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 occurred over the summers of 2008 and 2009 in Old Tampa Bay. It is probable that this will be a reoccurring event in the future. This species forms resting cysts which lie dormant in the sediments until conditions in the water column are favorable for blooms to occur. This pilot study looked at the abundance and distribution of *Pyrodinium bahamense* cysts in Old Tampa Bay sediments collected in May 2010 at the Environmental Protection Commission of Hillsborough County (EPCHC) surface water quality monitoring stations (Figure 1). The purpose of this study was to:

1. Map the distribution of *Pyrodinium bahamense* cysts in the sediments in Old Tampa Bay 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 sediments.

Additionally, we evaluated the monthly population trends of *Pyrodinium bahamense* in the water column of Old Tampa Bay from phytoplankton samples collected at the EPCHC surface water quality monitoring stations (Figure 1) during the 32 month period of January 2008 through August 2010. This time period encompasses the two summers of highest *Pyrodinium bahamense* bloom conditions (2008 and 2009). The phytoplankton data were correlated with corresponding water quality data to look at possible correlations between *Pyrodinium bahamense* blooms and surface hydrographic conditions and nutrient concentrations.

The dinoflagellate *Pyrodinium bahamense* was first described by Plate in 1906 from samples collected in the Bahamas (Balech 1985). This species is best known for its bioluminescence; this phenomenon has resulted in the establishment of several popular tourist destinations in Puerto Rico (Hernández-Becerril and Navarro 1996). *Pyrodinium bahamense* has been recorded in both the Atlantic and Indo-Pacific oceans and two geographic variations 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 forms 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 hyaline 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).

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 the west coast of Florida and in the Gulf of Mexico historically (Steidinger et al. 1967; Steidinger and Williams 1970; Licea et al. 2004) 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) and it has been noticeably absent in the EPCHC water monitoring records going back to the mid-1970's. Steidinger et al. (1980), however, shows scanning electron micrographs (SEMs) of specimens reported to be from Tampa Bay. The first known occurrence of *P. bahamense* in significant numbers at the EPCHC monitoring sites were in September and October 2000 at sites in Old Tampa Bay and Middle Tampa Bay respectively (Boler n.d.). There have been several periodic blooms recorded at EPCHC monitoring sites in Old Tampa Bay since 2000 (Figure 3; Badylak et al. 2007; Badylak and Phlips 2008). This trend appears to be increasing in frequency and intensity in recent years, with blooms reoccurring during the summers of 2008 and 2009 (Figure 3).

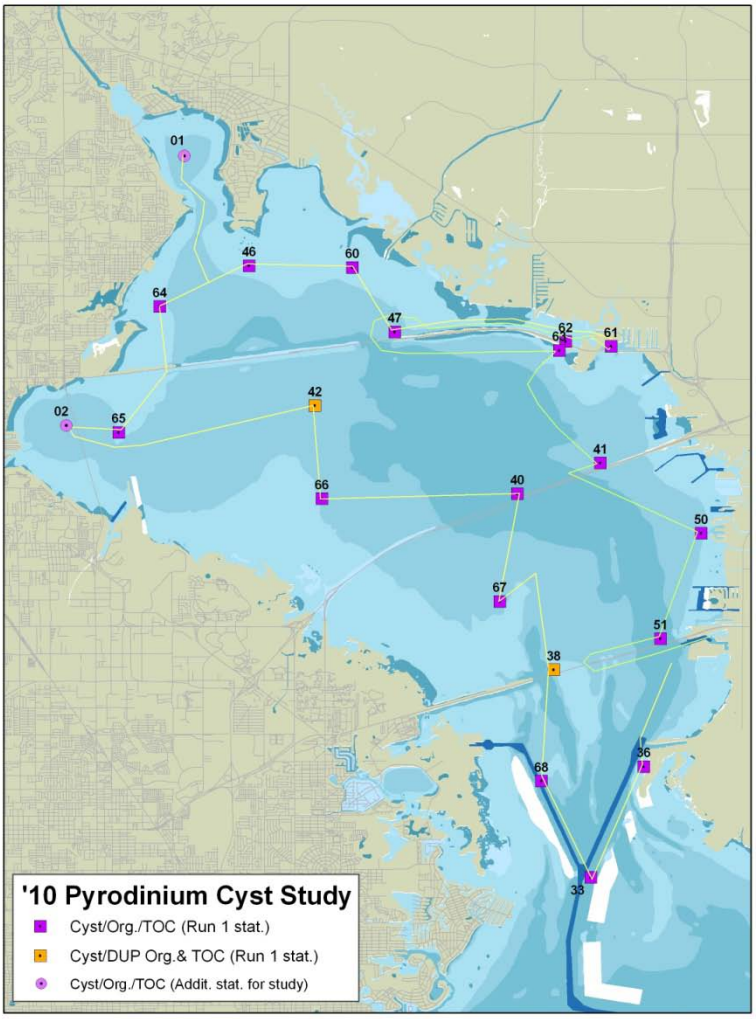

Figure 1 Sediment and water quality sampling stations in Old Tampa Bay. Monthly EPCHC water monitoring stations represented by squares, additional sampling sites for this study are represented as circles. Sites represented by orange colored symbols had duplicate sediment samples taken for QA/QC on silt/clay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

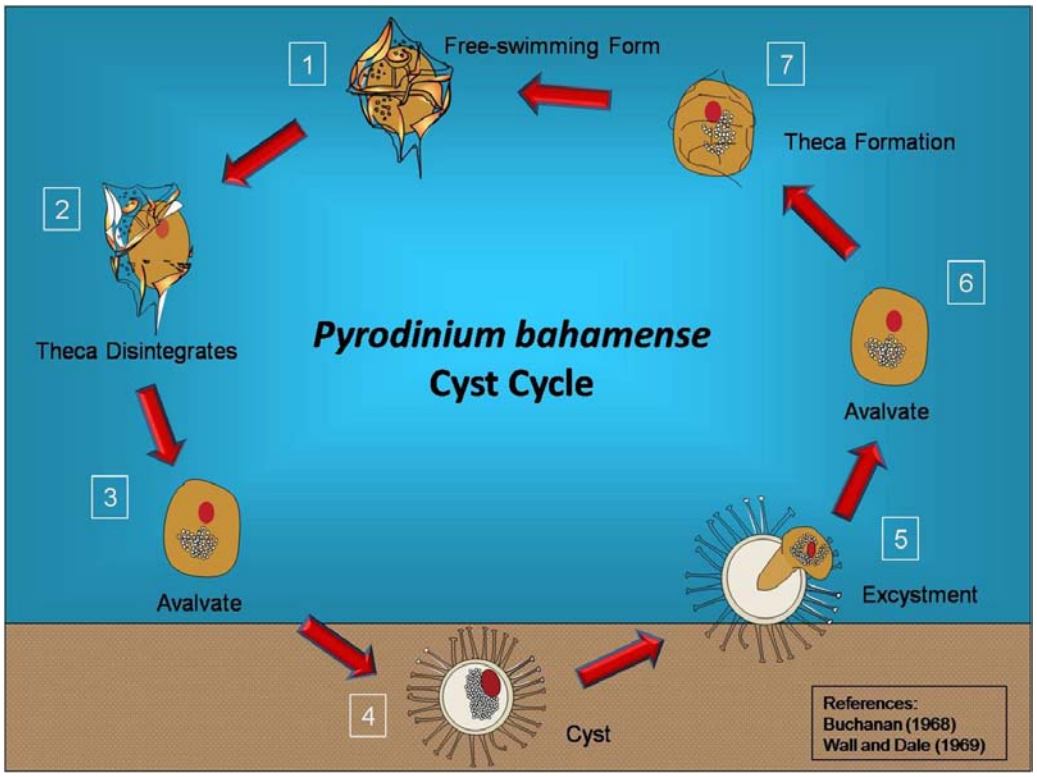

Figure 2 *Pyrodinium bahamense* cyst cycle.

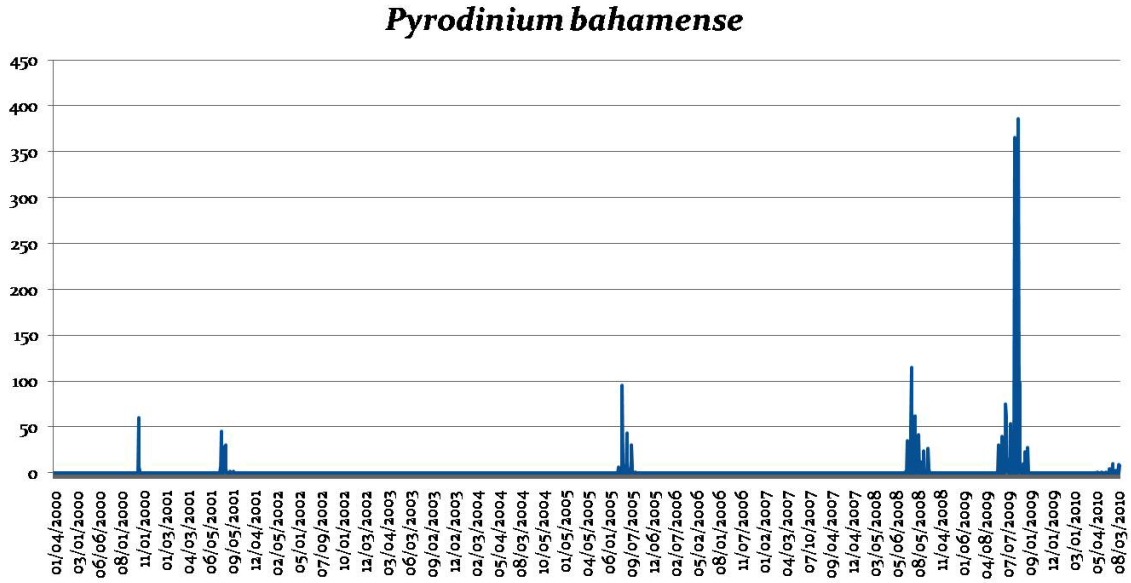

Figure 3 Trends in *Pyrodinium bahamense* cell counts (#/0.1 ml) at EPCHC Old Tampa Bay monitoring sites from January 2000 - August 2010

Methods

Sample collection:

Sediment samples were collected on May 4-5, 2010 at 19 EPCHC water monitoring stations in Old Tampa Bay plus two additional sites; one north of the Courtney Campbell Causeway in the vicinity of Safety Harbor and one west of the Bayside Bridge in the vicinity of the Clearwater WWTP discharge. The sediment sampling was scheduled to coincide with the EPCHC's monthly water quality monitoring sampling run to facilitate comparison of the cyst count results with the station water quality data. 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 two subsamples. Each subsample was placed in a HDPE plastic sample jar and refrigerated. One subsample was used for the dinoflagellate cyst extraction procedure. The other subsample was used for sediment composition analysis (% silt/clay and organic carbon content).

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.

Training and Quality Control and Assurance:

EPCHC scientists conducting the cyst extractions and counts were trained by scientists from the Florida Fish and Wildlife Research Institute (FWRI). To assure accuracy in cyst identification and counts, 6 of the 21 samples (28%) processed by EPCHC staff were cross checked by FWRI staff for QA/QC.

Sediment Analysis:

The percent silt/clay was measured following Tampa Bay Benthic Monitoring program protocols (Versar 1993). 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 Management, Analysis and Reporting:

Cyst count data was entered into the EPCHC's water monitoring database. Maps of the cyst abundance and distribution at the EPCHC water monitoring stations were generated using Arc-GIS. Data analysis consisted of principal components analysis (PCA) of the physical parameters and correlation analysis between the cyst abundance and the sediment characteristics and water quality parameter using the BIO-ENV procedure in PRIMER-E ver.6. Similar analyses were done to compare historical *Pyrodinium bahamense* cell counts with corresponding water quality data. Cyst count and *Pyrodinium bahamense* cell count data were square root transformed and the water quality and sediment data were log (n+1) transformed and normalized for analysis.

Results

Water quality and sediment characteristic:

Sample depths and surface hydrographic measurements are shown in Table 1. Sample depths at the 21 stations ranged from 1.0 – 8.9 meters with a median depth of 2.9 meters. Surface water temperatures ranged between 26 – 28°C and surface salinities ranged between 20.4 – 25.5 practical salinity units (psu) with a median value of 23.4 psu. Median pH and dissolved oxygen were 8.0 and 5.87 mg/l respectively. Nutrient concentrations at each sampling location are given in Table 2. Chlorophyll, turbidity, color and dissolved organic carbon measurements are summarized in Table 3.

Sediment characteristics are summarized in Table 4. The silt+clay content were generally low at most sites with a median of 3%. Three sites however had values that were > 75% silt/clay (Table 4; Figure 4A). These included the sites in Safety Harbor (PCS 01), west of the Bayside Bridge (PCS 02), and on the northeast side of the Courtney Campbell Causeway near Sweetwater Creek (PCS 61). There was a general trend of decreasing percent silt+clay towards the lower portion of Old Tampa Bay (Figure 4A). The sediment total organic carbon (TOC) ranged 0.1 to 5.6% with a median value of 0.2% (Table 4). Only four sites had TOC values greater than 1% and these sites corresponded to sites with high percent silt+clay (Figure 4B).

Stable isotope analysis results (Table 4; Figure 5) indicate unique δN^{15} signatures for different regions of Old Tampa Bay, with lower values in the upper and northern parts of the bay and increasing values towards the middle and lower sites. The δC^{13} values at most sites were near the median value of -24.5, falling between -30 and -20 (Table 4). Two sites, 10PCS38 and 10PCS42, however had low δC13 values relative to the other sites (Figure 5).

Principal components analysis (PCA) of the combined water quality and sediment parameters grouped the sites by their north-south locations within Old Tampa Bay, with the three sites having the highest silt+clay content falling as outliers (Figures 6 and 7). The first principal component axis accounted for 39% of the variability and was weighed by surface salinity and sediment $\delta^{15}N$ (Tables 5 and 6). The second principal component axis accounted for 18% of the

variability and was weighted by Kjeldahl and total nitrogen in the water column as well as by sediment total organic carbon and percent silt+clay content (Tables 5 and 6).

Pyrodinium bahamense cyst distribution:

The distribution of *Pyrodinium bahamense* cysts in Old Tampa Bay sediments was highest at the northwestern areas of the bay (Figure 8). There was a general decrease in the cyst density towards the lower part of Old Tampa Bay, and cysts were absent at two sites south of the Gandy Bridge (Figure 8). High cyst densities also tended to correspond with lower δN^{15} signatures (Figure 9).

BIO-ENV results comparing the cyst density with corresponding sediment and water quality parameters found the strongest correlation with combination of orthophosphate, dissolved total organic carbon and percent silt + clay ($\rho = 0.406$). The percent silt+clay had the highest single factor correlation with cyst density ($\rho = 0.309$).

Trends in Pyrodinium bahamense blooms in Old Tampa Bay: 2008-2010:

The *Pyrodinium bahamense* cell counts for the EPCHC water monitoring samples collected during the period from January 2008 – August 2010 appear to strongly correlate with surface water temperature over time (Figure 10). Highest cell counts corresponded to summer peaks in surface water temperatures in July 2008 and in August 2009, with apparent optimal temperatures of around 30°C (Figure 10). 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 11). Cell densities did not appear to strongly correlate with various forms of nitrogen (Figures 12 and 13). Cell counts decreased in the fall and were absent during the winter months. BIO-ENV results showed cell counts correlated with a combination of orthophosphate, chlorophyll-a and surface pH ($\rho = 0.542$). Total phosphorus had the strongest single factor correlation ($\rho = 0.435$), followed by orthophosphate ($\rho = 0.412$). There was an apparent lag time of approximately one month between maximum cell densities and peaks in the orthophosphate and total phosphorus levels (Figures 14 and 15).

Table 1 Sampling station depth and surface hydrographic measurements

Site	Depth (m)	Surface Temperature (°C)	Surface Salinity (psu)	Surface pH	Surface DO (mg/L)	DO Saturation (%)
10PCS01	2.70	27.92	20.42	7.92	5.87	84.6
10PCS02	3.20	27.26	22.42	7.85	5.34	76.1
10PCS33	8.90	26.74	25.50	8.01	5.87	85.4
10PCS36	4.60	27.07	24.80	8.05	6.33	92.3
10PCS38	2.70	27.39	24.46	8.21	7.07	103.3
10PCS40	4.90	26.15	23.72	7.97	5.18	73.8
10PCS41	3.80	27.18	24.17	8.10	5.87	85.4
10PCS42	3.60	26.94	22.76	7.93	5.11	73.3
10PCS46	2.30	27.67	22.29	7.92	5.74	83.3
10PCS47	3.70	27.48	23.19	7.95	5.56	80.9
10PCS50	3.00	26.76	24.37	8.16	6.93	100.1
10PCS51	5.10	26.57	24.65	8.10	6.46	93.2
10PCS60	1.40	27.93	22.79	8.00	6.16	90.0
10PCS61	2.60	27.17	22.69	7.93	4.93	70.8
10PCS62	1.30	28.00	22.16	7.86	5.12	74.6
10PCS63	1.00	26.55	23.38	7.77	4.36	62.4
10PCS64	1.30	26.77	22.26	7.96	6.42	91.6
10PCS65	2.20	27.17	22.83	8.01	5.84	84.3
10PCS66	2.90	27.30	23.19	8.01	5.78	83.8
10PCS67	2.70	27.09	24.03	8.11	6.62	96.0
10PCS68	5.70	27.42	25.10	8.12	6.74	99.0
Minimum	1.00	26.15	20.42	7.77	4.36	62.4
Maximum	8.90	28.00	25.50	8.21	7.07	103.3
Median	2.90	27.17	23.19	8.00	5.87	84.6
Average	3.31	27.17	23.39	8.00	5.87	85.0

Table 2 Sampling station nutrient concentrations.

Site	Ammonia (mg/l)	Kjeldahl Nitrogen (mg/l)	Organic Nitrogen (mg/l)	Total Nitrogen (mg/l)	Ortho Phosphates (mg/l)	Total Phosphorus (mg/l)
10PCS01	0.036	0.632	0.596	0.632	0.042	0.121
10PCS02	0.037	0.578	0.541	0.578	0.055	0.137
10PCS33	0.049	0.356	0.307	0.356	0.057	0.119
10PCS36	0.050	0.448	0.398	0.448	0.062	0.156
10PCS38	0.048	0.584	0.536	0.584	0.050	0.143
10PCS40	0.055	0.477	0.422	0.477	0.048	0.130
10PCS41	0.050	0.954	0.904	0.954	0.051	0.143
10PCS42	0.045	1.489	1.444	1.489	0.048	0.169
10PCS46	0.048	1.239	1.191	1.239	0.048	0.174
10PCS47	0.056	1.006	0.950	1.006	0.049	0.124
10PCS50	0.045	0.752	0.707	0.752	0.051	0.123
10PCS51	0.057	0.984	0.927	0.984	0.058	0.155
10PCS60	0.051	1.287	1.236	1.287	0.049	0.147
10PCS61	0.050	0.885	0.835	0.885	0.047	0.130
10PCS62	0.056	0.850	0.794	0.850	0.058	0.147
10PCS63	0.054	0.791	0.737	0.791	0.053	0.128
10PCS64	0.048	0.886	0.838	0.886	0.048	0.142
10PCS65	0.052	0.999	0.947	0.999	0.059	0.176
10PCS66	0.050	0.904	0.854	0.904	0.054	0.164
10PCS67	0.062	0.636	0.574	0.636	0.050	0.167
10PCS68	0.050	0.620	0.570	0.620	0.054	0.133
Minimum	0.036	0.356	0.307	0.356	0.042	0.119
Maximum	0.062	1.489	1.444	1.489	0.062	0.176
Median	0.050	0.850	0.794	0.850	0.051	0.143
Average	0.050	0.827	0.777	0.827	0.052	0.144

Table 3 Sampling station chlorophyll , turbidity, water color and dissolved TOC values for May 2010.

Site	Chl a (µg/l)	Chl b (µg/l)	Chl c (µg/l)	Total Chl (µg/l)	Turbidity (NTUs)	Color (345)F.45 (PCUs)	Dissolved TOC (mg/l)
10PCS01	6.8	0.4	0.8	8.0	4.5	11.3	7.25
10PCS02	5.4	0.0	0.7	6.1	3.3	8.6	6.50
10PCS33	4.0	0.1	0.6	4.7	1.6	7.4	4.85
10PCS36	4.6	0.0	0.8	5.4	3.7	7.0	5.62
10PCS38	5.7	0.0	1.1	6.8	2.8	6.4	6.61
10PCS40	7.8	0.0	1.4	8.5	3.6	6.4	6.11
10PCS41	6.2	0.1	1.1	7.4	2.9	6.2	6.15
10PCS42	7.9	0.5	1.0	9.4	5.5	7.4	6.52
10PCS46	10.4	1.0	1.4	12.8	7.5	8.9	7.00
10PCS47	8.1	0.8	0.9	9.8	4.1	7.3	6.12
10PCS50	4.4	0.1	0.7	5.2	2.9	6.3	6.21
10PCS51	5.6	0.0	1.0	6.6	3.2	6.7	5.82
10PCS60	9.9	1.0	1.2	12.1	6.6	7.7	6.05
10PCS61	10.2	0.0	1.5	11.7	2.5	9.3	6.83
10PCS62	11.8	0.3	1.5	13.6	5.4	9.1	6.66
10PCS63	3.9	0.0	0.3	4.2	2.3	8.0	5.73
10PCS64	8.0	0.7	0.7	9.4	4.5	9.3	6.27
10PCS65	6.4	0.0	0.9	7.3	5.3	7.0	6.53
10PCS66	9.4	0.0	1.4	10.8	8.0	7.1	6.21
10PCS67	7.4	0.0	1.3	8.7	3.5	6.4	6.46
10PCS68	4.7	0.0	0.8	5.5	1.8	6.1	5.81
Minimum	3.9	0.0	0.3	4.2	1.6	6.1	4.85
Maximum	11.8	1.0	1.5	13.6	8.0	11.3	7.25
Median	6.8	0.0	1.0	8.0	3.6	7.3	6.21
Average	7.1	0.2	1.0	8.3	4.1	7.6	6.25

Table 4 Sampling site sediment characteristics

Site	%Silt/Clay	%TOC	$\delta^{15}N$	$\delta^{13}C$	C:N ratio (mass)	C:N ratio (molar)
10PCS01	75.99	3.5	4.31	-23.67	8.95	10.44
10PCS02	88.75	4.5	4.71	-22.69	9.52	11.11
10PCS33	3.03	0.2	6.69	-27.36	18.00	21.00
10PCS36	1.60	0.7	5.36	-26.65	73.50	85.74
10PCS38	2.06	0.2	6.52	-39.77	24.46	28.53
10PCS40	3.01	0.2	5.29	-23.07	35.57	41.50
10PCS41	3.06	0.7	4.86	-24.14	10.54	12.30
10PCS42	5.03	0.2	5.09	-39.77	23.87	27.85
10PCS46	4.86	0.2	4.21	-23.87	32.40	37.80
10PCS47	4.10	0.3	4.95	-31.41	18.94	22.09
10PCS50	2.20	0.1	5.36	-22.01	14.68	17.12
10PCS51	2.13	1.6	5.28	-25.42	8.59	10.03
10PCS60	2.50	0.1	4.75	-22.46	10.61	12.38
10PCS61	94.10	5.6	3.81	-22.22	8.99	10.48
10PCS62	8.37	0.6	4.39	-24.50	11.90	13.88
10PCS63	1.29	0.1	5.15	-30.96	22.50	26.25
10PCS64	2.88	0.2	4.71	-28.84	14.38	16.78
10PCS65	1.98	0.1	4.75	-25.34	13.10	15.28
10PCS66	5.27	0.4	5.08	-22.72	7.81	9.12
10PCS67	3.50	0.2	4.85	-22.38	9.75	11.38
10PCS68	2.72	0.2	5.92	-30.09	21.73	25.36
Minimum	1.29	0.1	3.81	-39.77	7.81	9.12
Maximum	94.10	5.6	6.69	-22.01	73.50	85.74
Median	3.03	0.2	4.95	-24.50	14.38	16.78
Average	15.16	0.9	5.05	-26.63	19.04	22.21

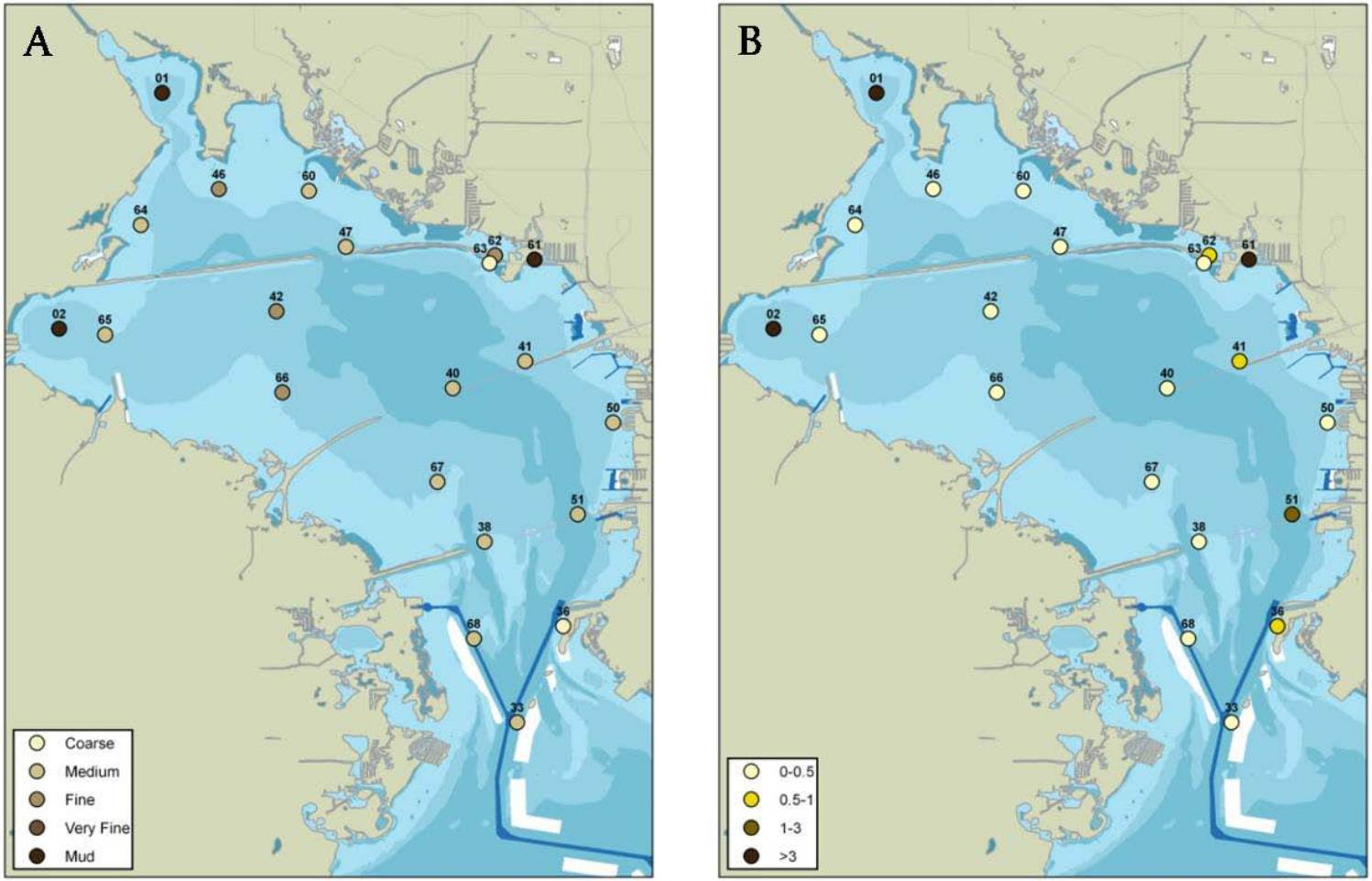

Figure 4 (A) Distribution of sediment type based on % silt/clay content and (B) sediment total organic carbon as % TOC in Old Tampa Bay.

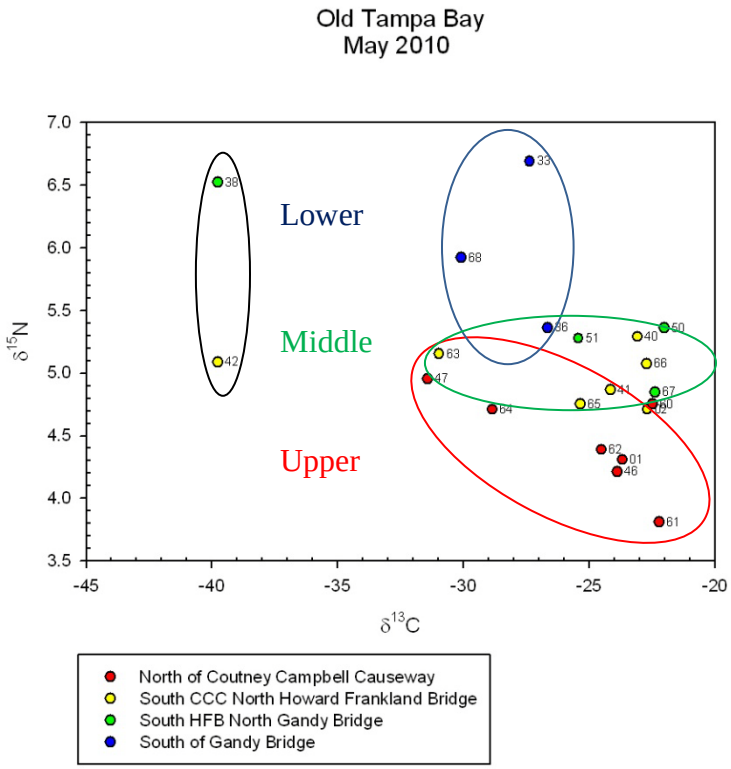

Figure 5 Stable isotope results coded by sample location within Old Tampa Bay

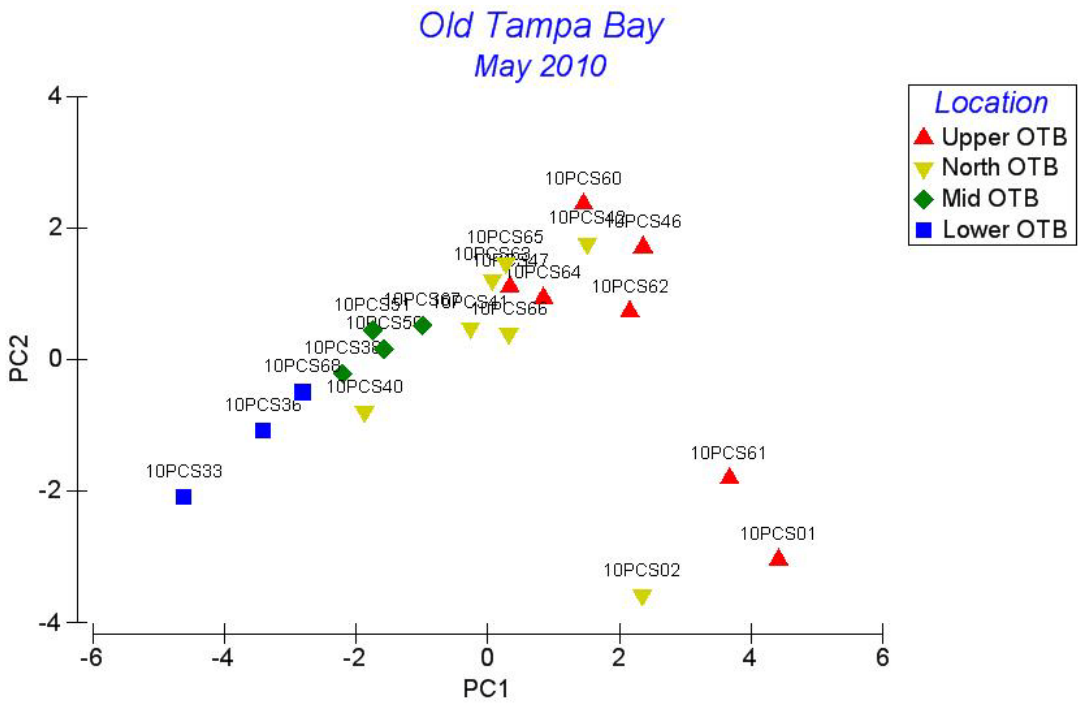

Figure 6 Principal Component Analysis (PCA) of water quality and sediment parameters.

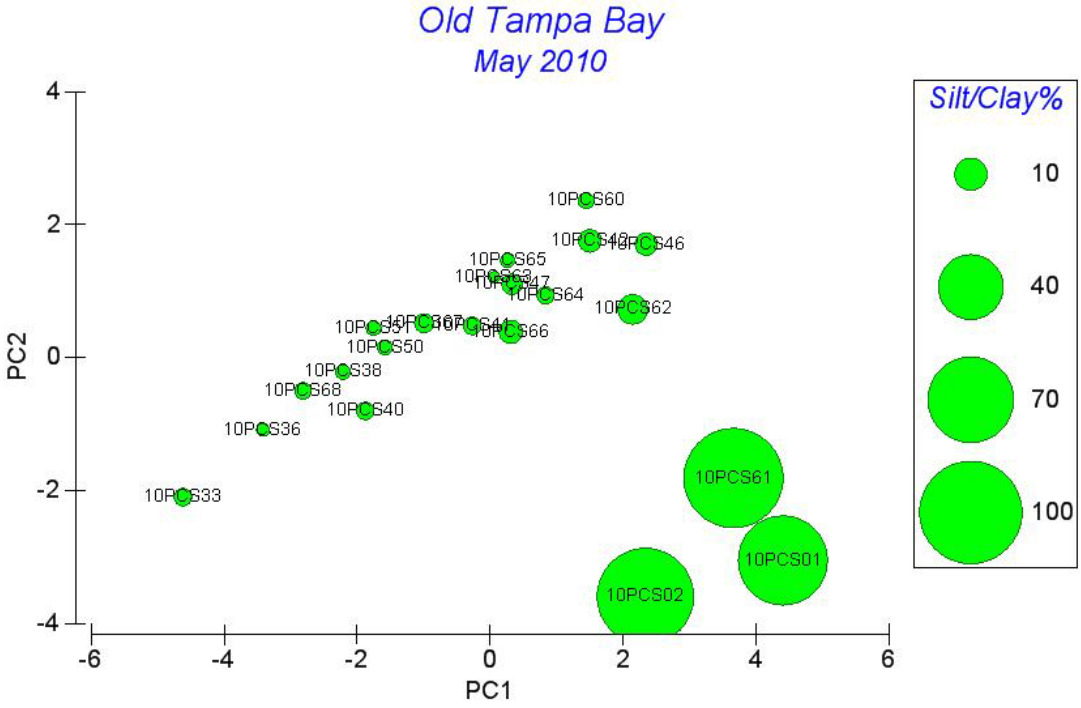

Figure 7 PCA bubble plot depicting percent silt+clay at all sites.

Table 5 Eigenvalues for PCA on water quality and sediment parameters.

Eigenvalues			
PC	Eigenvalues	%Variation	Cum.%Variation
1	5.49	39.20	39.20
2	2.53	18.00	57.30
3	1.80	12.90	70.10
4	1.07	7.60	77.80
5	0.88	6.30	84.10

Table 6 Eigenvectors for PCA on water quality and sediment parameters.

Eigenvectors (Coefficients in the linear combinations of variables making up PC's)					
Variable	PC1	PC2	PC3	PC4	PC5
Ammonia	-0.159	0.338	0.172	0.400	-0.121
Kjeldahl Nitrogen	0.227	0.461	-0.068	0.110	0.278
Organic Nitrogen	0.232	0.454	-0.073	0.103	0.279
Ortho Phosphates	-0.226	-0.001	0.200	0.424	-0.497
Dissolved TOC	0.341	0.017	-0.252	-0.177	-0.067
Silt/Clay%	0.302	-0.423	-0.024	0.106	0.068
Sediment TOC	0.224	-0.449	-0.013	0.304	0.108
δ15N	-0.374	-0.046	-0.067	-0.160	0.050
C:N ratio (molar)	-0.195	0.059	0.232	-0.653	-0.121
Depth	-0.264	-0.279	-0.068	0.084	0.404
Temperature	0.222	0.032	-0.324	-0.015	-0.585
DO	-0.199	0.014	-0.618	-0.026	-0.122
pH	-0.260	0.039	-0.553	0.085	0.104
Salinity	-0.396	0.020	-0.022	0.188	0.119

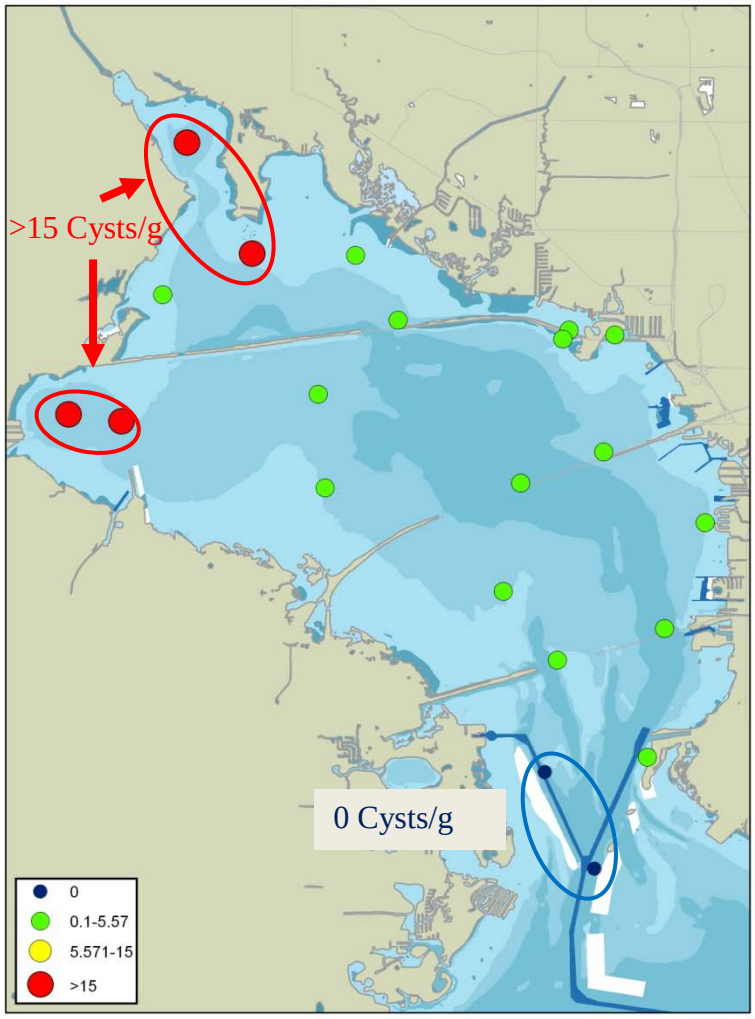

Figure 8: Distribution and density of *Pyrodinium bahamense* cysts in Old Tampa Bay.

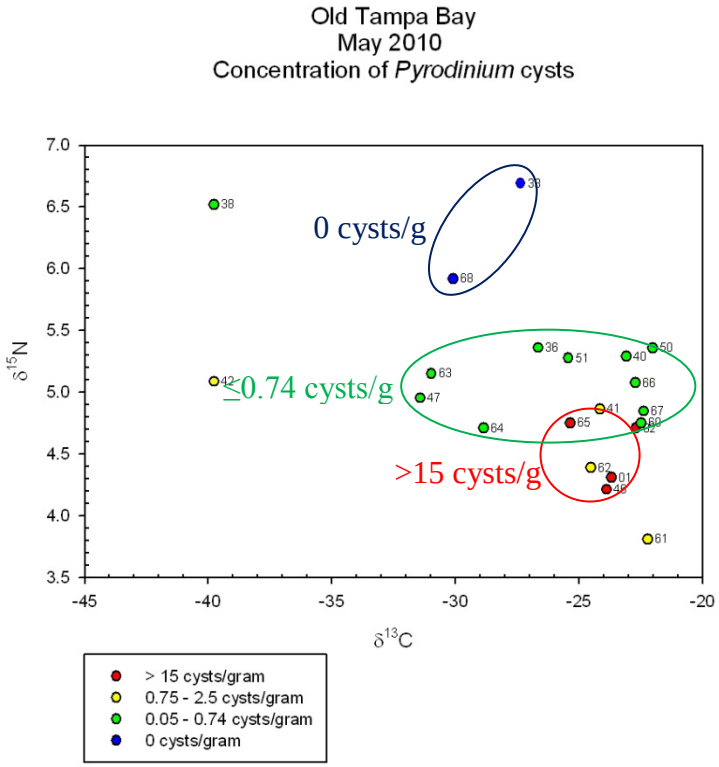

Figure 9 Stable isotope results coded by *Pyrodinium bahamense* cyst density.

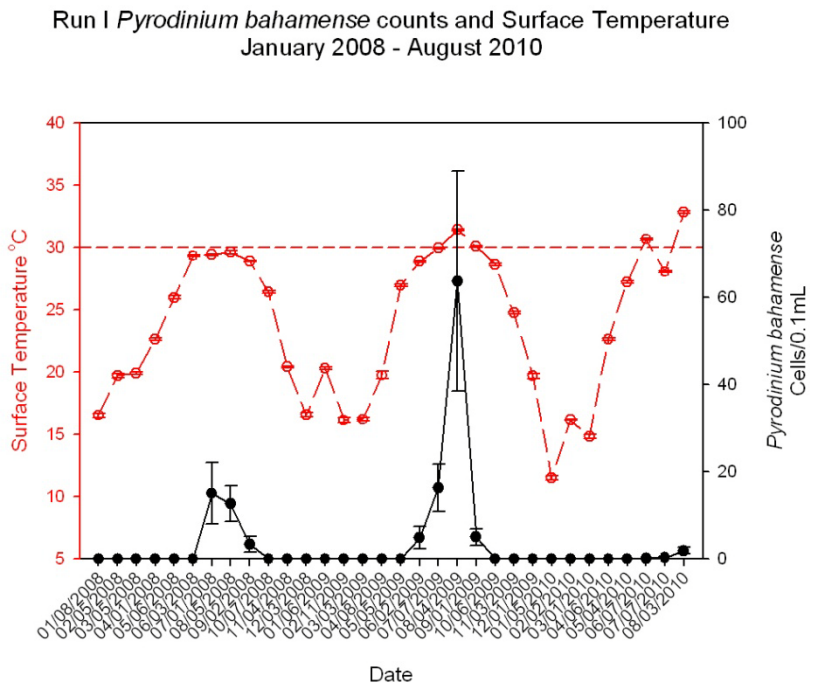

Figure 10 Monthly mean *Pyrodinium bahamense* cell counts and surface water temperatures at EPCHC Old Tampa Bay water quality monitoring stations from January 2008 – August 2010.

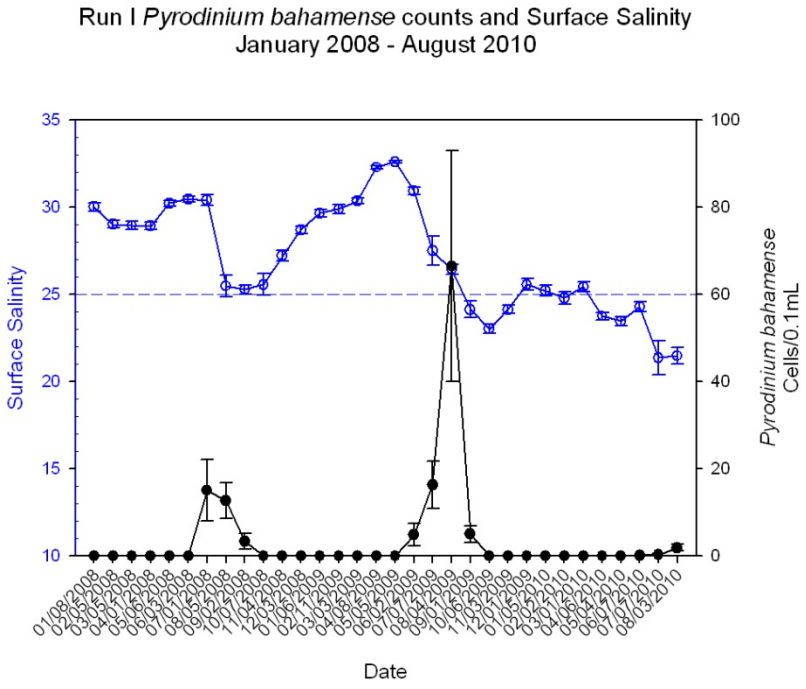

Figure 11 Monthly mean *Pyrodinium bahamense* cell counts and surface salinities at EPCHC Old Tampa Bay water quality monitoring stations from January 2008 – August 2010.

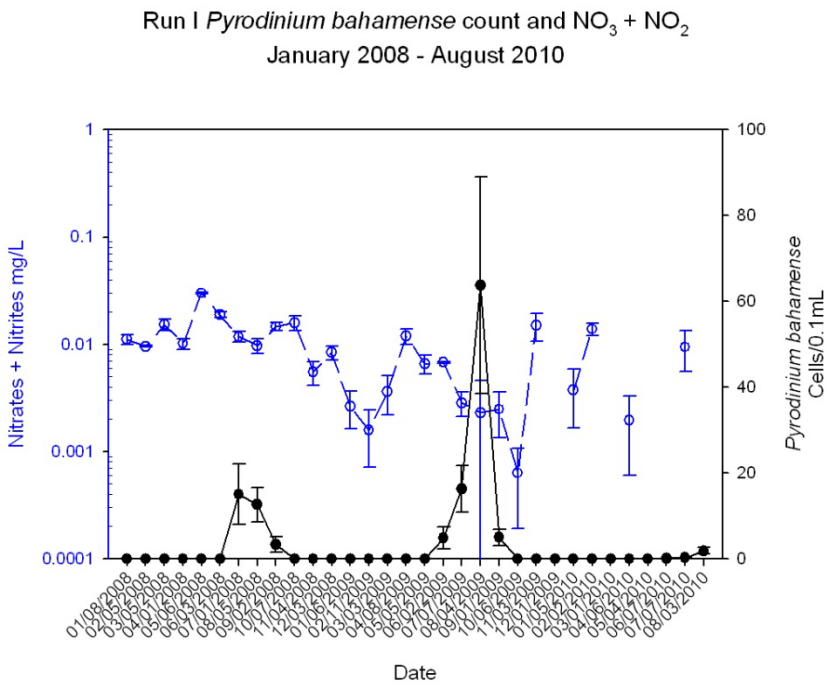

Figure 12 Monthly mean *Pyrodinium bahamense* cell counts and water sample NOx values at EPCHC Old Tampa Bay water quality monitoring stations from January 2008 – August 2010.

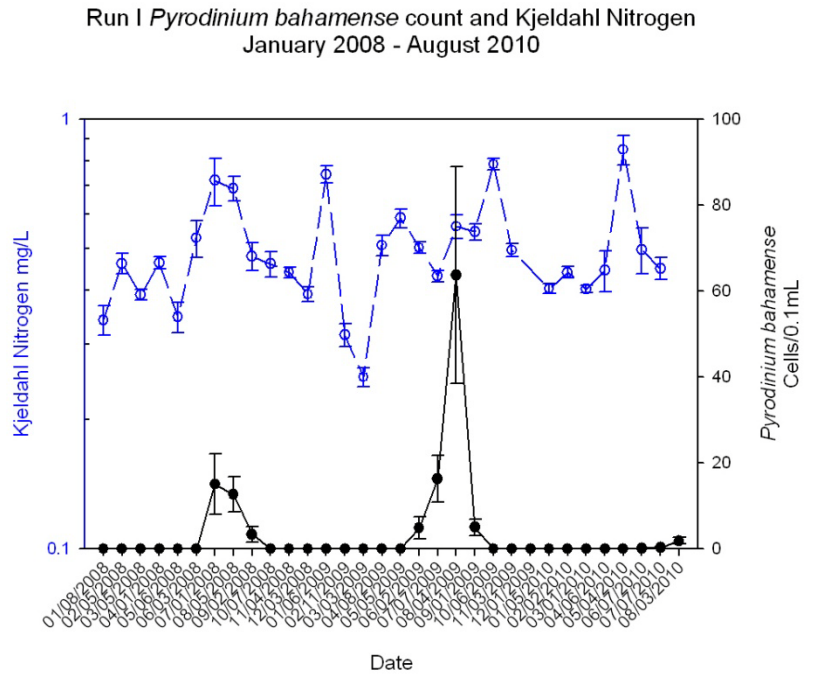

Figure 13 Monthly mean *Pyrodinium bahamense* cell counts and water sample Kjeldahl nitrogen values at EPCHC Old Tampa Bay water quality monitoring stations from January 2008 – August 2010.

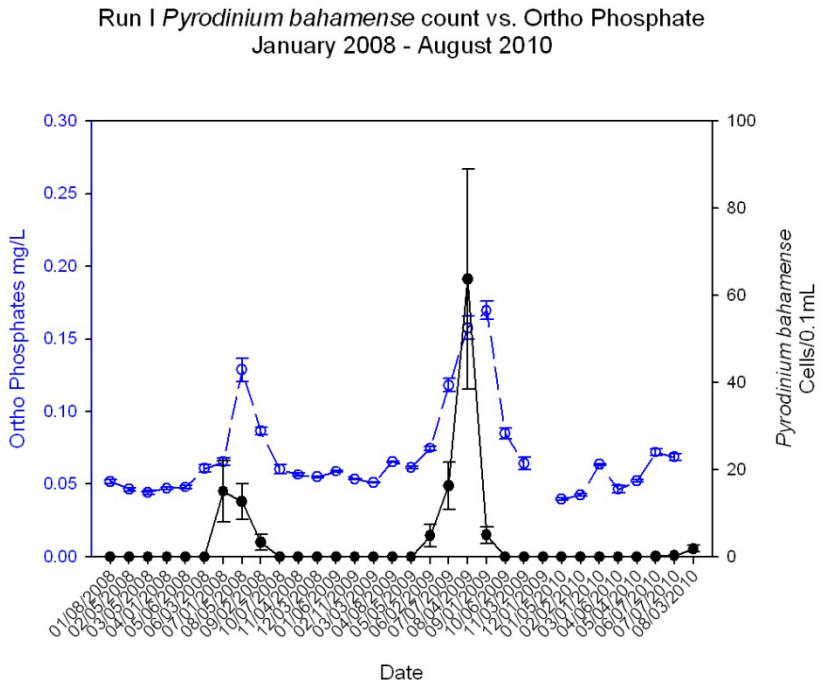

Figure 14 Monthly mean *Pyrodinium bahamense* cell counts and water sample ortho-phosphate values at EPCHC Old Tampa Bay water quality monitoring stations from January 2008 – August 2010.

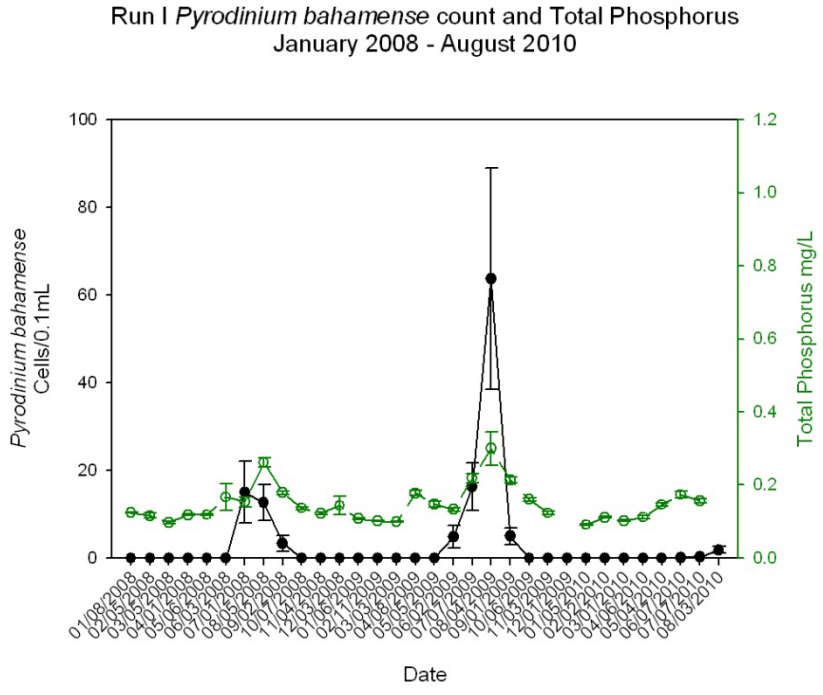

Figure 15 Monthly mean *Pyrodinium bahamense* cell counts and water sample total phosphorus values at EPCHC Old Tampa Bay water quality monitoring stations from January 2008 – August 2010.

Discussion and Conclusions

The occurrence of *Pyrodinium bahamense* blooms in Old Tampa Bay has shown an increase in frequency and intensity over the past decade. These blooms have tended to start in the early summer in the northwest area of Old Tampa Bay around Safety Harbor, near the west side of the Courtney Campbell Causeway, and the Bayside Bridge (EPCHC unpublished data). The distribution of *Pyrodinium bahamense* cysts also shows highest concentrations in the sediments in these areas. The presence of the Courtney Campbell Causeway spanning the bay from east to west, and of the Bayside Bridge isolating Largo Inlet on the west side of the bay are major impediments to water flow in these areas of Old Tampa Bay. The restricted flow has the effect of increasing the residence time of surface waters and causing the deposition of silty, high organic sediments. These conditions also favor the settling of dinoflagellate cysts in the sediments, which serve as a seed bank for future blooms.

The lower $\delta^{15}N$ signatures in the sediments in these areas indicate the possible deposition of nitrogen from fertilizers coming in from terrestrial runoff (Peebles et al. 2010). Additionally, the outfall from the City of Clearwater WWTP discharges near the Bayside Bridge may contribute to the nutrient load in this area. The overall trend of higher $\delta^{15}N$ values at the central and lower Old Tampa Bay sites reflect a greater marine influence due to tidal flushing in those areas (Peebles et al. 2010). The potential flux of nitrogen from the sediments to the overlying water column may further stimulate bloom conditions when temperature and salinity reach optimal conditions.

Analysis of the *Pyrodinium bahamense* population trends from January 2008 – August 2010 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* was 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 (May/June). It is possible that the start of the

rainy season resulted in a pulse of nutrients from terrestrial runoff which could have fertilized the bloom.

Despite the lower $\delta^{15}N$ values at the sites of high cyst densities, there was no clear correlation between different nitrogen nutrients (NO_2 + NO_3, Kjeldahl nitrogen) and *Pyrodinium bahamense* cell counts during the January 2008 – August 2010 time period. This may have been due to the variability of nitrogen values over time, with high values occurring when other water quality conditions were not favorable for bloom conditions. Total phosphorus and orthophosphates did appear to correlate with high *Pyrodinium bahamense* cell counts, even though phosphate is not considered to be a limiting nutrient in Tampa Bay. There was an apparent lag time between peak *P. bahamense* cell counts and orthophosphate, with maximum orthophosphate values occurring approximately one month after the bloom reached its zenith. The fact that orthophosphate levels followed the peak cell counts suggests that the increase in orthophosphate was due to the decomposition of the *P. bahamense* cells as the bloom started to decline, rather than being a contributing factor to the bloom. No clear link between *Pyrodinium bahamense* abundance and nutrients (specifically total phosphorus) was established in the study by Phlips et al. (2006), but they did note that highest cell densities occurred when total phosphorus exceeded 100 μg/L and total nitrogen was above 600 μg/L (Phlips et al. 2006).

Although *Pyrodinium bahamense* has been recorded on the west coast of Florida since the mid-20th Century, its occurrence in Old Tampa Bay appears to be a more recent phenomenon. The EPCHC water monitoring records go back to the mid-1970's, and *Pyrodinium bahamense* had not been recorded in the EPCHC phytoplankton count data prior to 2000. This may in part be due to misidentifications of this species in the past; however, *P. bahamense* is a relatively large dinoflagellate and has very distinguishing morphology so this does not seem to be a probable scenario. The EPCHC data since 2000 reveals at least five occurrences of high abundances of *P. bahamense* in Old Tampa Bay: October 2000, August/September 2001, August/September 2005, July-September 2008, and June-September 2009. Badylak et al. (2007) also recorded *Pyrodinium bahamense* densities up to 350 cells/ml at their sampling site in Old Tampa Bay during May-October 2002. The fact that *Pyrodinium bahamense* was not recorded at the EPCHC monitoring

sites during the corresponding time period may be due to differing sampling methods or to the patchiness of the cell distribution.

Low *Pyrodinium bahamense* cell counts were found during the summer of 2010 even though there were large blooms during the previous two summers. Surface water temperatures during the 2010 summer months were well within the optimal range to support a bloom and nutrient levels were also relatively high. One possible explanation may be the relatively low surface water salinities which were in turn due to high rainfall that summer. Heavy rainfall earlier in the year, during the normal dry season months, may also have washed in nutrients before water temperatures where high enough to trigger a bloom. This could have effectively eliminated the normal nutrient pulse experienced at the beginning of the summer wet season that might initiate a bloom.

The observed pattern of increasing frequency and intensity of *Pyrodinium bahamense* blooms since 2000 is expected to persist if the current trend of increasing summer temperatures continues due to climate change. Summer blooms in Old Tampa Bay may spread to other areas in Tampa Bay which have poor water circulation, potentially creating new cyst seed banks for future blooms. Findings from this pilot study are intended to provide baseline data for future monitoring of *Pyrodinium bahamense* blooms in Old Tampa Bay and for the management of future bloom events.

Acknowledgements

Funding was provided by the Tampa Bay Estuary Program (PO 6578). Dr. Karen Steidinger and Jamie Williams (Florida Wildlife Research Institute) provided training and QA/QC for the cyst extraction and counting procedures. Dr. Ernst Peebles and Elon Malkin (USF College of Marine Science) conducted the stable isotope analysis. Sara Markham did the silt/clay analysis and database management and Amanda Stewart did the sediment organic analysis. The EPCHC water lab processed the water chemistry samples. Barbara Goetting made the maps for this report and served as boat captain for the field sampling. Alex Roberts and Kieley Hurff assisted with the sampling preparations and field collecting.

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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 □m sieve into 150 ml beaker. Repeat with resulting filtrate using 90 □m sieve. Repeat again using 20 □m 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