Historical Pollution and Factors Influencing *Pyrodinium Bahamense* Algal Blooms in the Tampa Bay

Kaitlyn Newsome
Department of Soil and Water Sciences
University of Florida
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Introduction

Tampa Bay is a significant natural and economic resource for the state of Florida. As the state's largest open-water estuary, this waterbody serves as both a vital economic focal point for over 3,194,831 residents as well as provides a multitude of ecosystem services including tourism, fishing, and environmental habitats to numerous species of wildlife (Census Reporter 2019). From the 1950s-1980s, the Tampa Bay experienced dramatic water quality degradation resulting in numerous harmful algal bloom occurrences prior to the passing of key legislation in 1972 that facilitated the Bay's recovery. While water quality in the estuary has improved over the last five decades, non-point sources of nitrogen continue to pose a concern as they are now believed to account for over half of the total nitrogen load entering the watershed via urban and residential stormwater runoff resulting in extensive impacts to both natural and anthropogenic based systems and industries in the water body (TBEP n.d).

This paper aims to provide a historical overview detailing the importance of the Tampa Bay and the corrective actions implemented to-date prior to discussing harmful algal blooms (HAB), HAB generation cofactors, and excystment characteristics associated with a recently noted harmful algal bloom species in the Bay: *Pyrodinium bahamense*. While *Pyrodinium Bahamense* blooms have historically occurred on the western coast of Florida as well as in the Gulf of Mexico, it was not until recently that *P. bahamense* was confirmed in Tampa Bay. This dinoflagellate species is of primary health, economic and ecological concern in Tampa Bay due to its corresponding impacts on both overall organism health and biodiversity. In addition, this dinoflagellate species is identified as a primary harmful algal bloom species associated with extensive economic impacts in the area, contributing to an estimated 18 million dollars in commercial fishery losses ($27 million dollars today corrected using the July 2020 consumer price index for inflation) (Anderson et al 2000, BLS 2020). Review of the published literature and analyses associated with this algal species, indicate several bloom stimulation cofactors including: temperature, salinity, seasonal patterns, sediment resuspension rates, availability of inorganic nitrogen, and water residence times in specific regions of the Bay. These factors and causal relationships as related to *P. bahamense* will be discussed in greater depth.

<u>Background of Tampa Bay</u>

The Tampa Bay region is a commonly recognized, rapidly growing metropolitan area composed of several large cities including Tampa, St. Petersburg, and Clearwater (Figure 1). This area has experienced rapid population growth since the early 1950s. Recent migration data indicate that nearly 41,800 people moved to the area between July 1, 2018 and July 1, 2019 (Tampa Bay EDC 2020). The Tampa Metropolitan Statistical Area "MSA" includes Hillsborough, Pinellas, Pasco and Hernando counties, ranking fifth in the United States for net migration (Tampa Bay EDC 2020).

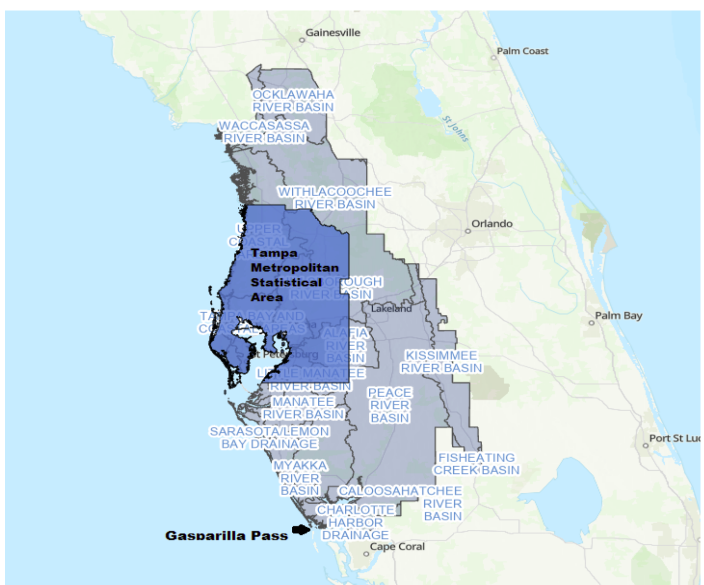


Figure 1: Overview of Tampa Bay Area (Source: SWFWMD ESRI GIS Tool)

From a geographical perspective, the Tampa Bay area is located adjacent to Tampa Bay from which its name was derived. As a result of extensive population growth and urban development, dredge and fill techniques were historically utilized in the 1950s and 60s to move material from shallow areas of the bay to extend the shoreline (Morrison, Yates n.d). This material was used to construct bridges, residential communities, power-plants, ports, and additional infrastructure (Janicki et al 1995). Approximately 9,800 acres of a shallow water area located within the Boca Ciega Bay, Old Tampa Bay and Hillsborough Bay were filled or channelized for urban and port development during this period (Morrison, Yates n.d.).

Many rivers and waterways feed into the bay including the Hillsborough, Alafia, Manatee, and Little Manatee Rivers. The Tampa Bay Watershed consists of approximately 6,400 square miles of coastal drainage and associated waters from the Gasparilla Pass to (and including) the Withlacoochee River Basin (Figure 1) (USGS 2019). Tampa Bay is Florida's largest open-water estuary acting as a vital habitat for a variety of keystone species as well as an economic engine for tourism and the State of Florida. Despite being declared a "dead" estuary in the 1970s, most of the Tampa Bay estuary has achieved notable recovery despite a population increase of over 1 million people since that timeframe (Greening et al 2016). One of the most notable environmental impacts associated with Tampa Bay during the 1970s was related to wastewater discharges and high nitrogen load influxes into the bay from wastewater treatment plants. Specifically (from the 1950s to the 1980s) past water quality declines resulting in Harmful Algal Blooms (HABs) were associated with the loss of nearly half (19,000 acres) of the bay's seagrasses as seen in Figure 2 (SWFWMD 1999). These blooms were later attributed to cyanobacteria (also known as blue-green algae) characterized by a blue-green tinted layer that normally occurs in nutrient-dense freshwater systems (TBEP 2019).

Following implementation of legislation (including the Wilson Grizzle Bill) and expansion of the Clean Water Act of 1972, major upgrades and technological advancements were applied to sewage treatment plants to reduce nitrogen loads in wastewater effluents (TBEP 2019, SWFWMD 1999). The establishment of the State's Surface Water Improvement and Management (SWIM) programs aided in addressing seagrass conservation on a state level by promoting nutrient load reductions in an attempt to reduce eutrophication impacts to the bay (Dawes et al 2004). In a 2014 technical report published by Sherwood and Kaufman, seagrasses were noted to cover 40,295 acres of the Tampa Bay (Sherwood, Kaufman 2016). In a subsequent evaluation performed in 2018, researchers mapped approximately 40,618 acres of seagrass, nearly doubling the acreage mapped in the early 1980s (Figure 3) (TBEP 2019).

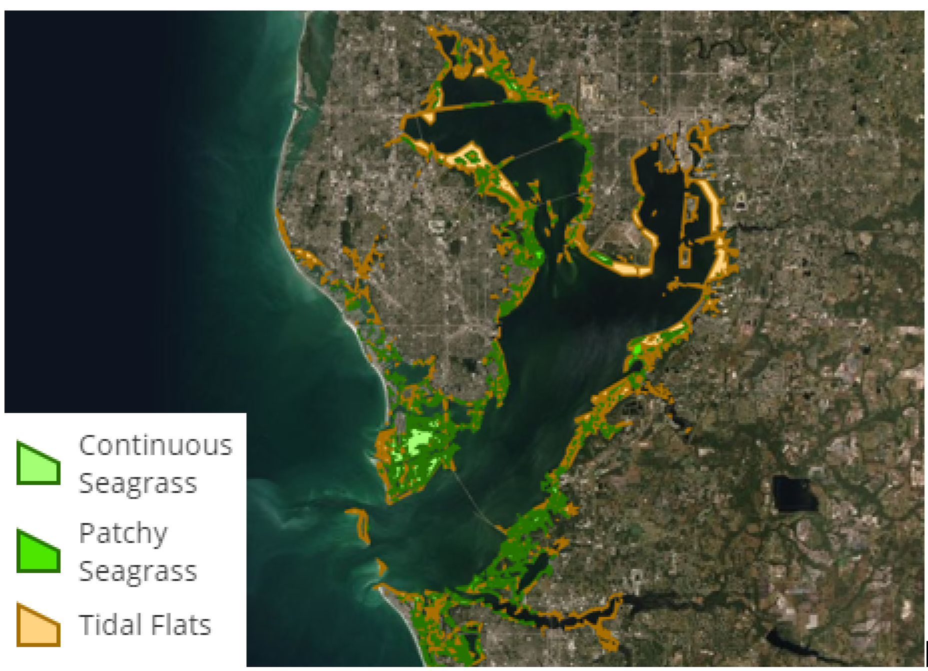


Figure 2: Seagrass Distribution in 1988 (Source: SWFWMD n.d.)

Figure 3: Seagrass Distribution in 2014 Following Implementation of SWIM. (Source: SWFWMD n.d.)

Since the 1970's wastewater treatment plant upgrades, dominant sources of nitrogen entering into the Bay have shifted to primarily nonpoint source runoff/leaching and atmospheric deposition. The Tampa Bay Estuary Program estimates that 60% of the nitrogen that entered Tampa Bay since 2010 was derived from non-point sources associated with urban and residential stormwater runoff (TBEP n.d). Areas such as the Manatee River continue to experience little progress in seagrass restoration due to an influx of nonpoint source-based nitrogen from the highly urbanized watershed surrounding Tampa Bay (Sherwood and Kaufman, 2016).

In addition, one-quarter of the current nitrogen load entering Tampa Bay is attributed to air pollution caused by power plants and automobiles (TBEP n.d, Fig. 4). Emissions from nitrogen oxides (NO_x) are primarily in the form of Nitrous Oxide "NO". Combustion conducted under high temperatures results in the conversion of N_2 gas to reactive NO_x. Per the Zeldovich Mechanism (a chemical mechanism used to describe the oxidation of nitrogen and NO_x formation), NO_x is produced and limited by the amount of free oxygen in air at temperatures above 1300 degrees Celsius (EPA 1999). Temperatures at or below 760 degrees Celsius result in either reduced or minute NO_x generation rates (EPA 1999). Anthropogenic sources now account for over 80% of USA NO_x emissions with a portion of the NO_x concentration being removed from the atmosphere via deposition as nitrate (NO_3^-) (WISC 2016). Multiple power plants are located in the Tampa Metropolitan Statistical Area "MSA" including Crystal River (Hernando County), Bartow (Pinellas County), Big Bend (Hillsborough County), Bayside (Pinellas County), Anclote (Pasco County) and Shady Hills Generating Stations (Pasco County) as well as three independent waste-to-energy plants located in Hillsborough, Pasco and Pinellas County. These emission sources account for approximately 9,765 tons per year of NO_x generation locally.

2019 Nitrogen Oxides Emissions at Electric Power Plants

Plant Name	Aggregated Fuel Group	Generation (k	Fuel Consumption for Electric Generation (MMBtu)	Fuel Un	CEMS Reported Plant Level NOx Emissions (Tons)	Selected NOx Emissions (Tons)
Crystal River	GAS	3,975,941,000	2,658,402	Mcf	2,944.30	9.73
Crystal River	GAS	6,746,584,000	70,588,100	Mcf	2,944.30	2,584.77
Crystal River	COAL	4,298,914,900	44,098,926	Tons	2,944.30	349.18
Crystal River	PET	22,699,100	238,521	Barrels	2,944.30	0.62
P L Bartow	GAS	1,807,683,000	901,516	Mcf	734.74	1.44
P L Bartow	GAS	4,008,538,000	45,249,131	Mcf	734.74	724.38
P L Bartow	GAS	19,879,524	319,965	Mcf	734.74	5.98
P L Bartow	PET	3,377,476	56,213	Barrels	734.74	2.94
Big Bend	GAS	3,682,000	55,907	Mcf	2,276.50	16.50
Big Bend	COAL	1,121,880,294	13,118,340	Tons	2,276.50	1,044.96
Big Bend	GAS	2,724,439,706	32,563,374	Mcf	2,276.50	1,215.05
H L Culbreath Bayside Power Station	GAS	2,763,725,000	0	Mcf	362.83	0.00
H L Culbreath Bayside Power Station	GAS	5,077,588,000	58,069,095	Mcf	362.83	361.08
H L Culbreath Bayside Power Station	GAS	21,206,000	239,634	Mcf	362.83	1.75
Anclote	GAS	2,278,498,000	25,640,356	Mcf	1,335.31	1,335.31
Pasco Cnty Solid Waste Resource Recovery	GAS	363,774	6,969	Mcf		0.66
Pasco Cnty Solid Waste Resource Recovery	OTHER	185,159,226	3,470,675	Tons		469.58
Hillsborough County Resource Recovery	OTHER	230,406,211	4,934,251	Tons		429.06
Pinellas County Resource Recovery	GAS	12,110	245	Mcf		0.02
Pinellas County Resource Recovery	OTHER	444,934,890	8,996,487	Tons		1,167.40
Shady Hills Generating Station	GAS	252,481,558	2,760,994	Mcf	44.12	43.88
Shady Hills Generating Station	PET	490,442	5,475	Barrels	44.12	0.24

Figure 4: NO_x Emissions In The Tampa Bay Metropolitan Statistical Area for CY 2019 (EIA 2019)

Nutrient Pollution

While Harmful Algal Blooms occur naturally, nutrient pollution associated with human activities can exacerbate the problem leading to more frequent and severe blooms as a result of eutrophication (EPA 2019). Eutrophication is defined as "an increase in the rate of supply of organic matter to a given ecosystem" (Nixon 1995), which is controlled by nutrient availability, temperature, and sunlight. Numerous factors contribute to increases in the supply of organic matter to coastal aquatic environments, but the most common is attributed to infiltration of nutrients from agricultural crop production areas (Nixon 1995). Nixon's study and geographical analysis demonstrated that atmospheric nitrogen impacts (related to fossil fuel combustion) can also act as an important anthropogenic source aiding in the supply of nutrients to fuel eutrophication within a water body. Following an evaluation of nitrogen emissions by source, he observed that the geographical distribution of nitrogen emission intensity (as it relates to fertilizer), livestock waste, and fossil fuel combustion corresponded to many locations where coastal marine eutrophication had become a recent issue (Nixon 1995).

What Are Harmful Algal Blooms?

Algal Blooms are characterized as overgrowths of algae, which causes the waterbody to appear colored green, blue, red, or brown depending on the dominant species. In freshwater habitats, algal blooms naturally form in warm, immobile water where nutrients and sunlight are available. In marine and brackish waters however, algal blooms can form in response to changing environmental conditions such as when nutrients accumulate near the surface of the ocean (due to upwelling), increases in sea surface temperature, or changes in sea currents (CDC 2021). Some blooms appear as scum on the surface of water caused by specific algae species that, under favorable conditions, can produce dangerous levels of toxins in fresh or marine water creating dead zones, contaminating water and shellfish, killing animals, and sickening humans (NOAA n.d, EPA 2019). These blooms are referred to as Harmful Algal Blooms or "HABS". The generation and intensity of Harmful Algal Blooms is influenced by sunlight, warm temperatures, as well as available nutrients (nitrogen and phosphorus) (NIEHS 2021). HABS occur in fresh, marine, and brackish water bodies and are caused by numerous organisms including phytoplankton, cyanobacteria, benthic algae and macroalgae (NOAA 2019). Per the National Oceanic and Atmospheric Administration, dinoflagellates and diatoms (types of phytoplankton) are the most commonly found HAB species in marine and brackish waters. In the case of Tampa Bay, the most common algal blooms observed off the coast include those due to *Karenia brevis* (also known as “red tide”), Pseudo-nitzschia and *Pyrodinium bahamense*, a naturally occurring dinoflagellate with no recorded occurrences in the bay from 1983 through Summer 2000 (Boler n.d).

Harmful Algal Bloom Concerns

Harmful algal blooms are of national concern due to potential human health impacts and effects on aquatic ecosystems. They also impact the economic health of coastal communities dependent on fishing and tourism due to their ability to produce toxins (NOAA n.d). While not all algal species produce toxins, some species (under the right conditions) generate multiple toxin profiles characterized by different chemical structures and masses (Virginia Institute of Marine Science n.d). Several dinoflagellate genera including *Alexandrium*, *Gonyaulax*, *Gymnodinium* and *Pyrodinium* are recognized by their red or brown color and are associated with the production of saxitoxin, a Paralytic Shellfish Toxin “PST” (Vale 2010, Perini et al 2014, Costa et al 2015). Another Saltwater species, *Karenia brevis* (commonly referred to as red-tide) produces brevetoxin, a neurotoxin that targets both the nervous and respiratory systems (NIEHS 2021). Certain diatom species such as *Pseudo-nitzschia* can produce domoic acid, a neurotoxin that when ingested, can result in Amnesiac Shellfish Poisoning “ASP” (NIEHS 2021).

Economic impacts

An economic study conducted by Donald Anderson in 2000 estimated the annual economic impacts from HABs from 1987 through 1992. Commercial fishery impacts from HABs due to losses of fish and shellfish varied from $13 to $25 million dollars with an average annual impact of $18 million dollars in the United States (approximately $27 million dollars today corrected using the July 2020 consumer price index "CPI" value for inflation) (Anderson et al. 2000, BLS 2020). The study noted that during the period between 1987-1992, the average public health impacts from HABs ranged from $18 million to $25 million dollars, averaging $22 million over the six-year interval ($33 million dollars corrected). Anderson's research also revealed an average annual recreation-tourism economic impact of $6.6 million with recorded high values of over $29 million dollars in some states ($10 - $43 million dollars corrected).

In a 2007 public cost study by Morgan et al. (2007) investigating economic impacts associated with red tides in Florida, nine county administrators and 28 municipalities were selected within Okaloosa, Franklin, Gulf, Pinellas, Manatee, Sarasota, Charlotte, Lee and Collier counties. These counties were chosen due to common red tide bloom occurrence and tourism popularity (Morgan et al 2007). This study found that the majority of funds for red-tide related cleanup were generated by tourism tax dollars. Only two counties relied on county taxes and/or fee revenues. Overall, four counties and two cities were able to provide actual dollar amounts specific to red tide events on public beaches indicating a total of $653,890 spent over the 2004-2007 time period, with total expenses per event (including labor, equipment, supplies and vendor fees) ranging from $11,114 to $250,000 (Morgan et al 2007). Sarasota County for example, spent an average of $4.87 per linear foot of beach for the labor and equipment necessary to remove dead marine life resulting from a single red tide event that occurred from October 2006 through February 2007 (Morgan et al 2007).

Impacts on Organisms/ecosystems

While HABs impact coastal businesses, they may also affect humans and animals using the water bodies for recreation, crop production, and drinking purposes (CDC 2020). Organisms may be exposed to HABs by direct skin contact (during activities such as swimming), inhalation (droplets and mist), as well as ingestion of contaminated food or water (Koreiviene et al 2014). A common route of HAB toxin exposure to humans involves the consumption of contaminated finfish and shellfish, or through exposure to aerosolized Neurotoxic Shellfish Poisoning "NSP" toxin (Grattan et al 2016). Research conducted in 2015 by Lorraine Backer and others analyzed HAB-related illness data collected from 15 states (FL, IA, MD, MA, NC, NY, OR, SC, VA, WA, WI, CA, KS, MN and MT) from 2007-2011 totaling 4534 events. Freshwater sources accounted for 77% of the reports (3499 events) followed by brackish water (21%; 973 events), marine waters (2%; 82 events), and unknown water bodies (4%; 172 events) (Backer et al 2015). Backer's research noted toxin testing was performed in 3301 of the provided reports revealing microcystins detected as the most common toxin (2629; 80%) in samples followed by saxitoxins (311; 9%) and domoic acid (31; 1%) (the most common toxin found in marine samples).

Harmful Algal Blooms negatively impact ecosystems by reducing biodiversity within the subject area. Research conducted by Chai et al in 2020 focused on the impacts of *Prorocentrum donghaiense* (*P.donghaiense*) blooms in China, and revealed that the species diversity of eukaryotic plankton communities was significantly reduced during bloom occurrence (Chai et al 2020). Chai's research positively correlated species richness to RUE (ratio of phytoplankton biomass to total phosphorous) with cell density exhibiting notable negative correlation thereby reducing trophic niche habitation. In a separate study focused on a *Karenia brevis* bloom that occurred in 2005 along the Southwest Florida Coast, researchers analyzed data collected in independent fisheries from 1996 through 2006. A significant change in small and large bodied fish species was noted from Summer 2005 through Spring 2006 with pronounced declines in juvenile recruitment of fish species such as spotted seatrout, sand seatrout and red drum (Flaherty-Walia et al 2011). Flaherty-Walia et al indicated that while subadult and adult populations appeared consistent with previous year data, the community shifts and species-specific decreases appeared to be correlated with the *Karenia brevis* bloom. Perrault et al. (2020) collected plasma from foraging green turtles *(Chelonia mydas)* in Florida's Big Bend for analysis of toxins. They reported that 13 out of 21 turtles tested positive for one toxin, with domoic acid being most prevalent within the sample population (28.6%). Additional toxins were also detected including lyngbyatoxin-A, microcystins, nodularin, and okadaic acid (Perrault et al 2020). These results demonstrate that HAB toxins can still be detected within marine fauna following the dissipation of a bloom event indicating both continued environmental exposure and/or internal accumulation within tissues (Capper et al 2013, Perrault et al 2014).

While *Pyrodinium Bahamense* blooms historically occurred on the western coast of Florida as well as in the Gulf of Mexico, it wasn't until recently that *P. bahamense* was confirmed present in Tampa Bay. Per Karlen and Miller (2011), *P. bahamense* had been previously absent in Hillsborough County's Environmental Protection Commission (HCEPC) records dating back to the 1970s. The first reported occurrence of *P. bahamense* in the Bay was observed in September and October of 2000 in Middle and Old Tampa Bay (Boler n.d). Since 2000, numerous *P. bahamense* blooms have been detected in the Bay including those that occurred during the summers of 2001, 2005, 2008 and 2009 (Badylak et al 2007, Badylak and

Phlips 2009). Per a historical analysis performed by Karlen and Miller (2011), this trend appeared to be increasing in both occurrence and intensity over recent years.

Tampa Bay and *Pyrodinium bahamense Case Study*

Tampa Bay is a vital habitat to more than 200 species of fish and some of the most diverse colonial water bird nesting populations in North America, including nesting herons, egrets, gulls and shorebirds (FWS 2017). Additionally, sea turtles, dolphins, and up to one-sixth of Florida's West Coast manatee populations depend on Tampa Bay during the winter (FWS 2017). Given the importance of this natural resource and increased frequency of HABS, this study was designed to survey the literature relative to a primary algal species of concern in the Tampa Bay Region: *Pyrodinium bahamense*.

Pyrodinium bahamense is a tropical dinoflagellate (recognized for its noteworthy bioluminescence) that produces saxitoxin, a potent marine neurotoxin classified as a paralytic shellfish toxin (PST) which can result in paralytic shellfish poisoning (PSP) (Seliger et al 1970, Morquecho 2019, Cusick, Sayler 2013). This species was first described from New Providence Island (Bahamas) by Plate in 1906 prior to being later observed in Papua New Guinea (1972), Brunei and Sabah, Malaysia (1976), Central Philippines (1983), Northern Philippines (1987), Ambon, Indonesia (1996) among other locations (Maclean 1989, Wiadnyana 1996). There are two recorded varieties of *P. bahamense* - one primarily found in the Pacific Ocean (*var. compressum*) and the other which is found in the Atlantic Ocean and Gulf of Mexico, classified as *P. bahamense var. bahamense* (FWC n.d). This dinoflagellate species has been identified in periodic recurring blooms in coastal waters including Tampa Bay as well as the Indian River Lagoon (Badylak et al 2004, Karlen and Miller 2011, FWC n.d). Historically, it was believed that *P. bahamense* was nontoxic. However it was later reported that var. bahamense detected in

Florida waters can also produce a form of paralytic shellfish toxin “PST”, called Saxitoxin, which when ingested can result in Paralytic Shellfish Poisoning (Landsberg et al 2006, Usup 2011, FWC n.d). *P. bahamense* is characterized by a simple toxicity profile with many isolates producing only dc-STX, STX, neoSTX, B1 and B2 toxins (Usup 1994)[1] (Figure 5).

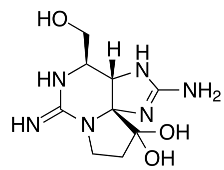
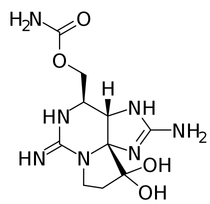
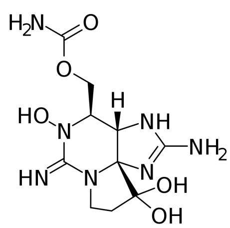
Toxin Name	Molecular Profile	Toxin Classification	Synonyms/Types
dc-STX “Decarbamoylsaxitoxin”		Neurotoxin; Binds and Blocks Sodium Channels	dcSTX-saxitoxin, decarbamoylsaxitoxin, decarbamylsaxitoxin
STX “Saxitoxin”		Carbamate Compound; Neurotoxin; Binds and Blocks Sodium Channels	50 types of structurally related toxins including itself, NSTX, GTX and dcSTX.
neoSTX “Neosaxitoxin”		Carbamate Compound; Neurotoxin; Binds and Blocks Sodium Channels	Parent Compound: STX; Neurotoxic Alkaloid

Figure 5: Saxitoxin and Related Derivative Forms

Landsberg et al. (2006) evaluated toxin profiles associated with blooms of *var. bahamense* in the Indian River Lagoon “IRL”. The primary toxins detected in puffer fish tissues, clonal cultures, and bloom samples from the IRL included STX, dc-STX, and B1 (Landsberg et al 2006). Paralytic shellfish poisoning is associated with the consumption of bivalves such as mussels, clams, and oysters contaminated by toxins in marine waters. The two most common toxins associated with this poisoning include saxitoxin and gonyautoxin which are produced and

[1] Analytical information and related molecular profiles were not available for B1 or B2 toxins.

subsequently bioaccumulated by microalgal dinoflagellates (Fox 2012). These toxins are heterocyclic compounds that block nerve and muscle action potentials by binding to voltage-gated sodium channels thereby blocking conductance and preventing impulse generation in nerves and skeletal muscles (Fox 2012, Hambright et al 2014). Paralytic shellfish poisoning is more severe than neurotoxic shellfish poisoning resulting in many neurological symptoms within humans including nausea, vomiting and/or diarrhea. Symptoms such as ataxia, vertigo, dysphagia, dysarthria, dysmetria, blindness, tachycardia and burning sensations of the face and extremities can arise within minutes to hours after the ingestion of contaminated shellfish (Fox 2012, Nguyen et al 2020). Following initial poisoning, an individual may be administered supportive therapy. Approximately 6% of patients (may be as high as 44%) however, encounter respiratory depression or failure resulting in death within 12 hours of symptom onset (Fox 2012). Saxitoxin has a lethal human dose of 0.2 mg for the average adult and is 1000 times more toxic than Sarin nerve gas (University of Oslo 2014).

Saxitoxin impacts on animals have also been reported, with symptoms including incoordination, recumbency and death due to respiratory failure (Fox 2012). Mass animal mortality events attributed to either saxitoxin or PSP poisoning have been observed in Washington State (1942), Cape Cod Bay (1987), Cape Blanc, Africa (1997), and Costa Rica (2013, 2014, 2017) over the period of record (McKernan and Scheffer 1942, Reyero et al 1999, Geraci et al 2011, Barrientos et al 2019).

General Life Cycle of *Pyrodinium Bahamense*

The life cycle of *P. bahamense* is similar to many other dinoflagellate species. It has a heterothallic sexual cycle that produces a large spiny spherical cyst (Usup 2012). Per Karlen and Campbell (2012), *P. bahamense* exists in the thecate, free-swimming stage during the summer months. When water quality conditions become unsuitable, the cell protoplast detaches from the cell wall and emerges from the theca (Figure 6) (Karlen and Campbell 2012). At this stage, the protoplasm may enter the "Gymnodinoid" stage where it forms a motile cell (Buchanan 1968). As the avalvate protoplasm compacts, it forms a sphere surrounded by a translucent wall thereby creating a cyst (Karlen and Campbell 2012). In the cyst stage, *P. bahamense* is considered non-motile and begins accumulating in the bottom sediments of the water column (Karlen and Campbell 2012). Excystment may occur under optimal temperature and salinity conditions allowing for the protoplasm to detach and exit the cyst wall, becoming a Gymnodinoid once again. Thecal plates begin developing as the avalvate protoplasm enters the water column returning to its free-swimming thecate form once again (Karlen and Campbell 2012).

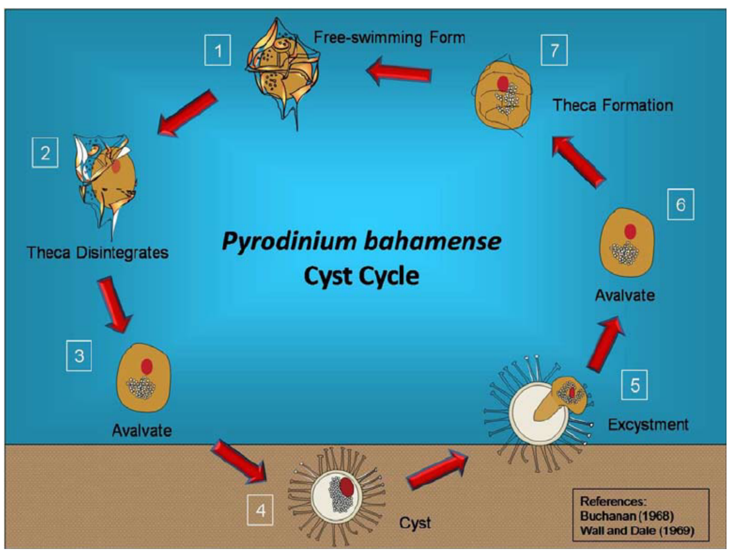


Figure 6: Pyrodinium bahamense cyst cycle (Karlen and Miller 2011)

Pyrodinium Bahamense Cyst Formation and Germination

One of the most notable factors associated with the *P. bahamense* life cycle includes the ability to produce resting cysts. These cells are characterized by the ability to survive in sediment creating "seedbeds" that are viable for extended periods of time. Consequently, these cysts may germinate later, releasing vegetative cells that can trigger subsequent blooms when external conditions are favorable (Anderson 1988). Cyst germination may only occur after dormancy is alleviated and external environmental requirements including temperature, light and oxygen are deemed suitable (Lopez et al n.d). More than 10% of the approximately 2000 identified marine dinoflagellate species produce cysts within their life cycle (Bravo and Figueroa 2014). Dinoflagellate cysts are considered to be particularly important due to their association with algae bloom location, timing, survival, disbursement, and toxin source (if a toxic species). However, data establishing defined relationships between these factors is limited. *P. bahamense* cysts (in their vegetative stage) have been detected in a broad range of locations including the Mexican Pacific Coasts, the southern Gulf of Mexico and along the Atlantic coast of the Mexican Caribbean Sea (Morquecho 2019).

A 2019 study performed by Lopez et al examined *Pyrodinium bahamense* cysts collected from the Tampa Bay Estuary in an effort to further understand dormancy patterns. *Pyrodinium bahamense* cysts that were immediately incubated following field collection displayed a seasonal pattern of dormancy and germination, similar to cell abundance patterns in the water column (Lopez et al 2019). Both newly deposited (surficial) and older (buried) cysts were confirmed to display similar germination patterns thereby suggesting that a common mechanism regulates cyst development and cycling (Lopez et al 2019). In addition, Lopez et al. (2019) noted that exposure of cysts to extended periods of cool and warm temperature altered the dormancy cycle for *P. bahamense* with cool temperatures preceding the cyst's emergence from dormancy.

An earlier 2017 experiment conducted by Lopez et al. (2017) analyzed cysts collected from August 2015 through September 2016 in the Tampa Bay Estuary. Both surface and sediment core sample-based cysts collected from Old Tampa Bay in August 2015, November

2015, and September 2016 were dormant and unable to germinate indicating seedbeds were not releasing elevated levels of vegetative cells during these periods (Lopez et al 2017). Lopez et al. (2017) later found that by February 2016, cysts collected from the field were beginning to germinate and by April 2016 - the time in which cells were first detected in the water body - mass excystment began to occur. Extended warm temperature exposure as observed later in the year however, appears to result in a return to primary or secondary dormancy in non-dormant cysts (Lopez et al 2017, 2019). Overall, Lopez et al's findings support the hypothesis that a seasonal cycle in cyst germination drives *P. bahamense* bloom occurrence in Tampa Bay with a secondary focus highlighting the induction of return to dormancy via extended warm temperature exposure as an important regulator in bloom cycles. These findings also potentially explain the delay from initial mass excystment to later HAB occurrence in Tampa Bay.

A separate germination and morphology study performed by Morquecho et al. (2014) used samples from Isla San Jose in the Gulf of California to evaluate *P. bahamense* morphological characteristics as well as optimal temperature and salinity conditions for cyst germination. They confirmed that morphological features and size of cysts agreed with previous descriptions, primarily with morphotypes previously found in the North Atlantic. Optimal salinities for excystment were observed to range from 20-35 parts per thousand (Morquecho et al 2014). Morquecho et al. also identified that the highest cyst germination rates occurred from 20 to 35 degrees Celsius with a peak noted from 25-30 degrees Celsius. Karlen and Campbell (2011) noted that the blooms that occurred in the summers of 2008-2009 within Tampa Bay were preceded by a drop in salinity (5-7 PSU), indicating a period of heavy rain at the beginning of the rainy season. Presumably, this rainy period flushed nutrients into the system from the adjacent land areas through leaching and surface runoff. Additional research conducted by Phlips et al.

(2006) further identified the optimal salinity range as it specifically related to Florida demonstrating a favorable broad salinity range of 10-45 PSU, with blooms only observed at levels above 20 PSU. Phlips et al. (2006) noted that *P. bahamense var. bahamense* cells were only detected at temperatures above 20 degrees Celsius, with blooms noted only when temperatures were recorded above 25 degrees Celsius. This data supports the previous historical understanding that *P. bahamense* var bahamense occurs in tropical and subtropical regions and highlights how these geographical distribution patterns could begin expanding as global temperatures increase. In addition, this data demonstrates the potential for the beginning of the rainy season to act as a fertilizing event for bloom initiation following the initial “surge” of urban and agricultural runoff that occurs from the adjacent watershed in early summer.

In addition to cyst dormancy and germination cycles, research has been performed to investigate cyst distribution patterns as it relates to *P. bahamense* blooms in the Tampa Bay. Karlen and Campbell (2012) mapped the distribution of *P. bahamense* cysts in Old Tampa Bay sediments to identify future bloom locations following a recent bloom in August 2011. Water quality and sediment features interconnected with the distribution of identified cyst beds were also investigated as part of the study. Sediment samples were collected during November and December 2011 from 25 sampling locations prior to cyst extraction and sediment analysis (Karlen and Campbell 2012). *Pyrodinium bahamense* cysts were identified at all 25 sites with densities ranging from 0.4 cysts/gram of sediment (Site ID: 11PCS38 - NW of Gandy Bridge) to 2,236.3 cysts/gram (Site ID: 11PCS03 - between the Howard Frankland Bridge and Courtney Campbell Causeway (Karlen and Campbell 2012). Following the analysis of sampling data, Karlen and Campbell determined that these two locations also corresponded to peak cell counts observed during both the August 2009 and 2011 bloom events. When compared to circulation models, notable surface current gyres and long water residence times were noted in these locations indicating potential cyst movement and deposition by tides, currents, and other transport mechanisms (Meyers and Luther 2008, Karlen and Campbell 2012). These findings suggested that winter time sediment resuspension and movement may be a vital factor in the distribution of cysts in Old Tampa Bay (Karlen and Campbell 2012).

In response to seasonal cycling, *P. bahamense* algal blooms have been most commonly noted during the summer rainy season with the highest occurrences observed during periods of high sunlight availability; most notably June - September (Grasso et al 2016, Karlen et al 2012, Marot 2017, Morquecho 2019). Cell density data was collected by Morquecho (2019) in an effort to characterize *P. bahamense* spatial and population variability within the Mexican Pacific and the Gulf of Mexico. Following analysis of the collected data, Morquecho noted a seasonal and longitudinal pattern with abundance, seasonality and species distribution decreasing from tropical to subtropical areas (Morquecho 2019). Another study by Grasso et al. (2016) analyzed *P. bahamense* concentrations to evaluate seasonal variability in Mosquito Bay, Puerto Rico. They found significant patterns of *P. bahamense* seasonally, with rain events observed to result in short-term density increases at higher water depths. A historical analysis performed by Karlen et al. (2012) for *P. bahamense* bloom occurrences in Old Tampa Bay analyzed seven separate bloom events from 2000-2011 (Figure 7). Of the reviewed bloom events, one bloom was observed from May - August (2011); two blooms from June - September (2009, 2010), one bloom from July - September (2008); one bloom from August - October (2005); one bloom from August - September (2001) and one bloom occurred in October of 2000. In response to persistent *P. bahamense* bloom events noted in 14 of the past 17 years by the Hillsborough County Environmental Protection Commission (HCEPC) and the Florida Fish and Wildlife Conservation

Commission (FWC) summer blooms have been noted to last for 4 ½ months on average (Lopez et al n.d).

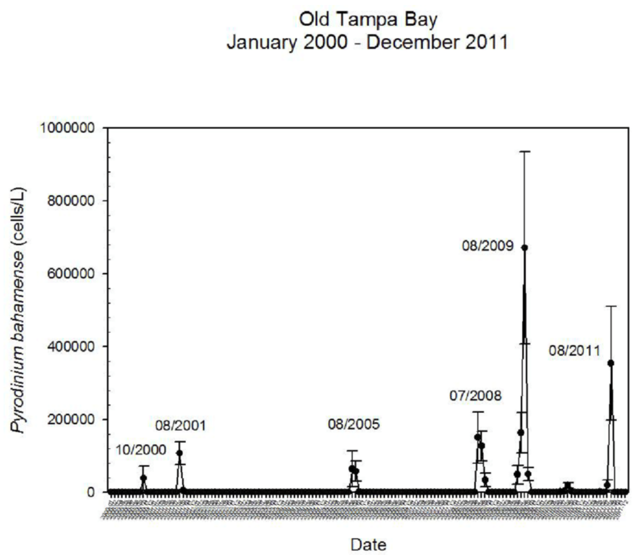


Figure 7: Recorded Pyrodinium Bahamense Concentrations Following Bloom Events Observed in the Old Tampa Bay from 2000-2012; Source: Karlen and Campbell (2012).

Another important factor to consider when evaluating *P. bahamense* is the correlation between peak biomass levels and nutrient concentrations in regions of high abundance (Phlips 2006). Water samples were collected three times per month from April 2002 through April 2003 from 3 sampling sites in Tampa Bay to investigate *P. bahamense* concentrations in response to changes in a multitude of water quality factors (Phlips et al. 2006). Of these sites, one sampling site was located in the mouth of the bay, one in the center and one in Old Tampa Bay - an area characterized by long residence time and low freshwater influx (Phlips 2006). During this period, sampling was also conducted on an additional 34 sites in the Suwannee River, 18 sites in Florida Bay, 7 sites in the Indian River Lagoon and 13 sites in North-Eastern Florida.

In addition to cell density, water samples were analyzed for nutrient levels to correlate to *P. bahamense* distribution and intensity. Phlips et al. (2006) found that the highest biomass was observed within inner Tampa Bay with cell densities of up to 380 cells per ml and cell biovolumes of up to 25 x 10^6 um per ml followed by the Indian River Lagoon at 777 cells per ml and cell biovolumes of up to 50 x 10^6 um per ml. (Phlips 2006). Over the course of study, Phlips noted that *P. bahamense var bahamense* represented over eighty percent of total phytoplankton biovolumes during both August and September 2002. Phlips (2006) also noted that *P. bahamanese* was observed in both the oligotrophic region of Florida Bay where mean total phosphorus concentrations were recorded at 8 ug/l as well as in Tampa Bay where total phosphorus concentrations exceeded 100 ug/l on multiple occasions. *P. bahamense* was also detected in both Sebastian and Ft. Pierce, Florida where total phosphorus was recorded at 330 ug/l demonstrating blooms can occur at both low and high TP levels (Phlips 2006). While Phlips (2006) was unable to clearly define a causal relationship between total phosphorus and *P. bahamense* abundance, a loose relationship was indeed established in Tampa Bay between peak density and TP concentrations (>100 cells/ml) during the summer in Old Tampa Bay (coinciding with location and timing of an active bloom).

This same study by Phlips et al. (2006) also investigated *P. bahamense* cell density as it related to total nitrogen concentrations observed during the subject period. While the observed relationship was less defined, they noted that *P. bahamense* was observed over a wide range of total nitrogen concentrations in the Indian River Lagoon, Tampa Bay and Florida Bay (Phlips 2006). In Tampa Bay, they observed a threshold total nitrogen concentration of about 600 ug/ml, similar to the threshold observed for total phosphorus. Overall, Phlips (2006) was unable to discern a clear trend among the sampling locations for mean total nitrogen, total phosphorus, or TN/TP ratios associated with *P. bahamense* with respect to the sampling data in question. Later studies performed by Karlen and Campbell (2011), were unable to identify a clear correlation between different nitrogen nutrients (NO_2 + NO_3, Kjedahl nitrogen) and *Pyrodinium bahamense* cell counts during the January 2008 - August 2010 time period. Total phosphorus however, did appear to correlate with high *P. bahamense* cell counts, even though phosphorus is not considered a limiting nutrient in the Tampa Bay (Karlen and Campbell 2011). Karlen and Campbell (2011) noted an apparent lag between peak *P. bahamense* and orthophosphate concentration, with maximum orthophosphate values detected approximately one month after the bloom's peak. This lag was attributed to natural *P. bahamense* cell decomposition processes occuring during the bloom as opposed to orthophosphate acting as a primary contributor to the bloom.

A study performed by Johansson (2014) attributed increases of total and dissolved organic nitrogen to rain induced nutrient loading to the bay segment from land runoff and atmospheric deposition. Johansson (2014) observed that ratios of total nitrogen and phosphorus in bay waters as well as the dissolved inorganic form ratios indicated that both total and inorganic nitrogen had been utilized to a greater extent than phosphorus forms by the time maximum *P. bahamense* abundance was observed. Ratios of total and specific forms of nitrogen and phosphorus indicated that inorganic nitrogen concentrations were very low relative to phosphate during the bloom period with inorganic nitrogen being utilized at greater proportions than phosphate during bloom development (Johansson 2014). Johannsson noted that the previous Phlips (2006) study performed was limited to the examination of total nutrient concentrations and that nutrient limitation assays conducted by the University of South Florida and the City of Tampa almost consistently showed that inorganic forms of nitrogen were the primary limiting nutrients (Vargo 1994, Johansson 2009) thereby agreeing with Johansson's findings that *P.*

bahamense growth (as analyzed via the averaged 2008, 2009, 2011 and 2013 bloom development data) was dependent on the availability of inorganic nitrogen (Johansson 2014).

Conclusion

While the Tampa Bay has made significant water quality improvement progress since the 1970s, it continues to be threatened by development of harmful algal blooms consisting of *Pyrodinium bahamense* as well as other species. Based on the reviewed, available studies published to-date, a developing correlation appears to exist and be supported for seasonality, temperature, salinity and inorganic nitrogen usage trends during *Pyrodinium bahamense* bloom development. Additional excystment and bloom generation factors that appear to demonstrate a causal relationship include sediment resuspension and water residence times as well. As the Tampa Bay Estuary continues to receive higher loads of non-point source nitrogen as a result of increasing population density in the Tampa Metropolitan Statistical Area (MSA), it will be imperative to fully account for all of the factors influencing formation of algal blooms (not just nutrient loadings) in order to formulate management plans for minimizing HAB impacts on this highly valued natural resource.

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