

PROJECT TITLE: Preliminary data analysis to test land-use influence on red tide in Choctawhatchee Bay, Florida

INTRODUCTION AND RATIONALE FOR PROPOSED WORK.

The coastal region of northwest Florida has been the site of a number of red tides associated with *Karenia brevis* blooms over the past decade, including a massive event in 1999 and 2000 that resulted in the closure of regional shellfish harvesting areas, widespread fish kills and dolphin mortality (NFWFMD, 2002). Ongoing monitoring efforts conducted in the region by the Florida Fish and Wildlife Research Institute (FWRI) between 2000 and 2006 have focused on several notable areas of concern, including Choctawhatchee Bay, the site of continuing observations of *K. brevis* cell densities as high as 2 million cells L⁻¹. We proposed to examine the relationship between land use patterns and the presence of *K. brevis* in coastal watersheds and regions adjoining coastal bays and bayous to better quantify how changing coastal land-use might contribute to the development of red tides by enhancing *K. brevis* growth and transition into blooms.

The northwest Florida coastal region includes a series of coastal embayments that are subjected to a broad range of environmental stressors, including urban and suburban discharge, agricultural inputs, and inputs from extensive military installments and nature reserves. The considerable spatial variability in land-use in this region has resulted in a spectrum of environmental impacts on, and biogeochemistry of, associated coastal environments. Choctawhatchee Bay, located in Okaloosa and Walton counties (30° 27' N, 86° 36' W; Fig. 1), epitomizes this heterogeneity in coastal land-use and water quality of associated coastal zones with large stretches of relatively undisturbed preserves adjoining subwatersheds of suburban development, including residential housing, commercial properties, and a golf course.



Figure 1. Choctawhatchee Bay location map. Source: Northwest Florida Water Management District (NFWFMD).

This dichotomy in land-use and apparent biogeochemical effects, including harmful algal bloom (HAB) production, is most striking in the bayous of western Choctawhatchee Bay, especially Cinco Bayou and Garnier Bayou (Fig. 2). A 2004 technical report by the Northwest Florida Water Management District (NFWFMD) classifies the majority of the Garnier Bayou watershed as “forested” or “wetland”, while the Cinco Bayou watershed is classified as “commercial” and “residential” (NFWFMD, 2004) The watershed for Garnier Bayou is largely undeveloped and encompasses a nature preserve associated with the Eglin Air Force Base (AFB)

facility, with a notable exception of Don's Bayou, a sub-bayou of Garnier Bayou, which drains a golf course facility. Cinco Bayou is located proximal to, and immediately south of, Garnier Bayou and the associated drainage is dominated by residential development and associated infrastructure (parks, roads, etc.).

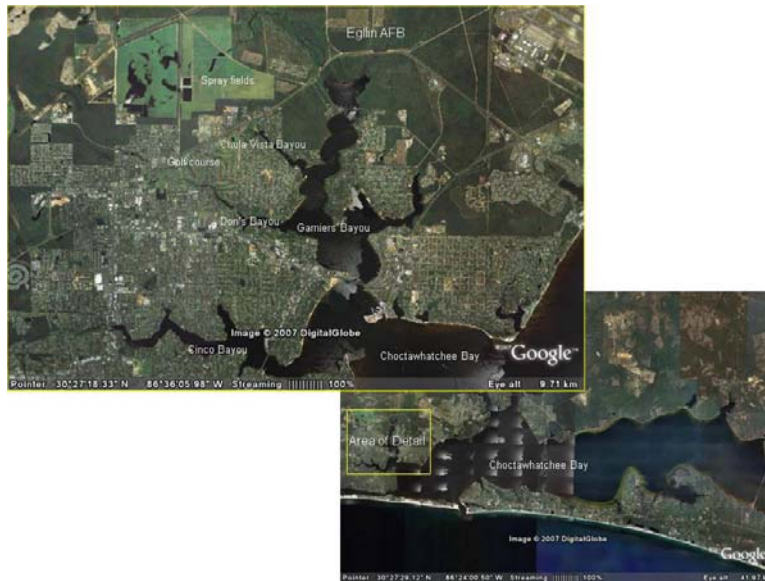


Figure 2. Western Choctawhatchee Bay site map with detail of Cinco Bayou, Garnier Bayou, and assorted site locations. Source: Google Earth.

Regional monitoring agencies, including FWRI and NFWFMD, identified red tides associated with *K. brevis* blooms as a regional issue of concern following the 1999-2000 season in which

Choctawhatchee Bay was affected by a series of red tides and mass mortality events involving fish, dolphins, and other wildlife.... From September 1999 through April 2000, approximately 144 bottlenose dolphins died in northwest Florida, generally coincident with the red tides (Table 8). Approximately 49 of these deaths were in Choctawhatchee Bay. Analysis performed by NOAA indicated the presence of red tide toxins in lung and stomach tissue in some of these animals. (NFWFMD, 2002)

Ongoing water quality monitoring in this region has identified considerable variability in the observation of *K. brevis*, the toxic dinoflagellate historically linked to red tide HABs throughout Floridian coastal environments on the Gulf of Mexico (e.g., Walsh et al. 2002; Brand and Compton, 2006; Hu et al., 2006). Of particular interest to this proposed study is the spatial variability revealed by historical observation of *K. brevis* in the western Choctawhatchee Bay bayous: in Cinco Bayou *K. brevis* concentrations may reach from 1,000 to over 2 million cells L⁻¹, contrasting with a general lack of detection of *K. brevis* in the proximal Garnier Bayou.

While the estuaries of NW Florida are monitored for *K. brevis* by FWRI, minimal research has been conducted in this region examining environmental conditions associated with bloom induction. We proposed to conduct a geospatial comparison of annual estuarine water quality and *K. brevis* cell density in these two bayous, to be paired with assay incubations, to assess the influence imparted by drainage basin land-use on estuarine biogeochemistry and resultant populations of *K. brevis*. These results would be disseminated using internet mapping services to inform the research and management communities, including regulators and regional planning agencies, as to the potential influence of drainage basin land-use on *K. brevis* populations in associated coastal and estuarine waters.

Controls on *K. Brevis* bloom dynamics

Growth rates of HABs have been linked to both biogeochemical and physical factors, including temperature, salinity, and turbidity. Various agents construed to be responsible for the spatial and temporal variability of *K. brevis* bloom events include coastal upwelling events (Lanerolle et al., 2006); nutrient-loading via submarine groundwater discharge (Hu et al., 2006), stormwater discharge (Neely et al., 2006), fluvial inputs, the establishment of water column stratification (Pitcher and Nelson, 2006), and the relative abundance of zooplankton grazers (Milroy et al., in press). Regardless of the actual mechanism (surface water, groundwater, or atmospheric), nutrient-loading is likely to be a major factor contributing to the destabilization of an aquatic system resulting in a HAB event.

Because nitrogen is typically a limiting nutrient in coastal and estuarine waters and is a common element in anthropogenic coastal inputs, nitrogen-loading has been identified as a particularly important biogeochemical variable affecting *K. brevis* growth rates and the development of harmful algal blooms. Furthermore, in a recent assessment of nitrate uptake by *K. brevis*, Sinclair and colleagues (2007a) noted a diurnal cycling in nitrate uptake with enhanced uptake in response to short-term (12-hour) exposure to low-nitrate environments. That study suggests that not only is overall nutrient-loading a likely cause of algal bloom development, but that the rate at which biogeochemical status changes from nutrient-limited to nutrient-replete might affect the ability of certain toxic algae to outcompete the resident populations and develop into HABs.

If nitrate limitation followed by exposure to nitrate-replete conditions favors algal growth, as suggested by Sinclair et al. (2007a), then coastal bayous are particularly likely candidates to favor the rapid growth associated with algal blooms. Coastal bayous experience considerable variability in nutrient concentrations due to their relatively small volumes and proximity to terrestrial sources of nutrients, including rivers and streams, atmospheric input, and submarine groundwater discharge (Paerl, 1997). The particularly dynamic environment of these coastal bayous may contribute to the establishment of rapid changes in nutrient concentrations as event-driven discharge replaces a significant volume of the bayou receiving waters with nitrogen-loaded waters.

Substantial surface and subsurface fluxes of water and inorganic nutrients from coastal storm events, including tropical storms, appear to be major contributing factors to the development of HABs in coastal and estuarine environments (Paerl et al., 2001; Hu et al., 2006). Storm fronts, particularly those that effect coastal zones with restricted surface water exchange,

contribute to a number of conditions that favor the development of HABs: rapid flushing of estuaries, nitrogen-loading from surface and subsurface inputs, water column stratification, and bottom water hypoxia (Paerl et al., 2001). A conceptual model of the interaction of these factors affecting HAB development and their relation to land-use is presented below (Fig. 3).

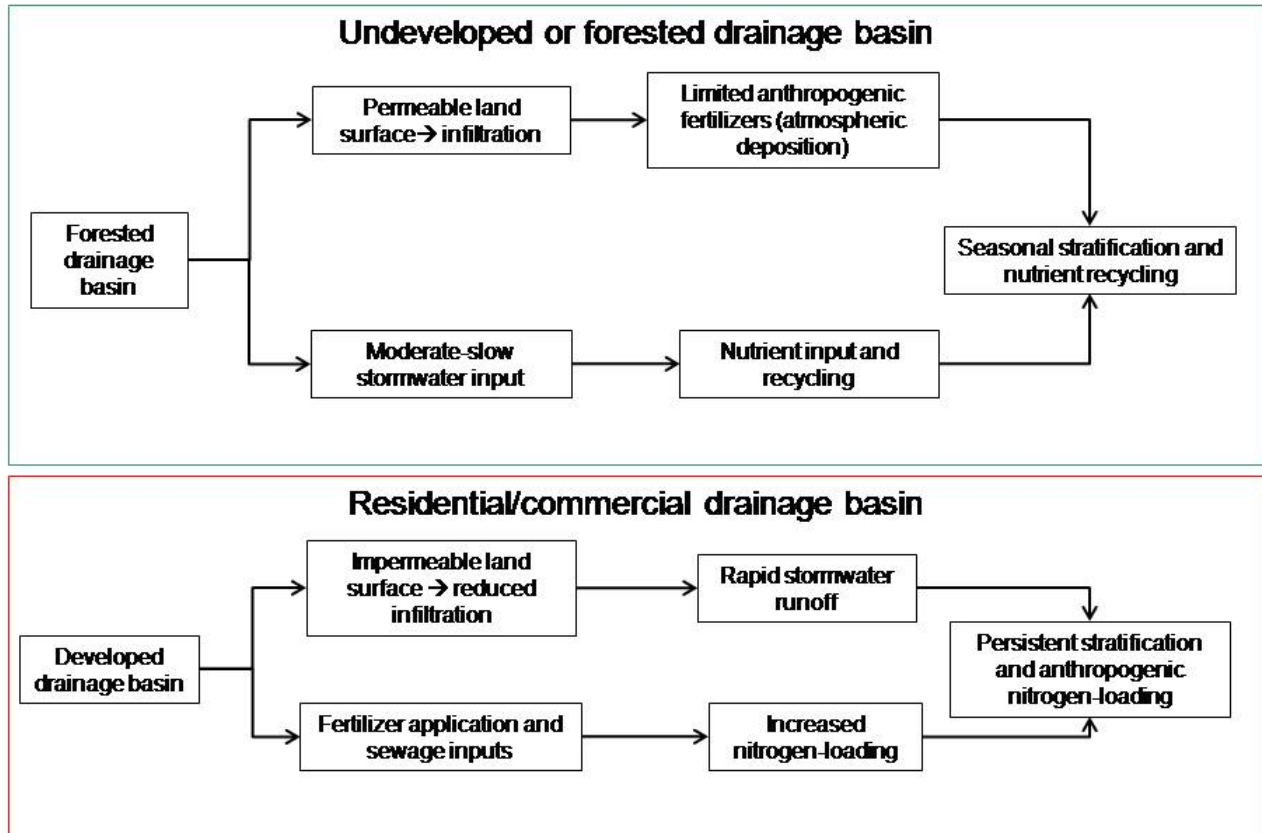


Figure 3. Conceptual model illustrating differences in physical and biogeochemical factors in forested/undeveloped (top) vs. developed (bottom) drainage basin and adjacent region.

Land-use in the associated drainage basin may affect the intensity of these effects via differential effects on nutrient-loading and transfer times between the watershed and receiving waters, with undeveloped sites more likely to delay the transfer of stormwater to the coastal zone and to transfer less dissolved inorganic nutrients to the discharged water. Developed regions may decrease the transfer time to coastal receiving waters due to the capping of permeable sediments by asphalt and other impermeable surfaces; water quality in these regions is likely to be affected by transfer of anthropogenic fertilizers and organic compounds from these regions.

While internal nutrient cycling can sustain excess algal growth, allochthonous nutrient loading is considered to be largely responsible for coastal eutrophication in general, and the generation of HABs, in particular. This nutrient-loading may take the form of surface water discharge from agricultural lands and residential developments or as submarine groundwater discharge from terrestrial aquifers. Interaction between meteoric water (i.e., precipitation) and fertilizers applied to residential, agricultural, and commercial (e.g., golf course) properties

degrades water quality, leading to elevated nutrient concentrations in the resultant surface water and groundwater, with nitrogen-loading being a particular concern.

Submarine groundwater discharge (SGD) is gaining increasing attention as an important mechanism for coastal nutrient-loading. Hu and colleagues (2006) hypothesize that SGD-related nutrient inputs were likely responsible for a 2005 *K. brevis* bloom in the Gulf of Mexico off of central Florida. Similarly, Swarzenski et al. (2006) attributed substantial loading of dissolved organic nitrogen (DON), ammonium (NH₄), and orthophosphate (PO₄) to SGD; the calculated nutrient-loading from SGD compared favorably to annual river nutrient fluxes.

Furthermore, *K. brevis* populations may be sensitive to photoinhibition. Using an ecological model to assess an historical Floridian *K. brevis* red tide, Walsh et al. (2002) noted the possibility of UV-B irradiation as a potential candidate for “cumulative biomass-dependent losses” responsible for observed algal mortality. Similarly, Sinclair et al (2007a and 2007b) attributed physiological changes related to nitrate uptake and growth rates in *K. brevis* to diel light exposure patterns.

Site description and history

Choctawhatchee Bay is located in the western Panhandle of Florida and spans both Okaloosa and Walton counties (Fig. 1). The area surrounding Choctawhatchee Bay exhibits considerable variability with respect to land use, with considerable portions bordered by undeveloped nature preserve, including a security buffer zone associated with the Eglin Air Force Base military installation, while other regions consist of considerable suburban development: housing developments, golf course, etc. More specifically, the Cinco Bayou drainage basin is dominated by residential development, while the majority of the Garnier Bayou drainage basin is located in the nature preserve associated with the Eglin AFB buffer zone. Notable exceptions to that generality are found in the southern portion of Garnier Bayou, which includes two sub-bayous that drain a golf course (Don’s Bayou) and a residential area and wastewater management spray fields (Chula Vista Bayou), as well as the Shalimar Yacht Basin on the southeastern edge of Garnier Bayou (Fig. 1).

Monitoring efforts conducted by the Florida Fish and Wildlife Research Institute (FWRI) between 2000 and 2006 revealed considerable spatial and temporal variability of *K. brevis* populations in the western Choctawhatchee Bay bayous as is summarized in Table 1.

Table 1. Summary of historical red tide monitoring in western Choctawhatchee Bay. Source: Florida Fish and Wildlife Research Institute.

Location	Sample inventory (2000-6)	<i>K. brevis</i> detections (>1000 cells L ⁻¹)	<i>K. brevis</i> range (cells L ⁻¹)
Cinco Bayou	24	15 (63%)	0-2,170,000
Garnier Bayou	38	2 (5%)	0-2,670
Garnier Bayou: Shalimar Yacht basin	6	4 (67%)	0-600,000
Western Choctawhatchee Bay	5	1 (20%)	0-229,600

Historical FWRI monitoring data for this area reveals several notable trends:

- Every detection of *K. brevis* occurred **between October and December** for the period 2000-2006
 - sampling conducted between January and September (40 samples throughout the study area) showed no *K. brevis*
- **Cinco Bayou** contained *K. brevis* at detectable levels (>1000 cells L^{-1}) in 63% of the samples collected between 2000 and 2006
- *K. brevis* was not detected in **Garnier Bayou** outside of two anomalous areas of concern:
 - the Shalimar Yacht Basin, where elevated levels were detected in four of six samples collected
 - a single site near the Eglin Parkway (SR 85) bridge over Garnier Bayou, where *K. brevis* was detected at low levels (1,300 and 2,670 cells L^{-1}) in two replicate samples collected on 6 December 2005
- Highest *K. brevis* concentrations (1.1 million and 2.17 million cells L^{-1}) were detected in two separate mid-estuary samples collected in Cinco Bayou on 26 October 2000.

In addition to the extended monitoring conducted between 2000 and 2006, a fall 1999 HAB event was identified by Florida FWRI throughout northwest Florida, including Choctawhatchee Bay (FWRI, 1999). *K. brevis* concentrations in western Choctawhatchee Bay were reported to be at the highest levels detected (over 1 million cells L^{-1}) and led to the closing of area shellfish harvesting areas between November 1999 and January 2000 coincident with the widespread regional red tides and associated fish and dolphin mortality discussed above.

Detection of *K. brevis*

Microscopic counts for the detection of *K. brevis* are time consuming and their accuracy requires a significant level of expertise (Millie et al., 1997). We employed newly developed molecular techniques for quantifying *K. brevis* cell densities based on the real-time reverse transcriptase (RT)-PCR method targeting the *rbcL* gene (large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBisCO gene) for *K. brevis* (Gray et al., 2003). This method has proven to be specific for *K. brevis* (e.g. no detected false positives against a wide variety of phytoplankton strains). The limit of detection (by dilution) is less than one *K. brevis* cell or less than 100 cells $liter^{-1}$ as the method is commonly used, providing much greater sensitivity than microscopic counts that have a limit of detection of 333 cells $liter^{-1}$ (Gray et al., 2003). The greater sensitivity of the RT-PCR method will allow us to determine whether *K. brevis* is truly absent from Garnier Bayou or whether the very low cell densities are never induced to bloom formation. Samples (typically 20–30 ml) were filtered onto polycarbonate filters and RNA extracted using RNeasy spin columns (Qiagen Corp., Madison, WI) The complete real-time RT-PCR protocol is detailed in Gray et al (2003). Briefly, PCR primers (forward primer, TGAAACGTTATTGGGTCTGT; reverse primer AGGTACACACTTTCGTAAACTA) with an internal fluorogenic probe are used to amplify a specific 91 base pair region of the *K. brevis rbcL* gene. Standard curves will be generated using samples with known cell densities of *K. brevis* culture cells. The PCR protocol used includes a 30 min precycling reverse transcription step at 45°, an initial denaturation step at 95° C for 10 min, and then 40 cycles of 95° C for 1 min, 55° C for 1 min, and 72° C for 1 min.

GOAL AND OBJECTIVES OF PROPOSAL.

Estuarine water samples will be analyzed by real-time reverse transcription (RT)-PCR to quantify the red tide (*K. brevis*) populations from water samples collected from two neighboring northwest Florida bayous during a recent (Oct-Dec 2007) red tide.

The objectives of the proposed work are as follows:

1. To determine short-term variability in the *K. brevis* population size of red tide organisms associated with documented fish kills in Choctawhatchee Bay, FL
2. To test recent RT-PCR methods on pre-existing samples collected before, during, and after a recent red tide event in Choctawhatchee Bay, FL.
3. To obtain pilot data for inclusion in extramural proposals targeted to assessing the influence of land-use practices on harmful algal blooms in northwest Florida estuarine and coastal waters.

PROJECT PERFORMANCE.

Water samples were collected from two western Choctawhatchee Bay bayous during the recent red tide season (October-December 2007). These two bayous (Garnier Bayou and Cinco Bayou) have a historical record of widely disparate properties with respect to red tides, with Cinco Bayou showing frequent red tides of intensities as high as 1 million cells L⁻¹, while the red tide-creating organism (*K. brevis*) is general not present at recordable levels in neighboring Garnier Bayou (FWRI, 1999).

Water samples (25 ml) were filtered onto 47 mm diameter 0.2 µm pore-size polycarbonate filters (Poretics; Osmonics Inc., Minnetonka, Minn.), folded, and inserted into 2 ml microfuge tubes and then stored frozen at -80°C for subsequent RNA extraction and analysis by a real-time RT-PCR method described by Gray et al. (2003).

A culture of *K. brevis* APC6 was obtained from the Florida Fish & Wildlife Conservation Commission and maintained at room temperature in f/2 medium (Guillard and Ryther, 1962) and 25µmoles m⁻² sec⁻¹ PAR on a 12-12 light – dark cycle. *K. brevis* standard curves were prepared by adding serial dilutions of known cell densities from cultures into 20 ml samples of Garnier Bayou water and then filtered onto 47 mm diameter 0.2 µm pore-size polycarbonate filters as above.

RNA was extracted from filters using a Qiagen RNeasy spin kit (Qiagen, Valencia, CA) using the manufacturer's protocol as modified by Gray et al (2003). Extracts were eluted in 40 µl of nuclease-free water and stored frozen until use.

Reverse transcriptase quantitative PCR (RT-QPCR) was performed using a TaqMan One-Step RT PCR Master Mix Reagent kit (Applied Biosystems, Foster City, CA). We used the PCR primer set described by Gray et al. (2003) which amplifies a 91 bp fragment of the *K. brevis* RuBisCO large-subunit gene (*rbcL*; forward primer, TGAAACGTTATTGGGTCTGT; reverse primer, AGGTACACACTTTCGTAAACTA; internal probe, FAM [6-carboxyfluorescein]-

TTAACCTTAGTCTCGGGTA-BHQ [Black Hole Quencher™]. RT-QPCR reactions consisted of 2µl of the target added to 23 µl of a onestep RT-PCR mixture prepared from 2 X RT-PCR TaqMan master mix (Applied Biosystems, Foster City, Calif.) containing each primer at a concentration of 1 µM, 2 mM MgCl, and a 0.5 µM concentration of the probe. The following cycling conditions were used: a precycling reverse transcription step of 45°C for 30 min; an initial denaturation step of 95°C for 10 min; and then 40 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Reaction mixtures were run on a Corbett Research (Mortlake, Australia) Rotor Gene RG-3000 Thermal Cycler. Data was analyzed using the Rotor-Gene 6 software.

The RNA extracted from the western Choctawhatchee Bay sites during the 2007-8 red tide season was amplified according to the methods described above. The results from RT-QPCR run are shown below (Figure 4). In the standards-only reactions (Figure 4A), replicate (or triplicate) cell number standards are shown to reach the cycle thresholds are reached between 22 and 35 cycles. Figure 4B displays the fluorescence profiles for both the standards and a series of unknown samples collected in the study area.

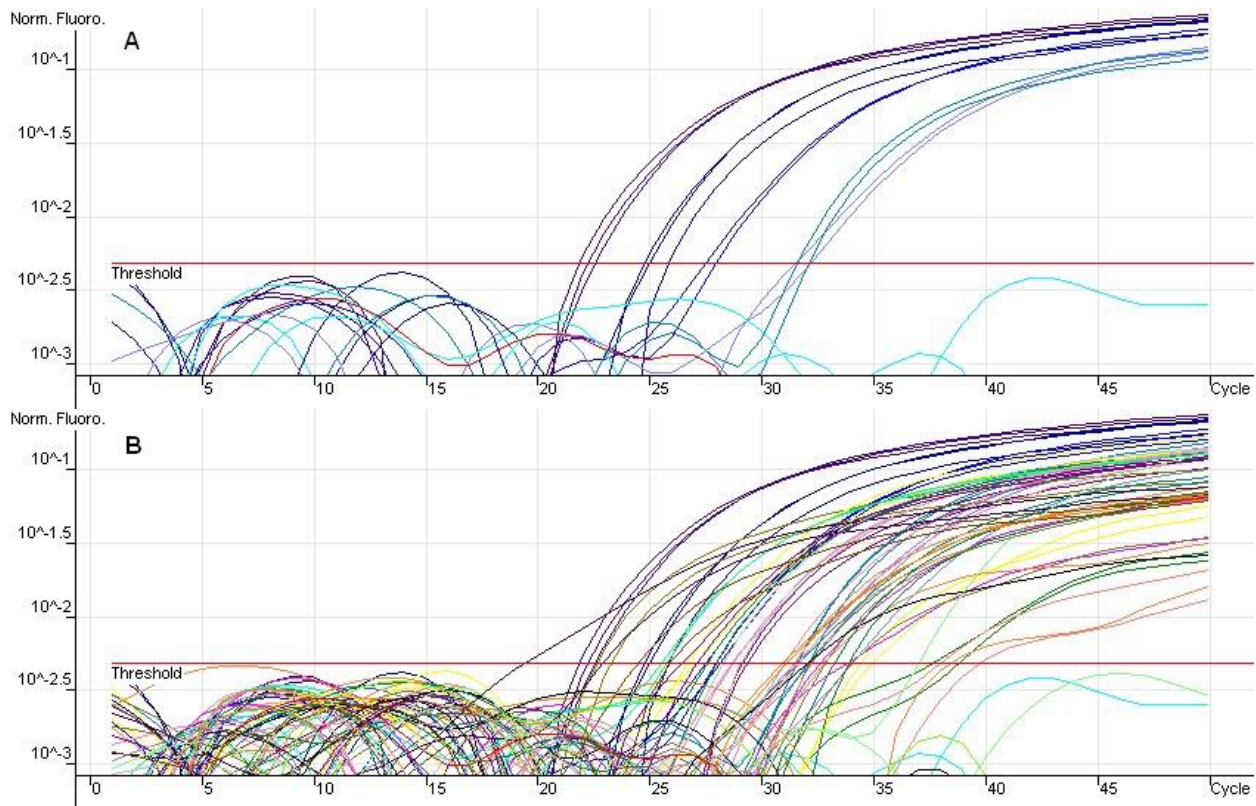


Figure 4. RT-QPCR quantification of *K. brevis* from western Choctawhatchee Bay samples. Figure A displays the quantification of standards only; Figure B displays both standards and unknown samples.

Standard curves of amplified *K. brevis* rbcL mRNA yielded linear relationships between number of cells extracted and the threshold cycle number for the QPCR reactions (Figure 5). Our complete analysis of all standard curves (data not shown) demonstrated that the response was linear through at least six orders of magnitude and technically able to detect less than one *K. brevis* cell per ml of water.

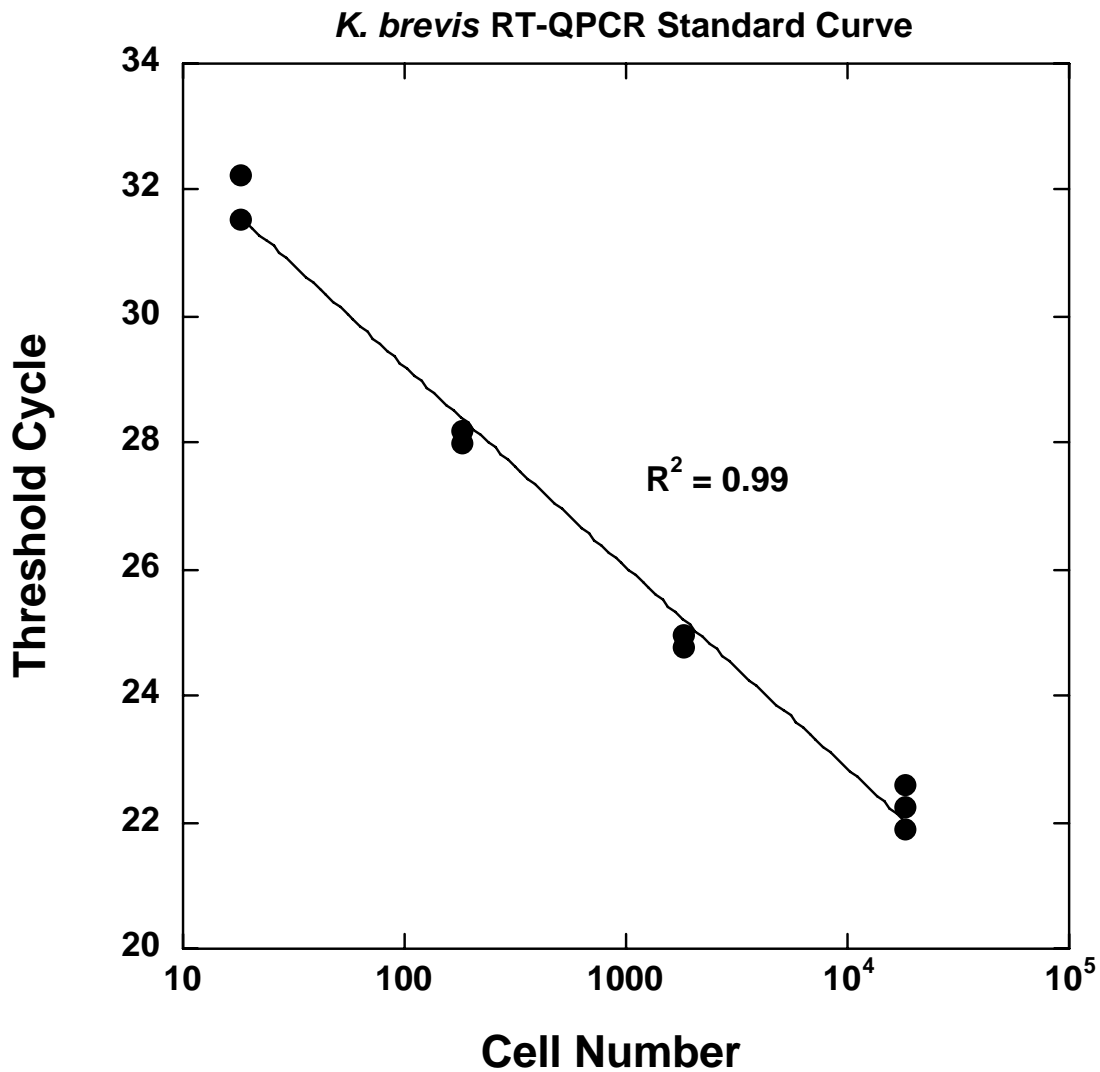


Figure 5. Standard curve for RT-qPCR analysis of *K. brevis*.

RESULTS, APPLICATIONS AND BENEFITS.

Surface water samples were collected biweekly from seven stations (C1-C7) along Cinco Bayou and nine stations (G1-G9) along Garnier Bayou (Figure 6) between 14 October 2007 and 6 January 2008. Water samples from selected stations (C1, C3, G1, G9) were processed for analysis of *K. brevis* concentrations via RT-qPCR.



Figure 6. Station map. Cinco Bayou stations are shown in red and are labeled 1 through 7, from west to east); Garnier Bayou stations are shown in green and range from 1 through 9 (north to south).

None of the Cinco Bayou samples revealed the presence of *K. brevis* during any of the sampling events. This result was surprising because previous monitoring by FWRI suggests that Cinco Bayou is more prone to red tides than is Garnier Bayou and the highest observed cell densities (up to 2.2 million cells L^{-1}) were previously observed at that site. The lack of *K. brevis* cells may have been due to the fact that the Cinco Bayou samples analyzed were collected from the freshwater endmember station (i.e., stations C1 and C3), which is not expected to have significant *K. brevis* populations, as previous studies have indicated a minimum salinity of at least 20 for Gulf of Mexico red tides (Magana and Villareal, 2006).

In contrast, the Garnier Bayou samples (G1 and G9) analyzed revealed both spatial and temporal variability of *K. brevis* populations between October 2007 and January 2008 (Figure 7). Analysis of the first sampling event revealed no detectable presence of *K. brevis* at station G9; no G1 samples were analyzed for *K. brevis* on this date. On 24 October 2007, the *K. brevis* levels at the freshwater endmember were greater than 1.5 million cells per liter, exceeding the FWRI designation of high (1 million cells per liter). During this sampling, *K. brevis* was detected at the mouth of Garnier Bayou (station G9) significant, though much lower levels (2480 cells L^{-1}).

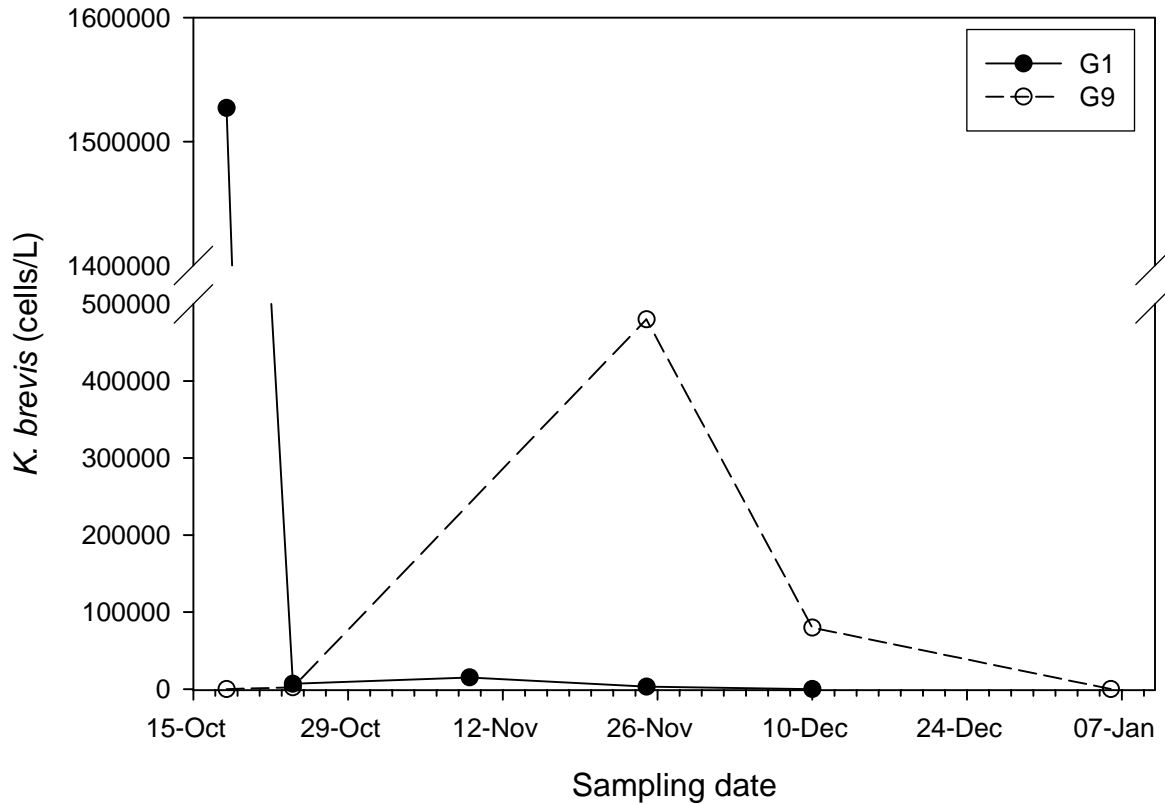


Figure 7. *K. brevis* concentrations in Garnier Bayou, 2007-8. Station G1 is at the northern, freshwater end of the estuary; station G9 is at the southern end of the bayou.

Following the 24 October 2007 peak, *K. brevis* levels declined quickly at station G1; 9 November 2007 sampling revealed a cell density of 7,100 cells L⁻¹. Subsequent sampling at this station on 25 November 2007 and 10 December 2007 showed lower, but detectable cell densities of 15,200 and 3,300 cells L⁻¹, respectively. The final sample analyzed from station G1 was collected on 6 January 2008 and revealed no detectable *K. brevis* (<100 cells L⁻¹).

Station G9 is located near the mouth of Garnier Bayou and at the confluence with Cinco Bayou on the western side of Choctawhatchee Bay. The observed trend at this site was quite different from that of the freshwater endmember station (i.e., G1). This station showed no evidence of the large 24 October 2007 event at G1, with very low levels (~2500 cells L⁻¹) recorded for that event. A substantial peak in *K. brevis* cell concentrations was detected at station G9 during the 25 November 2007 sampling event, when >450,000 cells L⁻¹ were detected.

These local results coincide well with data collected as part of the statewide monitoring effort accomplished by the Florida Wildlife Commission (Figures 8 and 9). Very high (>1 million cells L⁻¹) *K. brevis* were reported in the Choctawhatchee Bay region during the 14-19

October 2007 monitoring (Fig. 8A), coincident with the highest cell densities revealed from our RT-QPCR data (Figure 7).

FWC/FWRI detected high *K. brevis* populations in late October (Figure 8C); however, we did not sample at this time and so cannot confirm their observations here. The 9 November 2007 analyses were performed only on the upper bayou station(G1) samples and these revealed low *K. brevis* cell densities ($>7,000$ cells L^{-1}) that are in line with the regional FWRI observations (Figure 8D).

Our results appear to confirm the FWRI monitoring summary for the 25 November 2007 sampling event in which FWRI results suggest moderate *K. brevis* population of 100,000 to 1 million cells L^{-1} (Figure 9A), in line with our findings of a lower Garnier Bayou population of 480,000 cells L^{-1} (Figure 7).

FWRI did not sample Choctawhatchee Bay at the same time as our 10 December 2007 sampling event, though the immediately preceding FWRI sampling event (3-7 December 2007; Figure 9 B) is in line with our observations of moderate cell densities: 79,700 cells L^{-1} from our PCR analyses and a “LOWb” level (50,000-100,000 cells L^{-1}) in the FWRI sampling. Both the FWRI sampling (Figure 9F) and our own results indicate that the red tide event fully ceased by 6 January 2008.

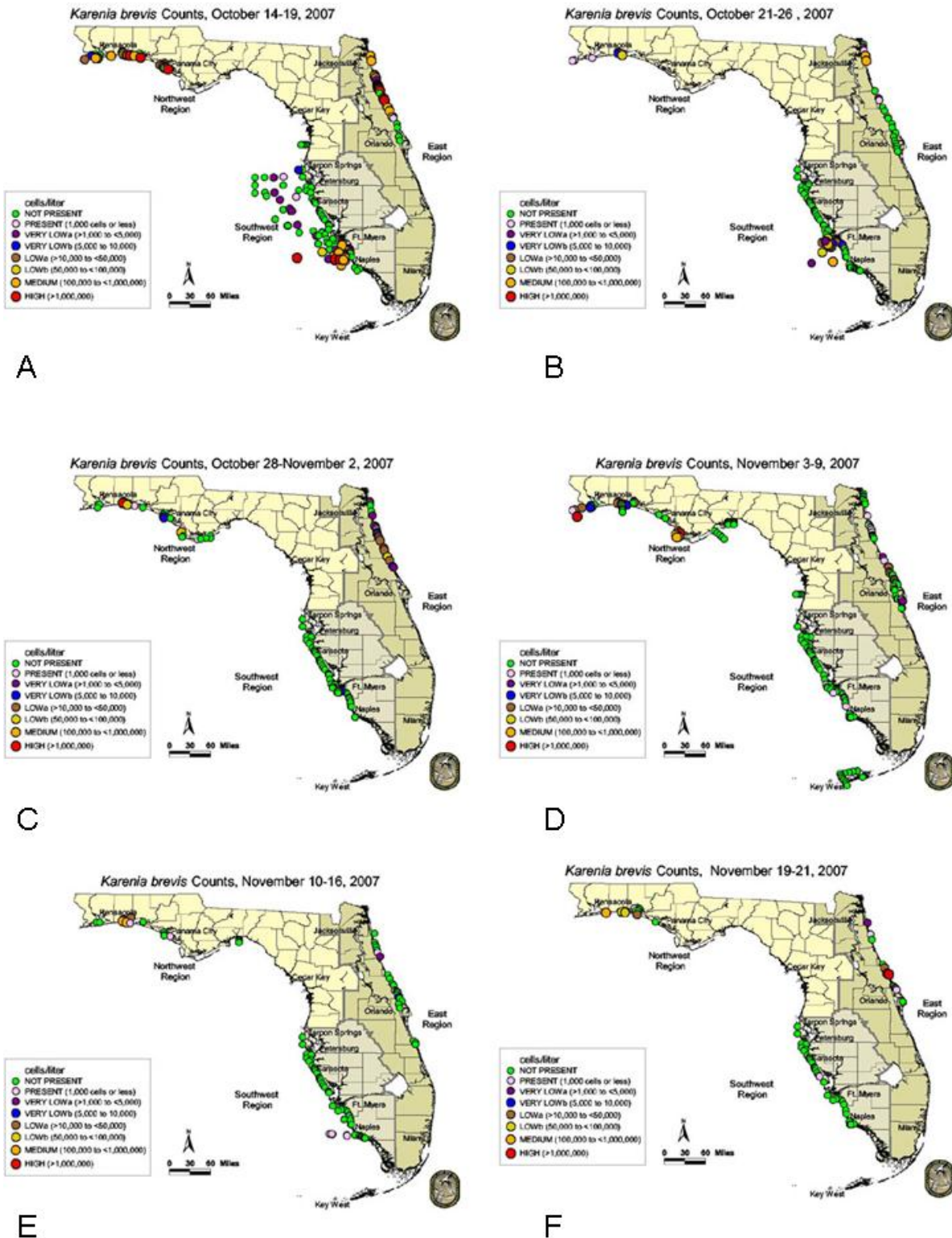


Figure 8. FWRI HAB maps I: October 14, 2007, - November 21, 2007. Source: Florida Fish and Wildlife Conservation Commission (http://research.myfwc.com/features/category_main.asp?id=2309)

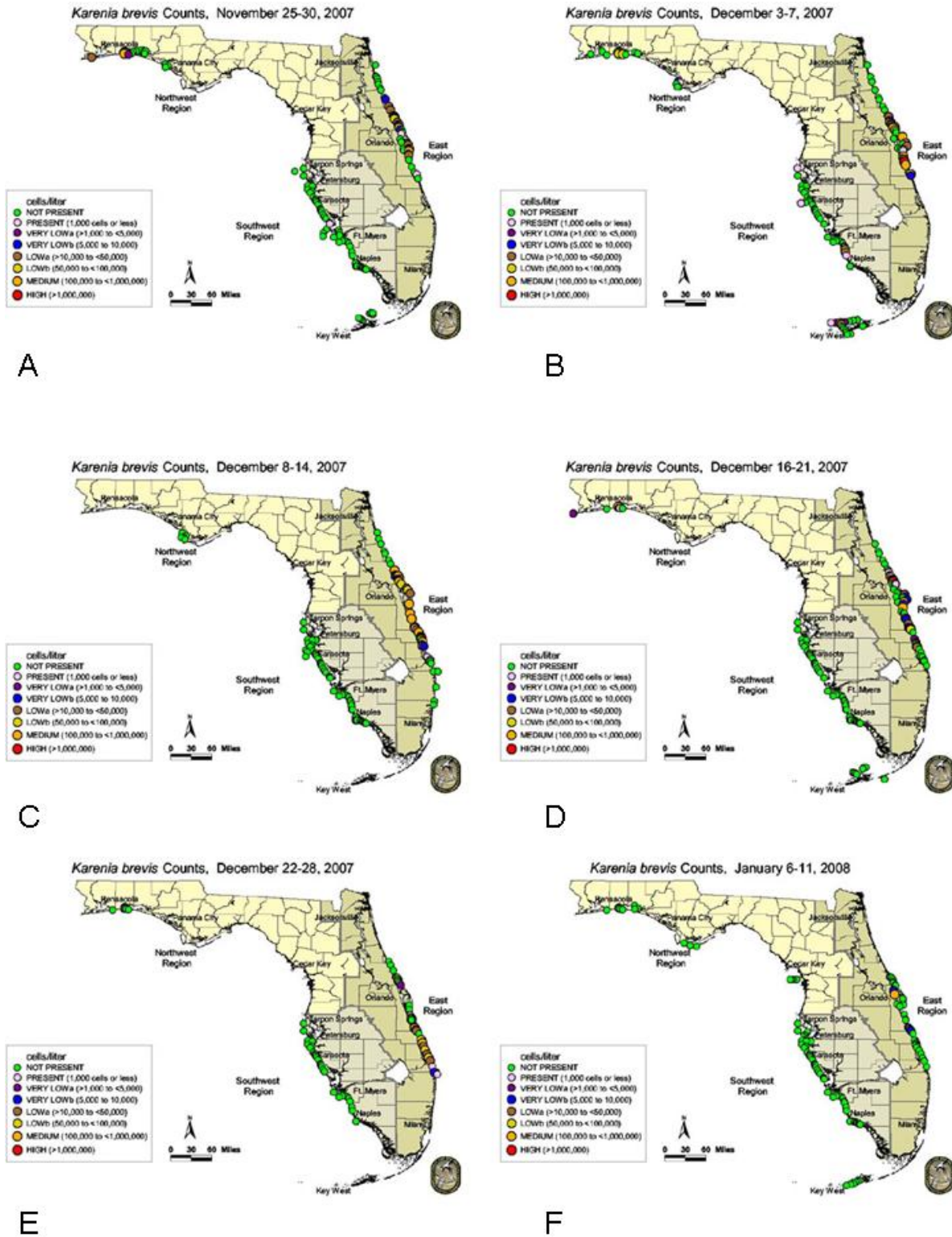


Figure 9. FWRI HAB maps II: November 25, 2007, - January 11, 2008. Source: Florida Fish and Wildlife Conservation Commission (http://research.myfwc.com/features/category_main.asp?id=2309)

The cell densities detected in Garnier Bayou during the 2007-8 bloom analyzed here are substantially higher than any levels previously detected by Florida Fish and Wildlife Research Institute sampling. The highest *K. brevis* cell densities previously recorded in Garnier Bayou were 600,000 and 229,600 cells L⁻¹, and were recorded in the southern portion of Garnier Bayou on 26 October 2000 and 5 November 2005, respectively (FWRI, 2006). Those previous high populations were in the vicinity of stations G8 and G9 (Figure 6). Significantly, previous FWRI monitoring did not reveal any *K. brevis* cell densities above 3,000 cells L⁻¹ in the upper reaches of Garnier Bayou, where our sampling revealed *K. brevis* cell densities of 1,500,000 cells L⁻¹, equivalent to the highest values previously recorded in Cinco Bayou.

It is likely significant that the 24 October 2007 sampling that revealed the highest *K. brevis* concentrations at station G1 were associated with an ongoing fish kill throughout Garnier Bayou (Chastain, personal communication). It is possible that the high *K. brevis* levels in the upper portion of Garnier Bayou were the result of meteorological forcing (i.e., wind-blown dinoflagellate and fish carcasses). Alternatively, these results may indicate that the red tide bloom that was responsible for contemporaneous fish kills actually started in the upper reaches of this bayou, which drains a largely forested watershed.

The current results, therefore, represent a potentially significant shift in our understanding of where red tides occur in western Choctawhatchee Bay, as well as the influence of land-use in coastal and estuarine watersheds on red tides. Based on the aforementioned FWRI monitoring data, which indicated that the highest *K. brevis* cell densities (1.1-2.2 million cells L⁻¹) were recorded only in Cinco Bayou, our working hypothesis was that the largely developed Cinco Bayou watershed was contributing significant inorganic nutrients and/or organic matter to “trigger” and support the observed red tides, while the considerably lower *K. brevis* populations in Garnier Bayou were a result of the largely forested watershed of upper Garnier Bayou and associated decreased loading of inorganic nutrients and OM. The current results require a wholesale review of those assumptions and indicate that the controlling factors of *K. brevis* population dynamics in this region are more complex than indicated by the previous data. We intend to explore this apparent research gap as part of an ongoing collaboration between the principal investigators.

By quantifying the *K. brevis* population size from the samples previously collected by Ms. Chastain, we have gained important insight into the development and intensification of *K. brevis* populations before and during the red tide events that affected western Choctawhatchee Bay during fall-winter 2007. Of particular interest to future work is the quantification of the *K. brevis* population in the period before a full red tide is observed. When assessed in conjunction with environmental data such as inorganic nutrients, salinity, and water temperature, these population numbers will provide critically important information as to how a normal dinoflagellate population can develop into a harmful algal bloom (HAB). As discussed above, these focused pilot data contradict our previous hypotheses linking land-use and red tides in this region. The focused sampling and evaluation of

One of our primary interests in this project is assessing the geographic influence on environmental controls on *K. brevis* populations as they relate to the formation of red tide HABs.

Jeffrey has considerable experience with UV photoinhibition effects on marine phytoplankton, while Schwartz is particularly interested in how submarine groundwater discharge might affect estuarine nutrient biogeochemistry. We will utilize the results generated from this project development research and apply existing research capabilities towards a new research project assessing the causes behind historical (and ongoing) geographical differences in red tide occurrences in two neighboring western Choctawhatchee Bay bayous. These results are important pilot data for future projects, for which we will pursue funds from extramural funding agencies, such as USEPA, FDEP, and NSF.

APPLICATION TO ONGOING AND FUTURE WORK

The quantification of *K. brevis* via qPCR that was completed during this project development period will be included in the master's thesis of Ms. Jennifer Chastain, a graduate student in the UWF Environmental Science program. Ms. Chastain will interpret these results in the context of broader environmental sampling for biogeochemical and physical parameters (e.g., DIN, PO₄, salinity, temperature, and light penetration).

The proposed research represents a pilot study to assist in a new collaboration between Wade Jeffrey and Matthew Schwartz in which the overall project goals are to coordinate Schwartz's submarine groundwater discharge and estuarine biogeochemistry research interests with Jeffrey's existing strengths in genetic and molecular techniques.

This pilot study provided fundamental data to this new collaboration and will significantly contribute toward the preparation of extramural funding proposals targeted to assessing how land-use practices in coastal northwest Florida may affect estuarine biogeochemistry and physical parameters currently considered to be the main causes of harmful algal blooms, particularly red tide.

8. LITERATURE CITED.

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