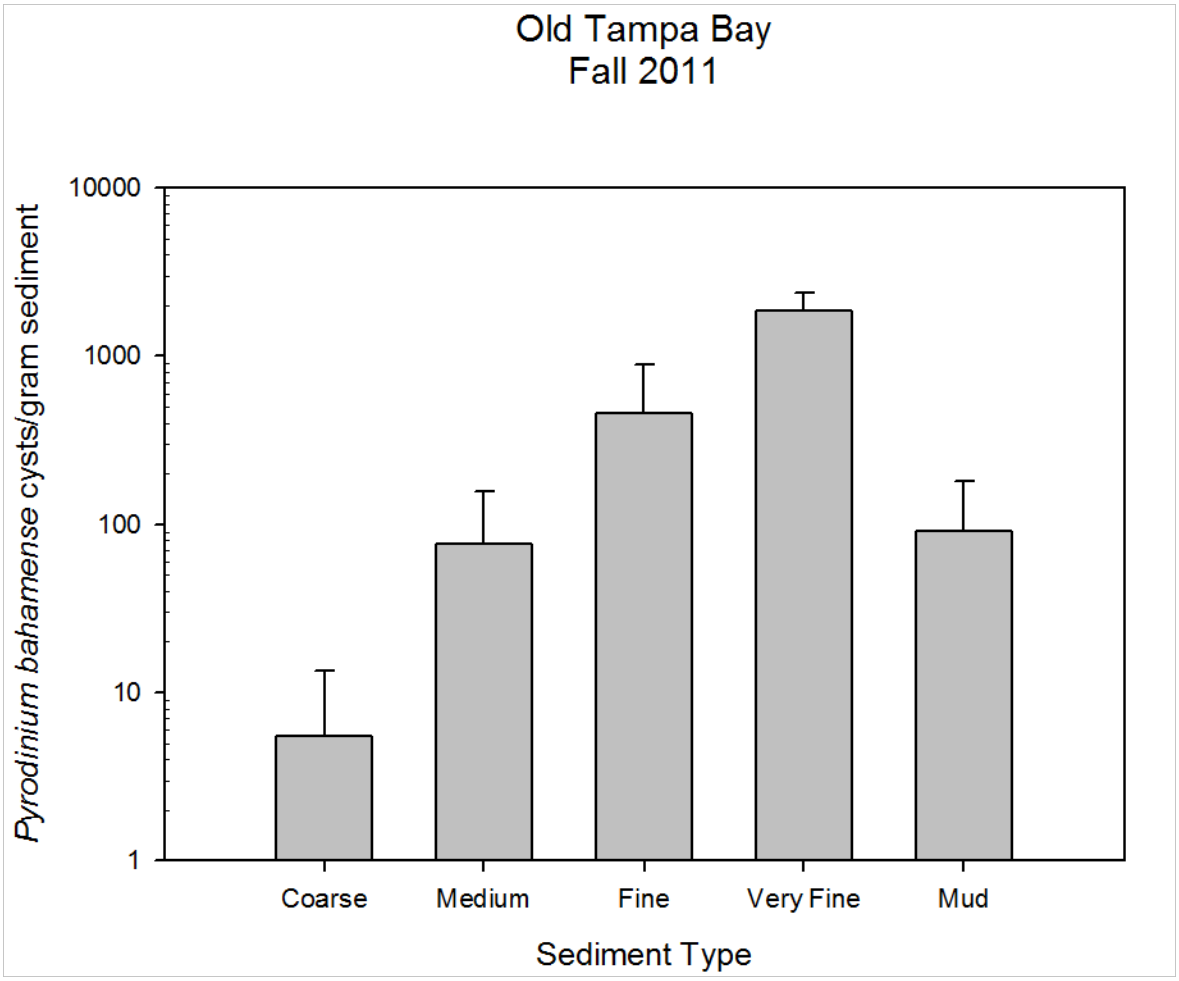

Figure 10. Mean density of *Pyrodinium bahamense* cysts by sediment type. Error bars represent 1 standard deviation about the mean.

Comparison with 2010 study results:

The density of *Pyrodinium bahamense* cysts in Old Tampa Bay was significantly higher in the fall of 2011 compared to the spring of 2010 ($p<0.001$; Mann-Whitney) -- by as much as two orders of magnitude (Figure 11). The 21 sites which were sampled in both studies all had higher cyst densities in the fall 2011 than in the spring 2010 with the exception of site 65 near the Bayside Bridge.

The distribution of cysts within Old Tampa Bay also shifted between years (Figure 12). The spring 2010 study had the highest cyst densities in the northwestern area of Old Tampa Bay which included Safety Harbor (PCS01) and in the vicinity of the Bayside Bridge (PCS 02 and 65). Cysts densities were very low or completely absent at sites south of the Gandy Bridge. The highest cyst densities observed in the current study were in the north central areas just south of the Courtney Campbell Causeway at sites 11PCS42 and 11PCS03 as well as at several sites along the eastern shoreline which included 11PCS06 near the southeast side of the Howard Frankland Bridge and 11PCS36 near Picnic Island, south of the Gandy Bridge.

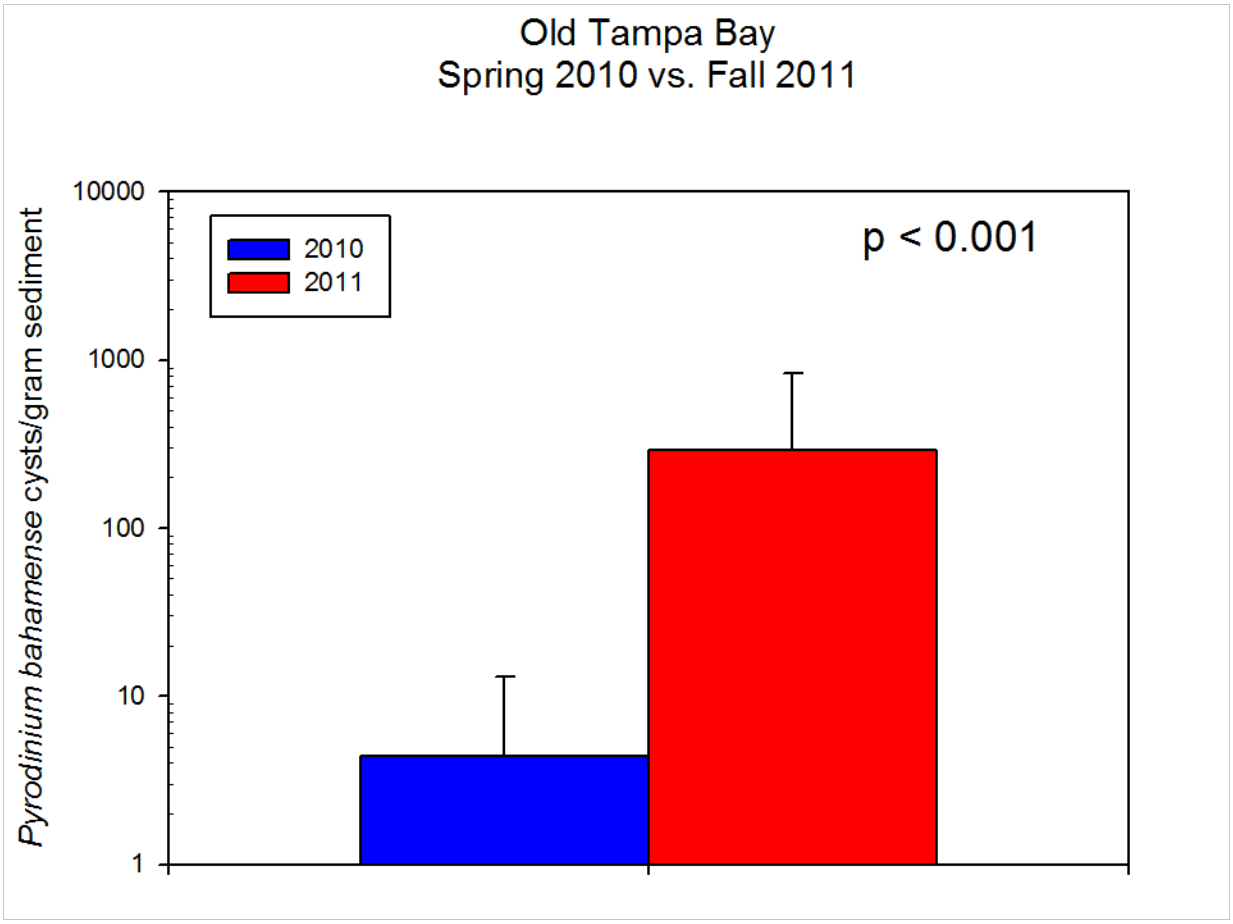

Figure 11 Mean number of *Pyrodinium bahamense* cysts/gram of sediment reported in spring 2010 and fall 2011.

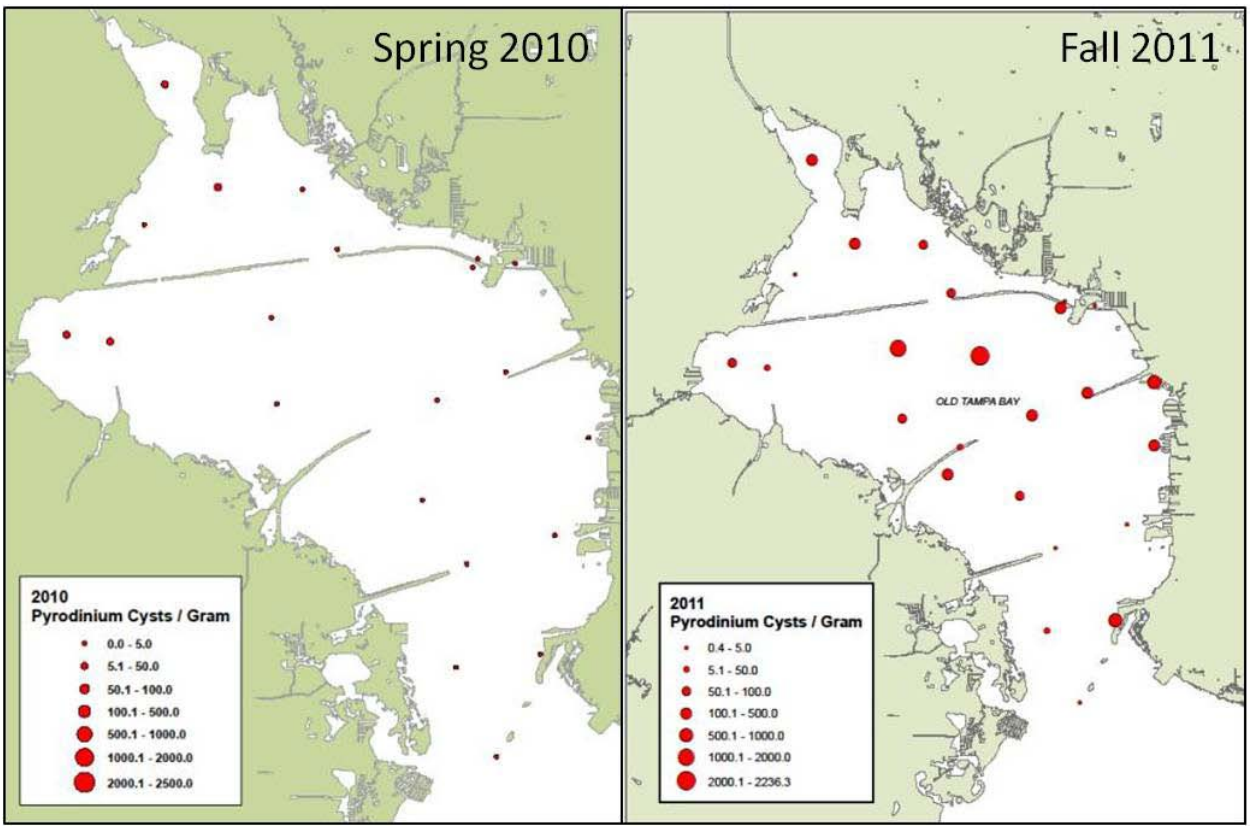

Figure 12 *Pyrodinium bahamense* cyst density and distributions from spring 2010 and fall 2011.

Trends in <u>Pyrodinium</u> <u>bahamense</u> blooms in Old Tampa Bay: 2000-2011:

Pyrodinium bahamense has been recorded at EPCHC monitoring sites during seven years since 2000 and only during the months of May – October (Figure 13). The first occurrence was in October 2000 with highest cell counts at station 65 on the west central side of Old Tampa Bay near the Bayside Bridge (Figure 13). The next records were in July and September 2001 with low cell counts present at most sites north of the Gandy Bridge. *P. bahamense* was absent from the EPC monitoring sites during 2002-2004. The next occurrence was in August – October 2005 followed by another two-year absence in 2006-2007 (Figure 13).

Pyrodinium bahamense has been recorded every summer from 2008-2011. A bloom was first observed in July 2008 with highest concentrations again at station 65 (Figure 13; 14A) and cells were recorded throughout Old Tampa Bay in August and September of that year (Figure 13; 14 B and 14 C). Low densities of *P. bahamense* were first observed in June 2009 at most sites

north of the Gandy Bridge with the highest concentrations at station 65 and 46 north of the Courtney Campbell Causeway (Figure 13; 15A). The cell densities increased in July 2009, particularly at sites 65, 42 and 43 north of the Howard Frankland Bridge (Figure 15B). The bloom peaked in August 2009 with the highest records at stations 42 and 63 between the Howard Frankland Bridge and south of the Courtney Campbell Causeway (Figure 15C) then reduced to low concentrations by September 2009 but extended throughout Old Tampa Bay (Figure 15D). *Pyrodinium bahamense* was detected from June – September in 2010 but below bloom concentrations (Figure 16A-D). The first record of *P. bahamense* in 2011 was in May along the east central area of Old Tampa Bay (Figure 13; 17A). This shifted to the west central area in June 2011 and then throughout Old Tampa Bay in low numbers by July 2011 (Figure 13; 17B and 17C). The bloom peaked in August 2011 with highest concentrations found at station 42 and station 67 between the Gandy and Howard Frankland bridges (Figure 13; 17D). The bloom conditions apparently subsided by the end of August 2011 and no *P. bahamense* cells were observed in September 2011 samples (Figure 13).

The *Pyrodinium bahamense* cell counts appear to be strongly correlated with surface water temperature over time (Figure 18A). Highest cell counts corresponded to summer peaks in surface water temperatures in July 2008 and in August 2009 and 2011, with apparent optimal temperatures for peak cell counts at about 30°C (Figure 18B). There was also an apparent relationship between peak *Pyrodinium bahamense* counts and decreasing surface salinity over time with peak cell counts occurring when the surface salinity dropped to around 25 psu (Figure 19A and 19B).

The BIO-ENV analysis (Table 3) indicated the strongest correlation with *Pyrodinium bahamense* abundance during the 2008-2011 time period was with the combination of surface pH and Ortho-phosphate (Spearman $\rho = 0.367$). The strongest single factor correlation was with ortho-phosphate (Spearman $\rho = 0.325$). Stepwise (backward) multiple regression analysis also showed a significant relationship between *P. bahamense* cell counts and ortho-phosphate and surface pH as well as with surface temperature, surface dissolved oxygen and Kjeldahl nitrogen. Simple Pearson correlations also show a similar relationship with these parameters with highly

significant ($p<0.001$) positive correlations found with ortho-phosphate ($\rho = 0.502$), pH ($\rho = 0.394$), temperature ($\rho=0.317$) and Kjeldahl nitrogen ($\rho=0.235$) (Table 4).

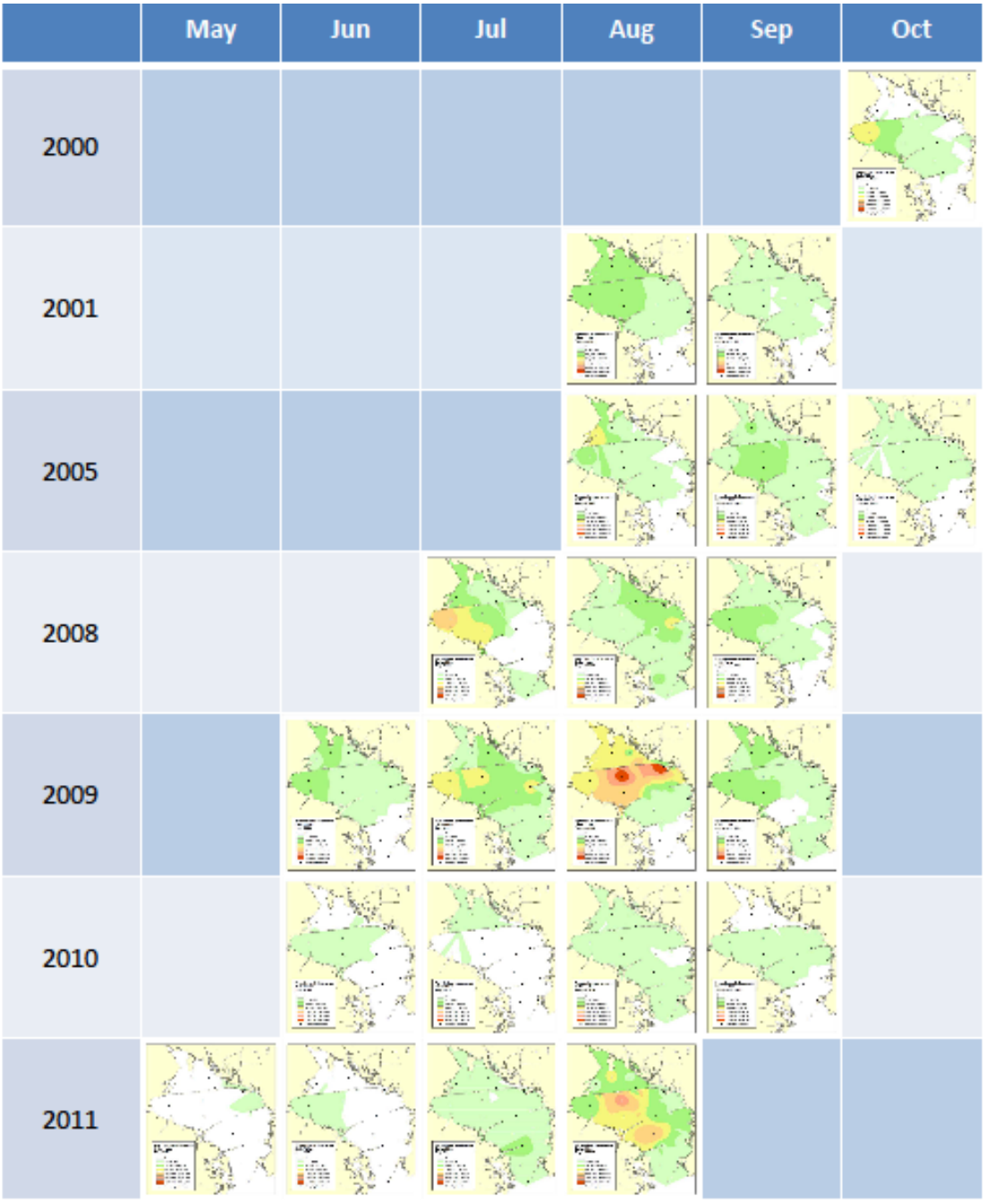

Figure 13. Old Tampa Bay wet season monthly *Pyrodinium bahamense* spatial distributions 2000 – 2011 (years where no *P. bahamense* were record omitted from figure).

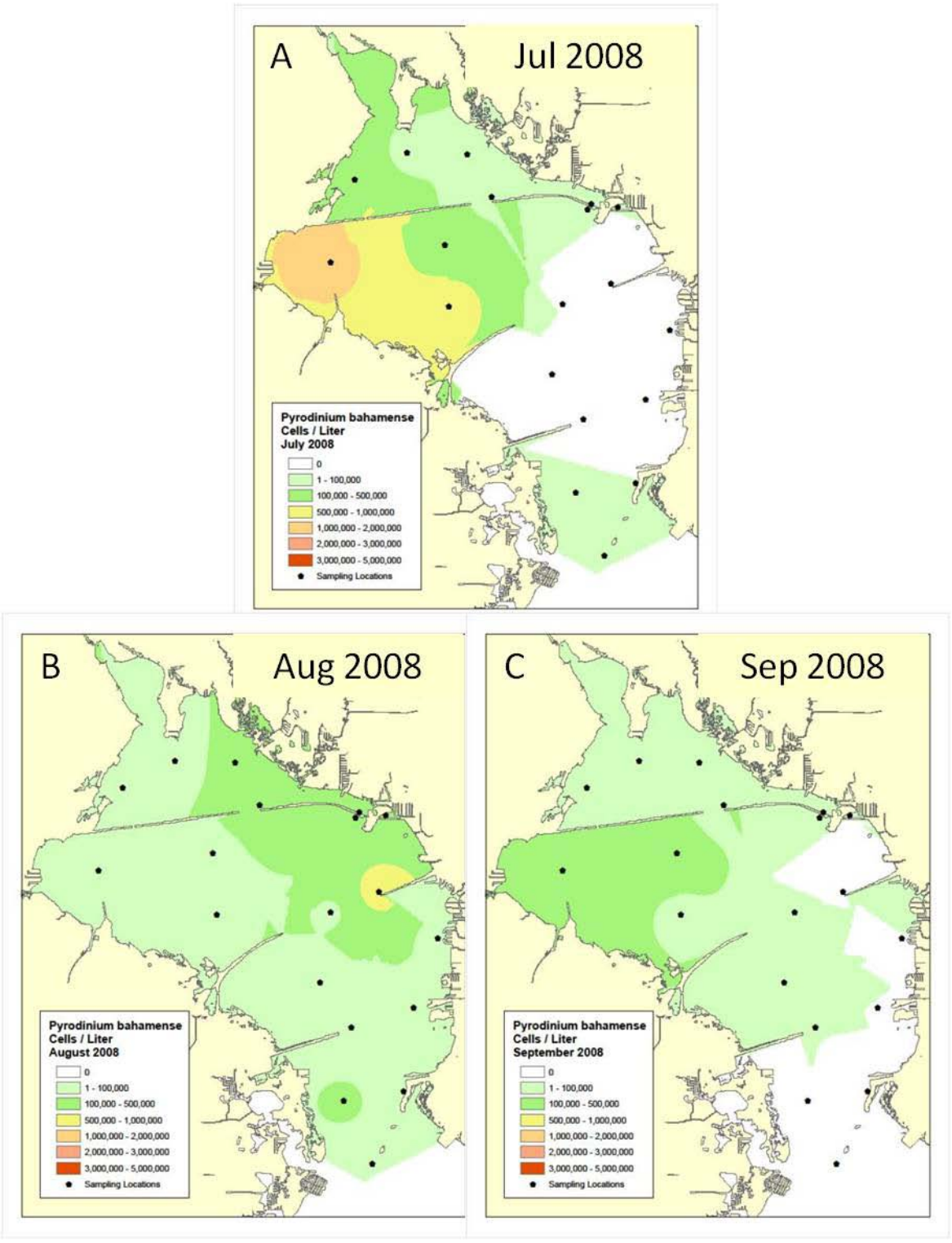

Figure 14. Summer 2008 *Pyrodinium bahamense* spatial distribution in Old Tampa Bay.

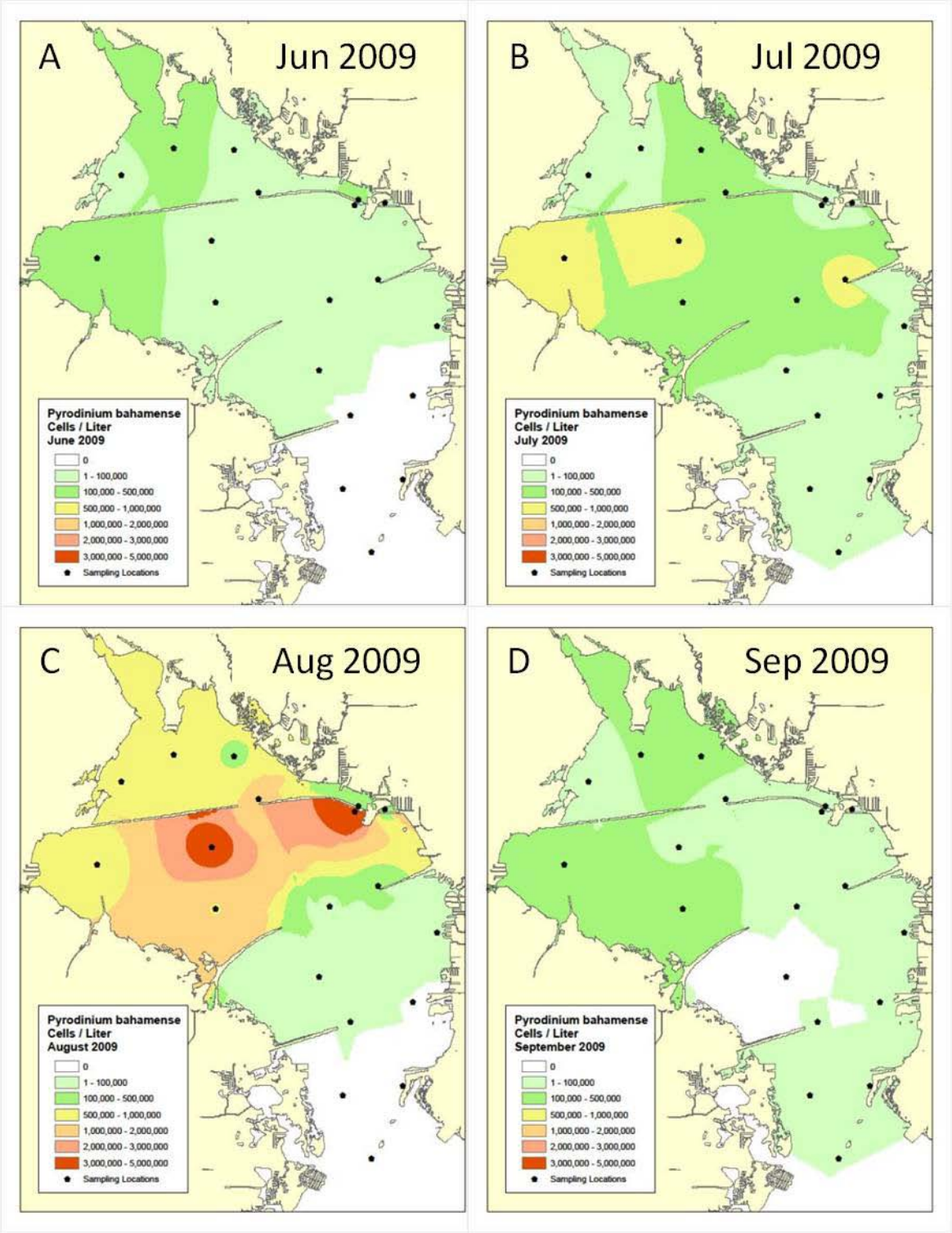

Figure 15. Summer 2009 *Pyrodinium bahamense* spatial distribution in Old Tampa Bay.

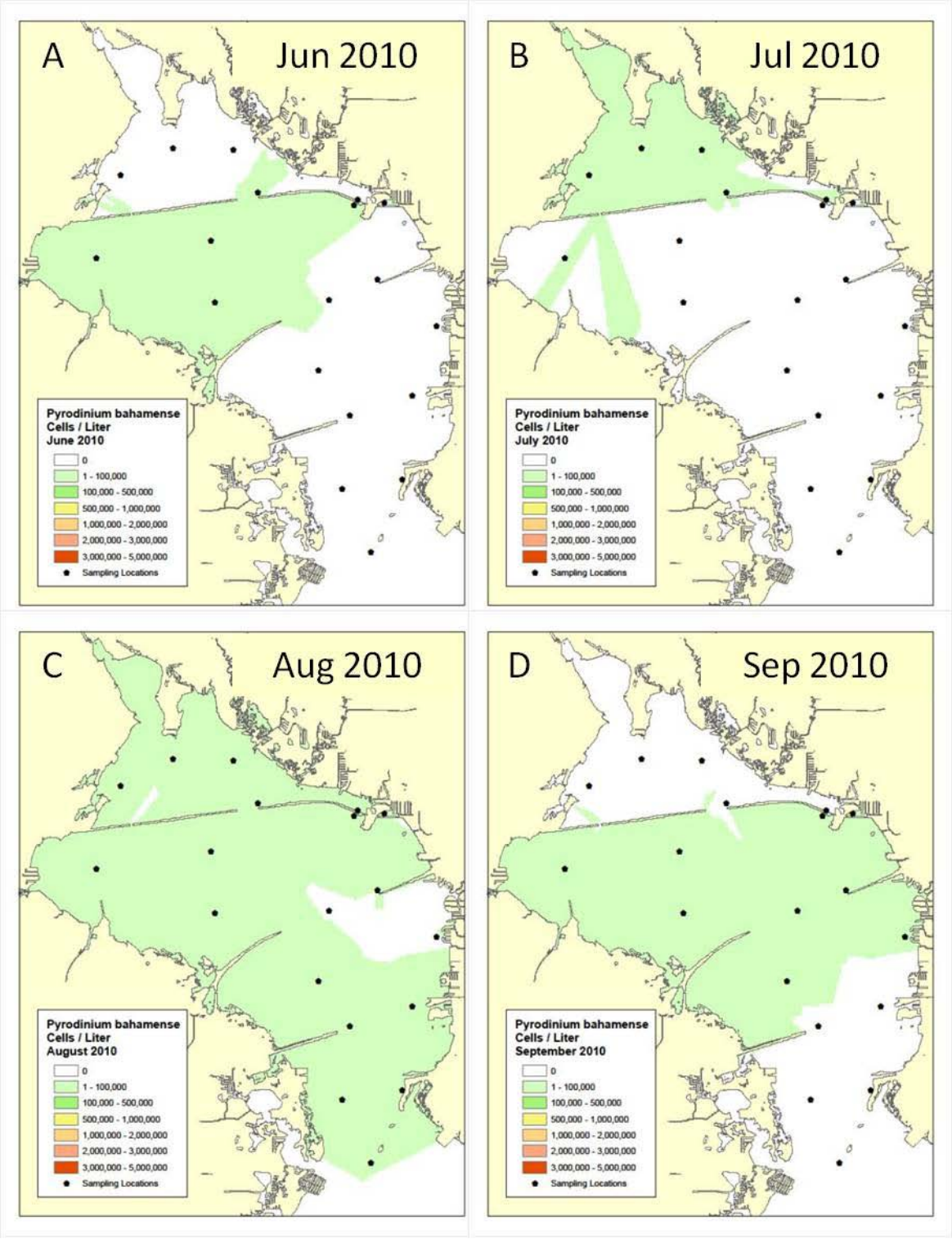

Figure 16. Summer 2010 *Pyrodinium bahamense* spatial distribution in Old Tampa Bay.

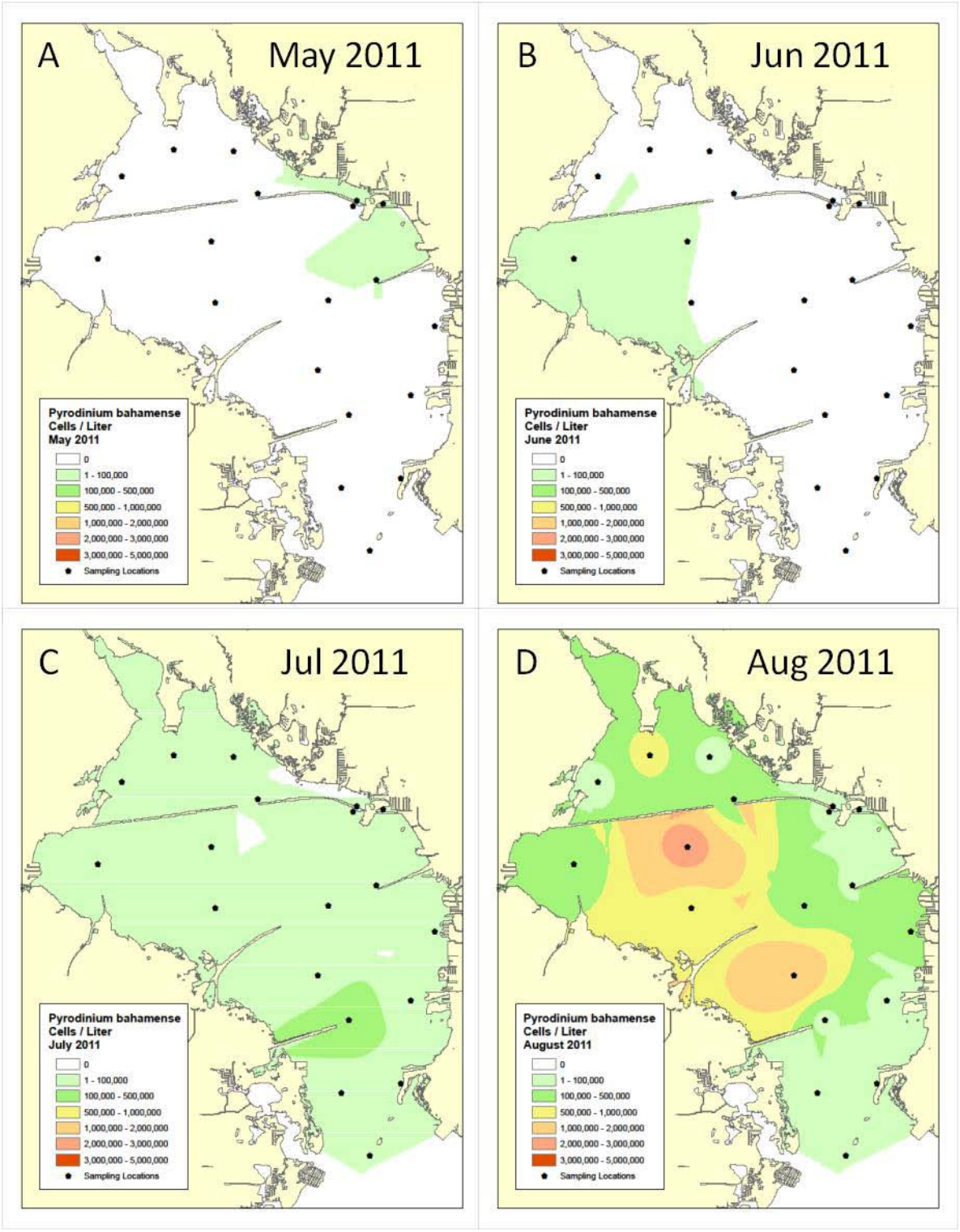

Figure 17. Summer 2011 *Pyrodinium bahamense* spatial distribution in Old Tampa Bay.

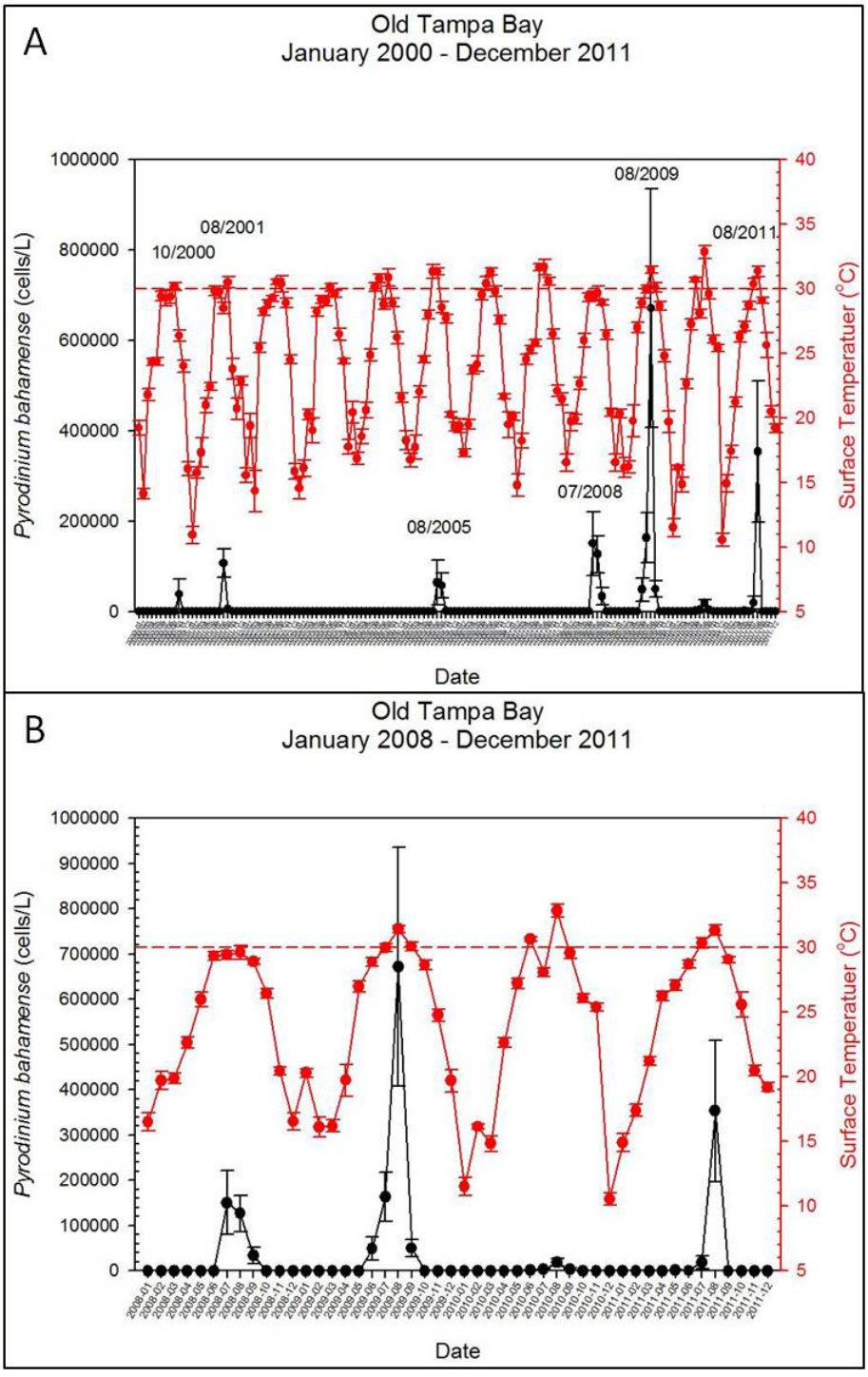

Figure 18 Monthly mean *Pyrodinium bahamense* cell counts (black) and surface water temperatures (red) at EPCHC Old Tampa Bay water quality monitoring stations from (A) January 2000 – December 2011 and (B) January 2008 – December 2011. Red dashed line indicates 30° C temperature optimum.

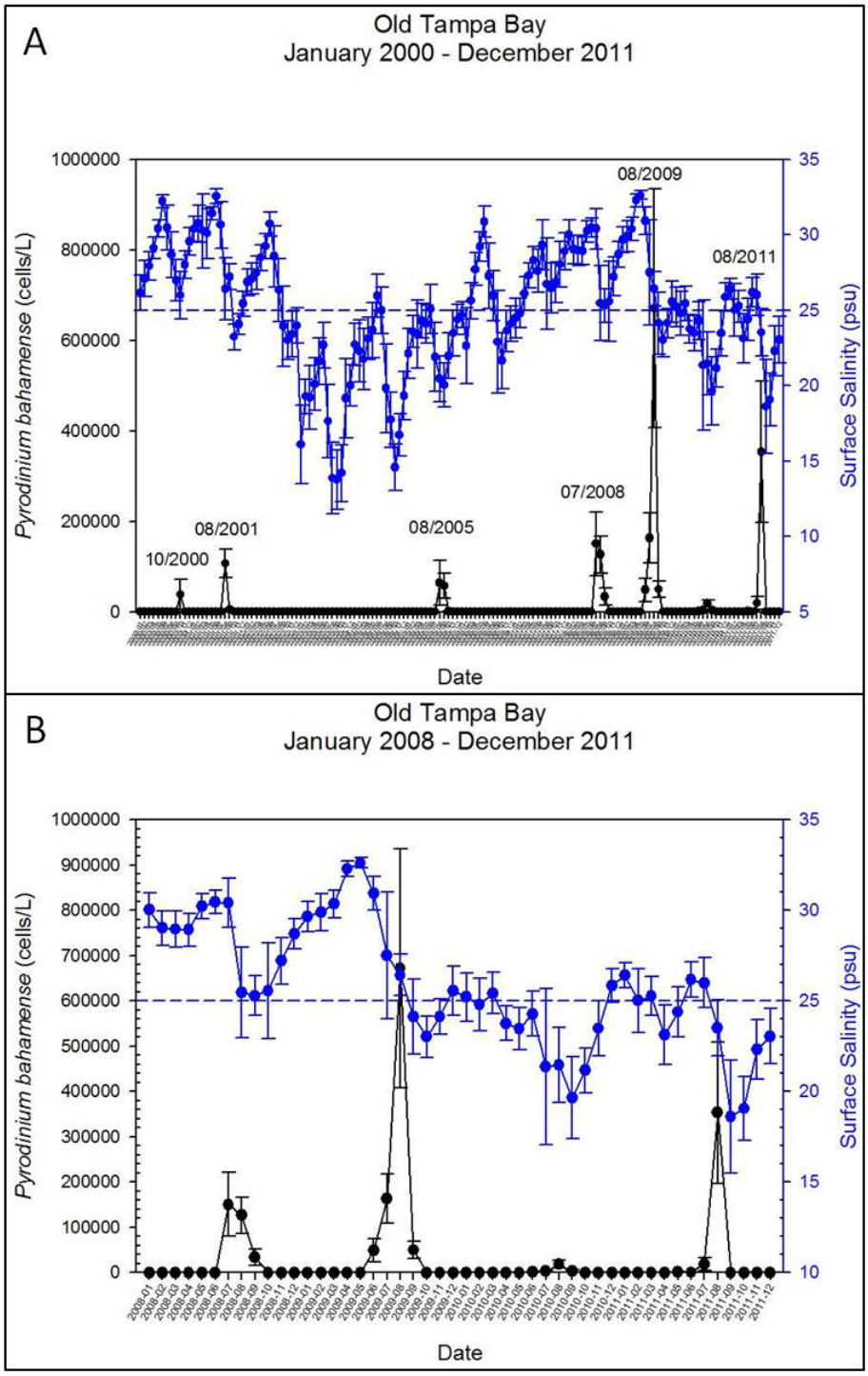

Figure 19 Monthly mean *Pyrodinium bahamense* cell counts and surface salinities at EPCHC Old Tampa Bay water quality monitoring stations from (A) January 2000 – December 2011 and (B) January 2008 – December 2011.

Table 3. BIO -ENV results for 2008-2011 Pyrodinium *bahamense* abundance and selected water quality parameters.

Parameters	Spearman Correlation
pH + Ortho-phosphate	0.367
pH + Ortho-phosphate + Kjeldahl nitrogen	0.358
Ortho-phosphate	0.325
pH	0.179
Dissolved Oxygen	0.067
Kjeldahl nitrogen	0.060

Table 4. Pearson correlation and significance (p - values) between the 2008-2011 *P. bahamense* cell counts and surface water quality and mid-depth nutrient measurements.

	Pyrodinium bahamense
Mid- depth Ortho-phosphate	0.502 ($p < 0.001$)
Surface pH	0.394 ($p < 0.001$)
Surface Temperature	0.317 ($p < 0.001$)
Mid-depth Kjeldahl nitrogen	0.235 ($p < 0.001$)
Mid-depth Nitrates + Nitrites	-0.069 ($p = 0.038$)
Surface Dissolved Oxygen	-0.061 ($p = 0.066$)
Mid-depth Ammonia	-0.0384 ($p = 0.247$)
Surface Salinity	-0.0119 ($p = 0.719$)

Discussion and Conclusions

The distribution of *Pyrodinium bahamense* cysts in the fall of 2011 was widespread throughout Old Tampa Bay with high concentrations in the north central region of the bay just south of the Courtney Campbell Causeway. This corresponded to the same area as the maximum bloom density that was observed over the summer. The highest cyst densities were also associated with very fine grained sediments. The diameter of *P. bahamense* cysts (50 – 65 µm) falls within the upper range for silts and the very fine grained sand sediment categories and so the cysts should behave as similar sized sediment particles in terms of where they would settle out of the water column.

Two major differences are apparent between the 2010 and 2011sampling periods. First the cyst densities were much higher in the 2011 samples. Second the distribution of greatest cyst densities within Old Tampa Bay shifted between the two years. The highest cyst densities in 2010 were found in the northwestern section of Old Tampa Bay while in 2011 this trend had shifted to the north central area. Several sites along the eastern shore and further south in 2011 also had high cyst densities relative to the 2010 period.

The difference in the cyst densities between 2010 and 2011 may have been due to changes in laboratory personnel and laboratory efficiency of cyst extraction during 2011. However, the same laboratory procedures were followed in both years.

The only other obvious difference was the time of year in which the samples were collected; late spring in 2010 vs. late fall in 2011. The 2011 sampling had occurred only a few months after the peak bloom in August 2011, while the 2010 sample collection took place nearly eight months after the 2009 summer bloom. The 2010 sampling was also was preceded by one of the most severe winters on record for the Tampa Bay area marked by several cold fronts and winter storm events. Sediment resuspension and transport within Old Tampa Bay is controlled primarily by wind driven waves during winter storms (Schoellhamer 1995). The winter storms in early 2010 may have resuspended the cysts that were deposited during the summer 2009 bloom

and transported them to other areas of Old Tampa Bay. In contrast, the 2011 samples were collected closer to the time of the previous summer bloom and before any major winter storms.

Pyrodinium bahamense cysts in May 2010 had the highest densities concentrated in the northwestern sections of Old Tampa Bay, particularly in Safety Harbor and near the Bayside Bridge area (Karlen and Miller 2011). These areas also corresponded to where past bloom events had initiated during the summers of 2008 and 2009. The highest concentrations of cysts in the fall of 2011were found in the north central section of Old Tampa Bay south of the Courtney Campbell Causeway and along the eastern side of the bay. Sites 11PCS42 and 11PCS03 had the highest cyst densities and corresponded with the peak cell counts during the 2011 bloom event and a prior bloom event in August 2009. Circulation models for Tampa Bay indicate that the portion of Old Tampa Bay between the Howard Frankland Bridge and Courtney Campbell Causeway is characterized by surface current gyres and long residence times (Weisberg and Zheng 2006; Meyers et al. 2007; Meyers and Luther 2008). These conditions may concentrate the *P. bahamense* blooms and subsequently result in the higher deposition of cysts at these sites after the bloom subsides. High cyst densities were also recorded along the east side of the bay and south of the Gandy Bridge even though the *P. bahamense* cell counts in these areas were relatively low during the 2011summer bloom. This suggests that the cysts are being moved and redeposited from other areas by tides, bottom currents and/or other sediment transport processes.

The occurrence of *Pyrodinium bahamense* blooms in Old Tampa Bay has increased in frequency and intensity since 2000 (Karlen and Miller 2011). Karlen and Miller (2011) stated that there were no recorded of occurrences of *Pyrodinium bahamense* in the EPCHC water monitoring database prior to 2000; however, three earlier instances of *P. bahamense* in Old Tampa Bay have been reported in the EPCHC monitoring database going back to1975. The first of these was in August 1975. A bloom occurred throughout Old Tampa Bay, but with highest cell concentrations north of the Courtney Campbell Causeway at station 46 and along the northwest section of the bay. The next occurrence was in August 1977 with a bloom extending north of the Howard Frankland Bridge and the Courtney Campbell Causeway with the highest cell counts around the Bayside Bridge at station 65. The third record was in September 1983

when low cell counts were reported in the west side of the bay between the Howard Frankland Bridge and Courtney Campbell Causeway.

There were no additional records found between September 1983 and October 2000 which may be in part due to misidentifications of this species in the past. This does not seem to be a probable scenario because *P. bahamense* is a relatively large and distinctive dinoflagellate. It is possible that temperature and/or salinity conditions during that time period were not optimal to support a bloom. Further analysis of water quality conditions during this time period is forthcoming.

Since 2000 there have been seven summers in which *Pyrodinium bahamense* has been found at the EPCHC monitoring sites. Several trends are apparent during this time period. First the frequency from year to year has increased over time, with occurrences in 2000 and 2001 followed by a three year break until the next bloom in 2005. Badylak et al. (2007), however, did record *Pyrodinium bahamense* in the summer of 2002. There were no recorded occurrences of *P. bahamense* for 2006 or 2007, but it has been found every summer from 2008-2011.

Second, the duration of the summer blooms has progressively gotten longer. The earliest records from 1975 – 1983 only recorded *P. bahamense* during a single month. The blooms from 2000 – 2005 ranged from 1 – 3 months and from 2008-2011 ranged from 3 – 4 months.

Third, the timing of when cells first appear has shifted to earlier in the summer. Earlier records up until 2005 reported the first *P. bahamense* cells being found between the months of August and October. The 2008 bloom first appeared in July. The earliest reports of *P. bahamense* in 2009 and 2010 were in early June, and in 2011, the first record was in May (Figure 13). This shift may be in response to higher surface water temperatures earlier in the summer or changes in seasonal rain patterns and corresponding surface salinities and nutrient inputs.

Analysis of the *Pyrodinium bahamense* population trends since 2000 suggests that blooms occur when surface water temperatures approach 30°C and surface salinities are around 25 psu. Phlips et al. (2006) also found that *Pyrodinium bahamense* blooms were temperature dependent with a lower temperature limit of 20°C and blooms occurring when temperatures exceeded 25°C. They also observed that *P. bahamense* had a wide salinity tolerance ranging

from 10 – 45 psu. The blooms which occurred during the summers of 2008 and 2009 were both preceded by a drop in salinity of 5 – 7 psu indicating a period of heavy precipitation at the start of the summer rainy season. The start of the rainy season most likely resulted in a pulse of nutrients from terrestrial runoff which could have initiated the bloom.

Low *Pyrodinium bahamense* cell counts were found during the summer of 2010 even though surface water temperatures during the 2010 summer months were within the optimal range to support a bloom. One possible explanation may be the relatively low surface water salinities which were in turn due to high rainfall that summer. The 2002-2004 and 2006-2007 time periods, when no *P. bahamense* cells were recorded, were also characterized by low salinities. This supports the hypothesis that a combination of physical water quality parameters such as temperature and salinity are controlling factors for the summer time blooms of this species.

The frequency and intensity of future *Pyrodinium bahamense* blooms may increase due to increasing summer temperatures and shifting climate patterns. Future blooms may also start to appear further south within Old Tampa Bay and possibly into Middle Tampa Bay as cysts are transported and redistributed. Findings from this study suggest winter time sediment resuspension and transport may be an important factor in the distribution of *P. bahamense* cysts in Old Tampa Bay, as well as, the distribution of summer bloom events. The effects of sediment transport processes within Old Tampa Bay on the redistribution of cysts and the potential for these processes to contribute towards the spread of bloom events to new areas of Tampa Bay warrants further investigation.

Acknowledgements

Funding was provided by the Tampa Bay Estuary Program (PO 6646). Dr. Karen Steidinger and Jamie Williams (Florida Wildlife Research Institute) provided training for the cyst extraction and counting procedures. We are grateful to the following EPCHC staff that helped support this study: Sara Markham did database management and Amanda Stewart did the sediment organic analysis; Barbara Goetting coordinated the field collections and served as boat captain for the field sampling and Joette Jernigan assisted with the sampling preparations and field collecting. Dr. Tom Dix (EPCHC) and Ed Sherwood (TBEP) assisted with editing the final report.

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APPENDIX A: FWC DINOFLAGELLATE CYST ISOLATION PROTOCOL

Modified[1] Dinoflagellate Cyst Isolation Protocol[2]

Supplies:

1-250 ml beaker

2-100 ml beakers

2-150 ml beaker

8-15 ml conical centrifuge tubes

1-6 or 12 well covered culture plate

16 ml of Sodium Polytungstate (SPT) @ 1.30 – 1.40 g/ml for each sample (Should be @ room temperature)

Seawater adjusted to appropriate salinity to match samples

250 μm, 90 μm and 20 μm sieves

Disposable pipettes (narrow and wide mouth)

Fill the 250 ml beaker to approx. 50 ml with tap water and place in freezer. Remove SPT from refrigerator to allow warming to room temperature.

Using a wide mouth disposable pipette remove ~ 60 ml of surface sediment/H_2O and place in a 100 ml beaker. When removing sample try to remove the upper most surface layer of sediment and avoid the heavier/courser sediments.

Place 100 ml beaker containing sample inside 250 ml beaker from freezer. Sonicate the sample for 7 min with the sonicator (Fisher model # 100) set to 5.

Pour sonified sample through 250 μm sieve into 150 ml beaker. Repeat with resulting filtrate using 90 μm sieve. Repeat again using 20 μm sieve (filtrate does not need to be saved). Back wash the concentrate into a 100 ml beaker and bring up to approx. 42 ml using seawater.

Using a large mouth disposable pipette mix the sample thoroughly and add 10 ml to a 15 ml conical centrifuge tube (total of 4 tubes).

Using a narrow mouth disposable pipette slowly and carefully add 4 ml of Sodium Polytungstate (SPT) at 1.30 –1.40 g/ml to the bottom of the tube under the sample.

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1 Originally modified by P. Scott and E. Truby and further modifications by K. Hayes and C.S. @ FMRI-St. Pete.

2 After Bolch, C.J.S., 1997. The use of sodium polytungstate for the separation and concentration of living Dinoflagellate cysts from marine sediments. Phycologia 36:472-8.

Weigh each sample to make sure they are equal and balanced prior to centrifugation.

Centrifuge for 10 min @ 3250 rpm (program 1) on the Eppendorf Centrifuge 5810 following centrifuge instructions.

Remove top 10 ml (down to the interface of seawater and SPT) and place this in a fresh 15 ml conical using a narrow mouth disposable pipette. This fraction should contain Dinoflagellate cysts.

Weigh and balance each sample again, and centrifuge for 3 min @ 2570 rpm (program 2).

Remove the top 8.5 – 9.0 ml and discard. Place the remaining sample in a covered culture chamber (6 well).

Unplug all equipment and clean area when finished.

Last update – 10/05/2002 KAH