PHASE II FINAL REPORT

Harmful Algal Blooms in Coastal Waters of Lee County, FL: Bloom Dynamics and Identification of Land-Based Nutrient Sources

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Background

Harmful Algal Blooms (HABs) have increased in their frequency of occurrence and spatial extent over recent decades throughout coastal waters of the world. HABs include both toxic phytoplankton species, such as red tides (*Karenia brevis*), as well as excessive biomass of macroalgae, or seaweeds. There is general agreement among scientists that increasing runoff of nutrient pollution from land-based sources is a major cause of the global increase in HABs (Glibert et al. 2005). The strength of the causal relationship and the ultimate source of the nutrients is highly variable and somewhat unique to each location, season, and HAB species.

During our Phase I studies evidence was presented indicating a link between the increasing intensity and duration of HABs and increasing land-based nutrient pollution from southwest Florida. In the case of red tide (*Karenia brevis*), an analysis of existing data (cell counts) from the 1950's and 1990's showed that these blooms have intensified an average of 15-fold since the 1950's, occur primarily in nearshore (not offshore) waters, and that the duration of the blooms has increased (Brand and Compton, in press). Newly acquired evidence from Lee County's coastal waters has indicated that the red drift macroalgal blooms that plagued area beaches in 2003 and 2004 are a recent phenomenon and indicative of increasing land-based nutrient pollution (Lapointe and Bedford, in press).

The Phase I macroalgal HAB study used tissue $\delta^{15}N$ analysis to identify potential land-based nitrogen sources supporting these blooms. Analysis of macroalgae from area beaches for $\delta^{15}N$ revealed values of + 5 to + 8 ‰, which closely matches the source values of wastewater nitrogen (Lapointe and Bedford, in press). Although local sewage sources along area beaches and municipal outfalls may contribute to this sewage signal, macroalgae from the Caloosahatchee River showed the highest $\delta^{15}N$ values (+ 8 ‰ to + 15 ‰), which suggest the importance of the river discharges during high flow periods in transporting wastewater nitrogen loads from eastern areas of Lee County, Glades County, and Lake Okeechobee, into coastal waters of Lee County.

The limited scope of our Phase I study would not allow us to resolve the relative importance of local nitrogen inputs from Lee County versus far-field sources from Caloosahatchee River discharges. Accordingly, we performed a Phase II program following months of high Caloosahatchee flows in August and September 2005 to develop a long-term water quality monitoring in collaboration with Lee County staff. A primary goal of the Phase II program was to identify what aspects of a long-term water quality monitoring program could be performed by Lee County staff compared to more specialized services required from expert outside consultants.

Materials and Methods

The Phase II research performed in August and September 2005 included examination of existing data files that began in Phase I of this research. We continued to examine hard scientific data collected from published scientific papers, unpublished scientific manuscripts, government reports, digital libraries, and websites. Existing data from satellite imagery and aerial photography was also used. Personnel at organizations such as University of South Florida, Florida Gulf Coast University, Florida International University, Mote Marine Laboratory, county environmental laboratories, the Conservancy of Southwest Florida, Sierra Club, and other non-governmental organizations were considered for information that could be useful in testing the hypotheses.

The water quality sampling design for Phase II involved three basic approaches: 1) collection of water samples along a gradient from various freshwater sources to offshore stations for analysis of a variety of water quality variables; 2) monitoring of macroalgal biomass and associated toxic microalgae, and species composition at selected sites along beaches and in nearshore coastal waters; 3) collection and analysis of macroalgae and toxic microalgae (*Karenia brevis, Microcystis aeruginosa*) for $\delta^{15}N$ isotopic signatures for comparison with published values for nutrient sources (agricultural canal discharges, sewage outfalls, stormwater outfalls, rainfall).

I. *Water Sampling and Analysis*: In collaboration with Lee County staff, water samples were collected in August and September from stations along Lee County's beaches, through San Carlos Bay, Pine Island Sound, Charlotte Harbor, Estero Bay, and to offshore areas. The water samples were analyzed by the Lee County Environmental Laboratory for salinity and nutrients (ammonium, nitrate, nitrite, orthophosphate, total dissolved nitrogen, total dissolved phosphorus). The water samples were also analyzed for phytoplankton composition and abundance, presence and abundance of toxic algae, turbidity, organics, and nutrient bioavailability by Larry Brand at RSMAS, University of Miami, Virginia Key, FL.

II. *Distribution and Abundance of Macroalgal Blooms*: Sampling stations for long-term monitoring of macroalgal biomass on beaches were established in collaboration with Lee County staff and other key contacts. A network of seven sites spanning Lee County's shoreline (Fig. 1) were sampled for water quality paramters (salinity and nutrients) and biomass of macroalgae using quadrats placed along replicate belt transects. Macroalgal samples were sorted, identified, wet weighed, and dried in a Fisher Isotemp lab oven to establish a wet weight-to-dry weight conversion factor. Biomass was quantified as grams dry weight per square meter (g/m^2). Digital photographic images were also collected at the stations to provide digital data for percent cover determinations using the randomized point-count method.

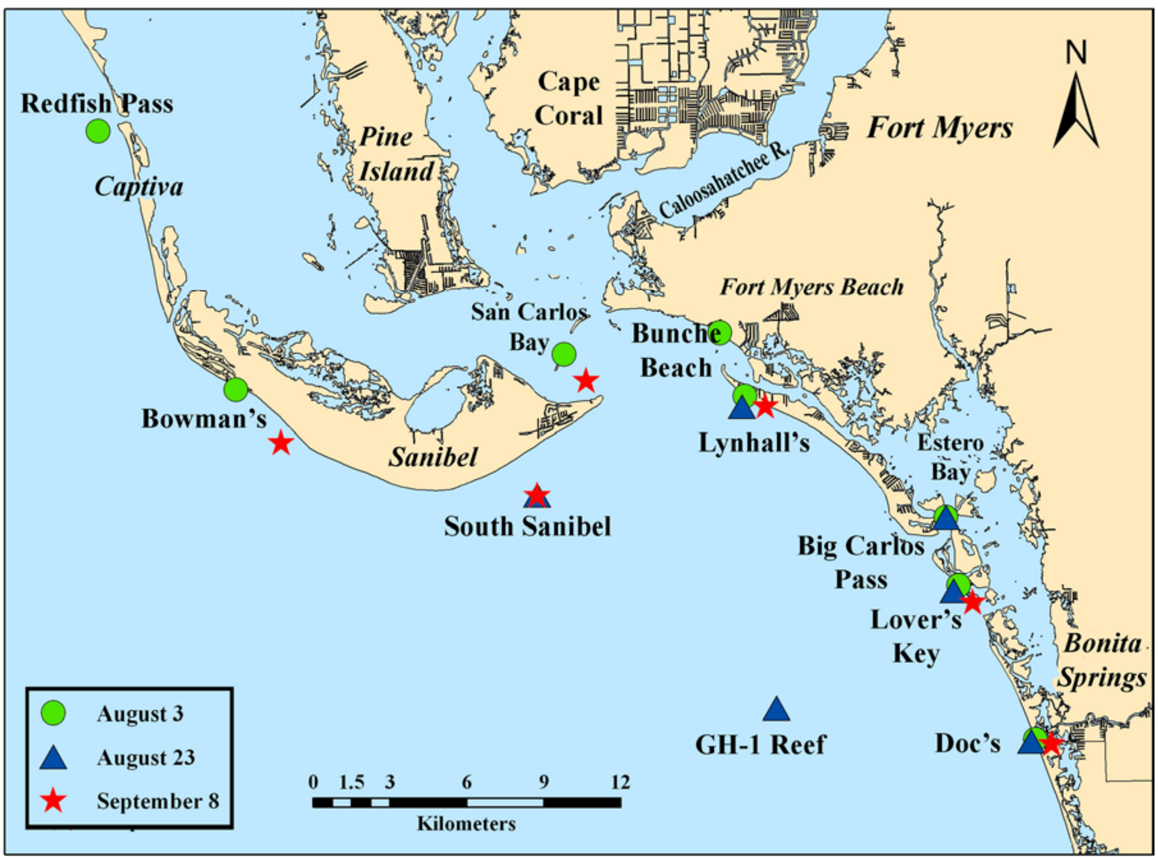

Figure 1. Map of the Lee County area showing sites sampled in August and September 2006

Digital aerial photography was also used to identify HABs in Lee County's coastal waters. On September 8th and 14th, 2005, we used a small aircraft flying at 500-1,000 ft to obtain digital photographic images of nearshore waters and beaches. These photos provided a planar section of coastline that can be used like a GIS image for overlay of important environmental parameters. The digital images were used to distinguish color class species and bare beach sand. This was achieved by measurement of spectral reflectance of visible and NIR wavelengths (400-800 nm) of macroalgae samples collected from beach sites. The benefit of this approach is that it allows a method to efficiently quantify the distribution and abundance of macroalgal blooms

III. $\delta^{15}N$ *and C:N:P Analysis of Macroalgae and Toxic HABs*: Samples of macroalgae from the seven monitoring stations described above (II) were processed and analyzed for $\delta^{15}N$ values and C:N:P ratios as in the Phase I program. In addition, samples of *Karenia brevis* and *Microcystis aeruginosa* blooms were collected from offshore southern Sanibel Island and the Caloosahatchee River estuary, respectively, for $\delta^{15}N$ analysis. These phytoplankton were filtered onto GF/F filters, dried in a lab oven, and analyzed for $\delta^{15}N$ values.

Results and Discussion

I. Water Sampling and Analysis: Water samples for phytoplankton and nutrients were collected in August and September 2005 by Lee County staff from their routine monitoring stations. Subsamples of these collections, as well as additional samples from May to July 2005 and from October 2005 onward, have been analyzed by Larry Brand for phytoplankton variables. These results and a discussion of the data are provided in Appendix I of this report.

Water samples were also collected at the beach sites for salinity and nutrient analysis on August 3rd, August 23rd, and September 8th. The August samples were analyzed by the Lee County Environmental Lab for ammonium, nitrate, total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), and the TDN:TDP molar ratio. Concentrations of ammonium and nitrate were below detection limits for these analyses (0.93 µM for ammonium, 0.71 µM for nitrate) at the beach sites on August 3rd (Table 1). On August 23rd, four of eight samples were below detection limits for ammonium and all samples were below detection limits for nitrate (Table 1). The September 8th samples were analyzed by the

Table 1. Water column concentrations (µM) of ammonium, nitrate, soluble reactive phosphorus (SRP), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP), and TDN:TDP molar ratios in samples collected August and September 2005 in Lee County, FL, USA.

Date	Site Name	Ammonium (µM)*	Nitrate (µM)*	TDN (µM)	SRP (µM)*	TDP (µM)*	TDN:TDP Ratio (M)
8/3/05	Bowman's Beach	*< 0.93*	*< 0.71*	28.91 ± 7.57	0.47 ± 0.02	*< 0.65*	44.8
	Sanibel Causeway	*< 0.93*	*< 0.71*	46.05 ± 1.51	0.98 ± 0.02	1.29 ± 0.00	35.7
	Bunche Beach	*< 0.93*	*< 0.71*	51.05 ± 1.51	0.71 ± 0.00	0.97 ± 0.00	52.7
	Lynhall's Beach	*< 0.93*	*< 0.71*	49.26 ± 6.06	0.69 ± 0.02	0.97 ± 0.00	50.9
	Lover's Key Beach	*< 0.93*	*< 0.71*	49.62 ± 4.54	0.36 ± 0.05	0.81 ± 0.23	64.9
	Doc's Beach	*< 0.93*	*< 0.71*	39.98 ± 4.04	0.42 ± 0.05	*< 0.65*	61.9
	8/3/05 Mean	*< 0.93*	*< 0.71*	44.15 ± 8.45	0.61 ± 0.24	1.01 ± 0.2	51.80
8/23/05	Bowman's Nearshore	1.14 ± 0.20	*< 0.71*	47.83 ± 1.01	0.21 ± 0.11	*< 0.65*	74.1
		< 0.93	*< 0.71*	53.90 ± 13.63	*< 0.13*	*< 0.65*	83.5
	Lynhall's Nearshore	1.00 ± 0.10	*< 0.71*	53.90 ± 7.57	0.47 ± 0.07	*< 0.65*	83.5
		< 0.93	*< 0.71*	34.63 ± 2.52	0.44 ± 0.02	*< 0.65*	53.6
	Lover's Nearshore	*< 0.93*	*< 0.71*	31.06 ± 3.53	*< 0.13*	*< 0.65*	48.1
		< 0.93	*< 0.71*	32.84 ± 3.03	0.87 ± 0.73	1.02 ± 0.53	38.1
	Doc's Nearshore	0.96 ± 0.05	*< 0.71*	34.98 ± 2.02	0.37 ± 0.02	*< 0.65*	54.2
		3.68 ± 0.15	*< 0.71*	34.27 ± 4.04	1.69 ± 0.34	1.94 ± 0.00	17.7
	8/23/05 Mean	1.70 ± 1.32	*< 0.71*	40.43 ± 9.74	0.68 ± 0.53	1.48 ± 0.46	56.60
9/8/05	Bowman's Nearshore	*< 0.21*	0.30 ± 0.03	23.00 ± 0.10	0.25 ± 0.00	1.02 ± 0.08	22.6
	San Carlos Bay	0.25 ± 0.05	2.40 ± 0.01	48.36 ± 0.21	1.34 ± 0.03	2.08 ± 0.08	23.3
	Lynhall's Nearshore	*< 0.21*	0.47 ± 0.06	33.72 ± 1.11	0.68 ± 0.01	1.48 ± 0.23	23.1
	Doc's Nearshore	*< 0.21*	0.44 ± 0.04	29.04 ± 0.05	0.23 ± 0.01	0.92 ± 0.01	31.6
	S. Sanibel	*< 0.21*	0.26 ± 0.02	141.28 ± 34.72	6.44 ± 0.57	16.51 ± 2.47	8.5
	9/8/05 Mean	*< 0.21*	0.77 ± 0.91	55.08 ± 49.09	1.79 ± 2.64	4.40 ± 6.78	21.81

* *Italics indicate values below analytical detection limits.*

Nutrient Analytical Services Lab at the Chesapeake Biological Lab, Solomons, MD, using more sensitive analytical methods. Despite much lower detection limits for ammonium (0.21 µM vs. 0.93 µM), concentrations of this nutrient were still undetectable at four of the five beach sites (Table 1), indicating the rapid assimilation of this preferred nitrogen form by nitrogen-limited algal blooms in the coastal waters. However, concentrations of nitrate were all above the detection limit (0.10 µM), with the highest concentration (2.40 µM) occuring in the relatively low salinity waters of San Carlos Bay. Regression analyses of SRP, TDN, and TDP concentrations in surface waters versus salinity in all three samplings were all significant ($P < 0.05$; Fig. 2), indicating that nutrient enrichment of coastal waters was a result of freshwater discharges from land.

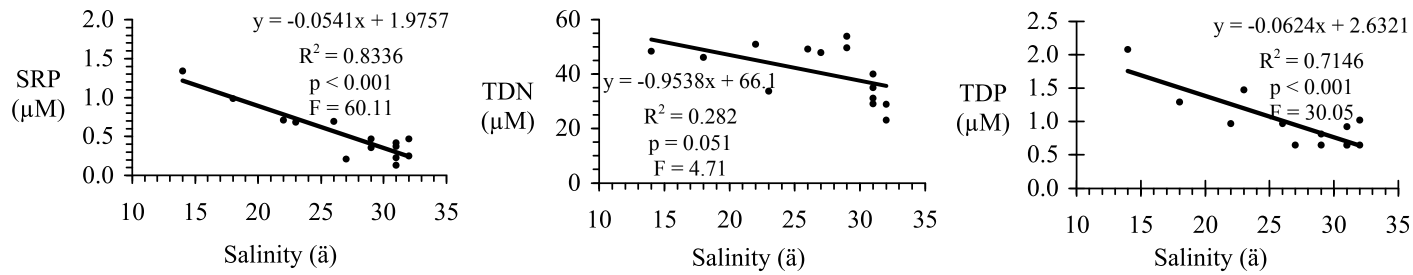

Figure 2. Regressions of soluble reactive phosphorus (SRP), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) versus salinity for surface water samples collected in August and September 2005, Lee County, FL, USA.

The TDN:TDP ratio varied between 8.5 and 83.5, with the lowest value associated with a red tide bloom in 30 ‰ salinity water off southern Sanibel Island on September 8th (Fig. 3). These results support the observations of Ketchum and Keen (1947) and Slobodkin (1953) that the nearshore red tides on Florida's southwest coast are associated with high phosphorus coastal waters associated with fresh water runoff of the Peace and Caloosahatchee rivers.

II. *Distribution and Abundance of Macroalgal Blooms*: Biomass of macroalgae collected from seven beach sites on August 3, 2005 are shown in Table 2. At Doc's, Lover's Key, and Lynhall's, a variety of macroalgae were present at biomass levels up to ~ 30 g dry weight / m^2. Although this was a relatively low level of biomass compared to red drift bloom events in 2003 and 2004, many of the same rhodophyte bloom species occurred in August 2005 (e.g. *Hypnea spinella*, *Agardhiella subulata*, *Gracilaria tikvahiae*, *Acanthophora spicifera*). In addition, blooms of chlorophytes that are well known nutrient indicators species also occurred on beaches in southern Lee County during the present study. These included blooms of *Enteromorpha* sp. at Doc's and *Ulva lactuca* (Sea Lettuce) at Lynhall's.

Figure 3. Image of red tide sampling off southern Sanibel Island, September 8, 2005.

Nearshore biomass of macroalgae was estimated along underwater transects at Doc's, Lover's Key, and Lynhall's on August 23rd. Collections by divers indicated that ~ 7-9 g dry weight / m^2 occurred on these soft bottom communities at this time (Table 2). These are very low levels of benthic macroalgae and incapable of supporting the massive red drift blooms that occurred in 2003 and 2005.

The low levels of macroalgal biomass in August 2005 are clearly related to the highly turbid conditions and low levels of downwelling light in Lee County's coastal waters at this time. Water sampling at the beach sites in August indicated relatively low salinities (18-32 ‰), largely a result of large discharges from the Caloosahatchee River (Franklin Lock) that occurred during this period (Table 3). These low salinities corresponded with high concentrations of chlorophyll *a* (2.6-16.7 µg/l) in August, as well as high turbidity (Secchi depths < 1.8 m) in August/September (Table 3). Stratification of the water column was apparent in the August/September samplings, with relatively lower salinity and higher dissolved oxygen in the surface layer (Table 3). Hypoxic conditions in bottom waters were apparent at Doc's on August 23rd and September 8th. A buoyant plume of water discharged from the Caloosahatchee River was clearly visible in our aerial survey on

Table 2. Algal biomass (g / m^2) and percent cover on beaches and in nearshore waters at sampling sites in August 2005, Lee County, FL, USA, .

Location	Species	% of Site Total	Beach Drift Biomass Wet Weight (g / m^2)	Beach Drift Biomass Dry Weight (g / m^2)	Beach Drift Zone Width (m)	Beachfront Biomass Dry Weight (g / m)	Beach Drift Zone % Cover	Nearshore Biomass Wet Weight (g / m^2)	Nearshore Biomass Dry Weight (g / m^2)	Nearshore % Cover
Doc's	*Hypnea spinella*	79.85	71.00	9.23		9.23				
	Enteromorpha sp.	15.97	14.20	1.99		1.99				
	Thalassia testudinum	2.50	2.22	0.51		0.51				
	Agardhiella subulata	1.12	1.00	0.12		0.12				
	Gracilaria tikvahiae	0.56	0.50	0.06		0.06				
	Doc's Total:	100	88.92	11.91	1	11.91	1.5	75	9	13
Lover's Key	*Thalassia testudinum*	9.15	87.67	20.16		80.65				
	Halodule wrightii	1.91	18.33	7.15		28.60				
	Gracilaria tikvahiae	1.58	15.11	1.81		7.25				
	Acanthophora spicifera	0.27	2.56	0.31		1.23				
	Hypnea spinella	0.08	0.78	0.10		0.40				
	Lover's Total:	100	124.44	29.53	4	118.14	14.2	58	7	6
Lynhall's	*Ulva lactuca*	44.95	23.22	6.27		100.32				
	Gracilaria tikvahiae	40.43	20.89	2.51		40.11				
	Gracilaria cervicornis	5.16	2.67	0.32		5.12				
	Hypnea spinella	4.09	2.11	0.27		4.39				
	Acanthophora spicifera	2.37	1.22	0.15		2.35				
	Thalassia testudinum	1.51	0.78	0.18		2.86				
	Halymenia floresia	1.51	0.78	0.09		1.49				
	Lynhall's Total:	100	51.67	9.79	16	156.64	12.8	-	-	-
Bunche	*Thalassia testudinum*	100	43.89	10.09	4	40.38	1.2	-	-	-
San. Causeway	*Thalassia testudinum*	100	94.44	21.72	4	86.89	19.8	-	-	-
Bowman's	*Thalassia testudinum*	100	27.56	6.34	2	12.68	3.0	-	-	-
Captiva	*Thalassia testudinum*	100	126.56	29.11	7	203.75	20.2	-	-	-

Table 3. Site locations, field data--temperature (ūC), salinity (ä), pH, Secchi depth (m)--and water column chlorophyll *a* (µg/l) and phaeophytin (µg/l) concentrations during August and September 2005 samplings, Lee County, FL, USA.

Date	ūLatitude	ūLongitude	Site Name	Temp. (ūC)		Salinity (ä)		pH		D.O. (ä)		Secchi Depth (m)	Chl a (µg/l)	Phaeo-phytin (µg/l)
				S	B	S	B	S	B	S	B			
8/3/05	26.4692667	-82.0285167	Sanibel Causeway	31.8	-	18	-	8.0	-	9.2	-	-	16.6	1.9
	26.4759833	-81.9674333	Bunche Beach	32.5	-	22	-	8.0	-	8.2	-	-	7.7	2.2
	26.4535667	-81.9579667	Lynhall's Beach	31.7	-	26	-	7.9	-	7.8	-	-	7.5	2.9
	26.3859000	-81.8754667	Lover's Key Beach	31.1	-	29	-	7.8	-	7.1	-	-	3.1	2.7
	26.3315167	-81.8463000	Doc's Beach	31.2	-	31	-	7.8	-	5.9	-	-	4.0	2.4
	26.4587000	-82.1574500	Bowman's Beach	32.0	-	32	-	7.9	-	6.1	-	-	2.7	1.5
8/23/05	26.44979	-81.95906	Lynhall's Nearshore	31.2	31.2	29	29	7.9	7.9	5.0	4.9	-	14.9	7.7
	26.38391	-81.87747	Lover's Nearshore	31.8	32.3	31	31	8.1	8.0	6.2	4.7	1.8	6.9	3.2
	26.33080	-81.84799	Doc's Nearshore	31.9	32.5	31	32	8.1	7.9	5.6	2.4	-	4.0	3.8
	24.42000	-82.04000	Bowman's Nearshore	32.1	31.2	27	28	7.8	7.9	5.8	6.1	-	21.8	9.1
9/8/05	26.46000	-82.02000	San Carlos Bay	29.7	29.5	14	22	8.2	8.0	6.8	6.2	1.1	9.0	6.9
	26.43000	-81.95000	Lynhall's Nearshore	29.5	29.9	23	26	8.0	8.0	6.2	6.7	1.4	7.3	3.0
	26.38000	-81.87000	Lover's Nearshore	29.8	30.0	30	31	7.9	7.9	4.9	3.6	1.6	-	-
	26.33000	-81.84000	Doc's Nearshore	29.6	29.8	31	32	7.0	7.6	3.4	0.7	1.5	12.9	3.8
	26.42000	-82.04000	Bowman's Nearshore	29.4	29.4	32	32	8.1	8.1	7.0	6.7	0.3	12.9	3.2
	26.44000	-82.14000	South Sanibel	30.2	29.8	30	32	8.3	8.2	12.6	6.6	1.1	336.6	249.5
			Mean (excluding South Sanibel):	31.0	30.6	27	29	7.9	7.9	6.3	4.7	1.3	9.4	3.9
			± 1 SD:	1.1	1.2	5	3	0.3	0.1	1.4	2.1	0.5	5.7	2.3

September 7th (Fig. 4). This aerial survey also revealed patches of discolored water indicative of *Karenia brevis* off Sanibel Island (Fig. 5), as well as blooms of *Microcystis aeruginosa* in the Caloosahatchee River and downstream estuary adjacent to Ft. Myers (Fig. 6).

Figure 4. Aerial photo showing Caloosahatchee River plume flowing southward along Fort Myers Beach, September 7, 2005.

Figure 5. Aerial photo of discolored water indicative of a red tide (*Karenia brevis*) bloom off Sanibel Island, September 7, 2005

Figure 6. Aerial photo of the *Microcystis aeruginosa* bloom in the Caloosahatchee estuary, September 7, 2005.

To develop a digital imaging system for the measurement of areal coverage by macroalgal blooms in shallow coastal waters and beaches, we have obtained spectral reflectance data on species of benthic algae growing in the shallow waters off Lee County. The optical instrumentation for this measurement is shown below (Fig. 7). Fiber optic light pipes focus on the algal specimens (c) and reflected and incident light enters at an angle of 40 degrees into the spectrometer (a). The measurements are referenced against a white standard of 98 % reflectance (d). Examples of the spectral data measured using specimens collected off Lee County on September 8th include *Agardhiella subulata, Botryocladia occidentalis*, *Gracilaria blodgetii*, *Gracilaria tikvahiae*, and *Rhodymenia divaricata* (Fig. 8A-E). These species form a composite of the biomass that bloomed in the shallow waters off Bonita Springs Beach in July 2004.

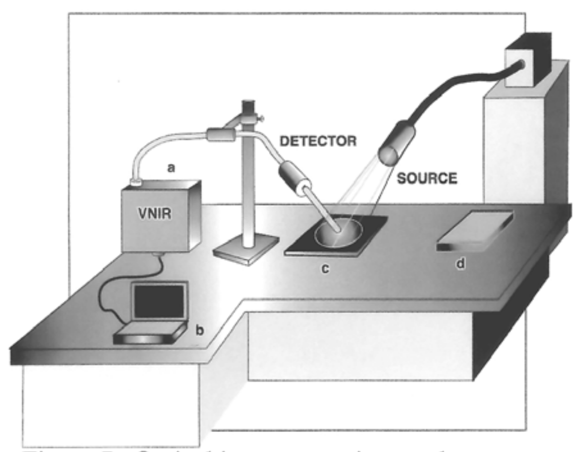

Figure 7. Optical instrumentation used to measure reflectance spectra of macroalgal and toxic phyto-plankton HABs in Lee County coastal waters.

Two regions of these spectra are important in making estimates of area coverage. Between 500 nm and 700 nm the reflectance is relatively constant for these species. This is because of the combined absorption of light at these wavelengths by chlorophylls and chromo-proteins. The reflectance from 700 nm to 750 nm increases markedly to a plateau at 900 nm in all species. Although the overall albedo differs from species to species, the relationship between the two spectral regions is relatively constant. This allows discrimation between algal substances versus reflection and absorption by water covering the benthic substrate. We have not made extensive measurements of the reflection from dry sand beaches in Lee County, but the few measurements we have made indicate that it is 80-90%. We have modeled the changes in reflectance with water depth covering the algal sand bottom (Fig. 9). Figure 9A shows the spectral transmission of light through one meter of water and the spectral reflectance of typical algae. The product of these two curves produces the reflectance curves shown in Figure 9B. The attenuation of light by water, especially at long wavelengths (700-1000 nm), is very strong and gives rise to reflectance spikes not apparent in algae in air.

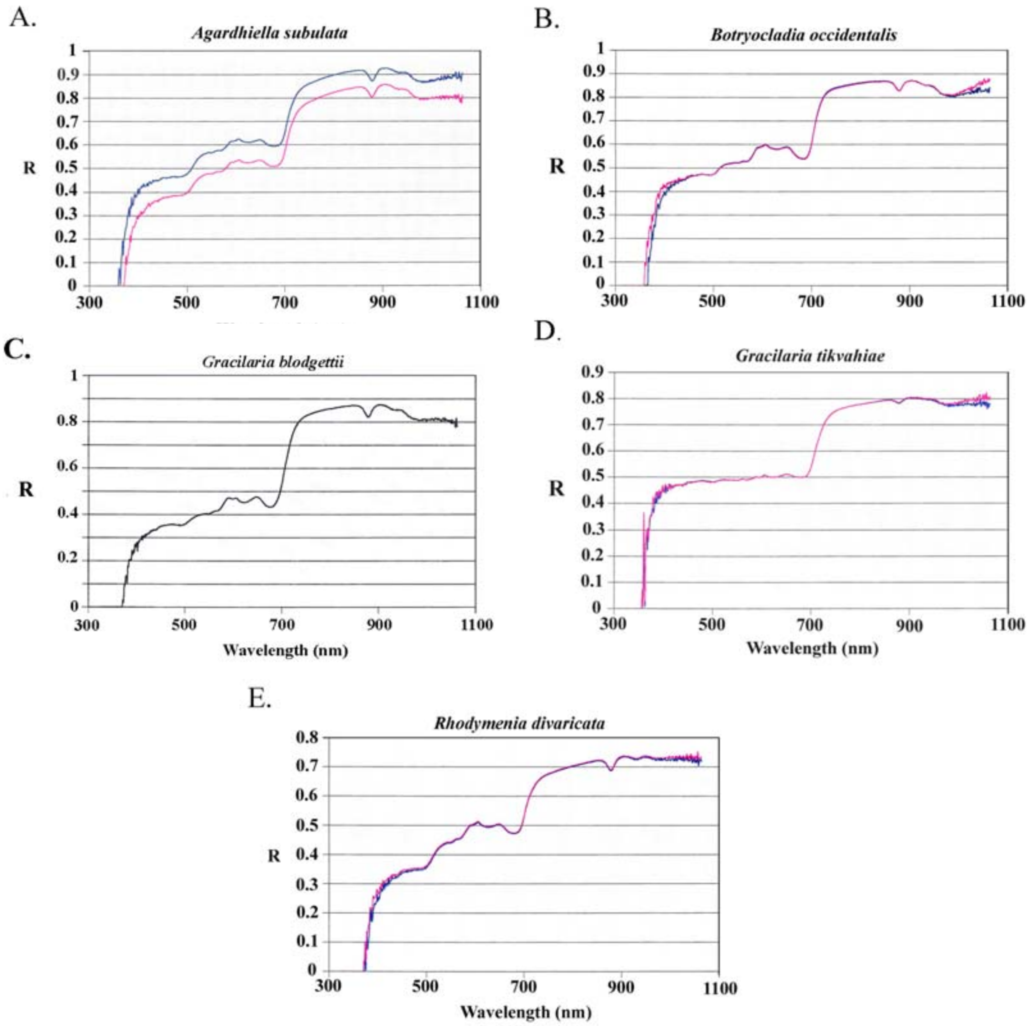

Figure 8. Reflectance spectra of red macroalgae from Lee County, FL, USA, showing reflectance spike above 700 nm. Species shown: A) *Agardhiella subulata*; B) *Botryocladia occidentalis*; C) *Gracilaria blodgettii*; D) *Gracilaria tikvahiae*; E) *Rhodymenia divaricata*.

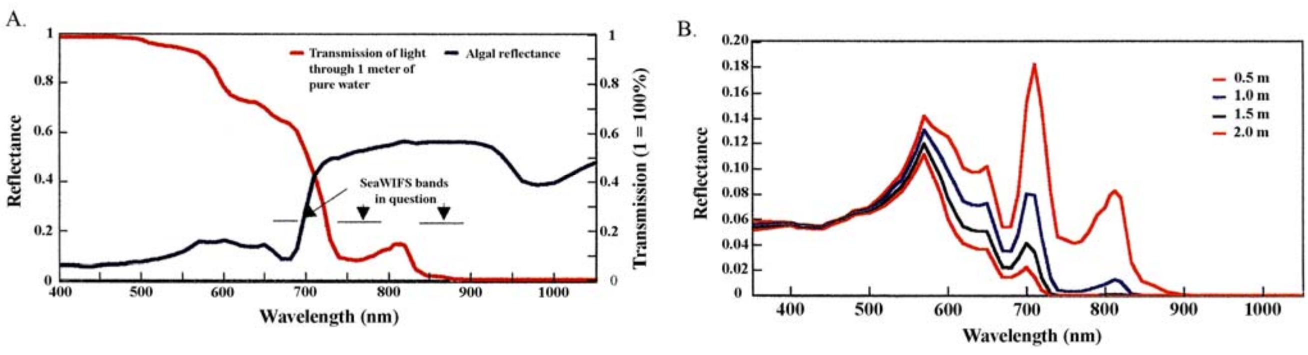

Figure 9. Model of how macroalgal reflectance may be used to monitor HABs in Lee County coastal waters: A) Spectral reflectance and transmission of light through 1 meter of pure water (in red) and macroalgal average (in black); B) Graph showing the product of the two plots in panel 'A' at depths of 0.5, 1.0, 1.5, and 2.0 meters.

III. $\delta^{15}N$ *and C:N:P Analysis of Macroalgae and Toxic HABs*: The C:N:P ratios in macroalgae collected from the seven beach sites on August 3rd were relatively low, indicating a high degree of both N and P enrichment. Along the beach gradient, the C:P and N:P values at Sanibel and Captiva islands were low compared to beaches in southern Lee County (Table 4, Fig. 10). Macroalgal C:P and N:P ratios from Sanibel and Captiva averaged 226.4 ± 41.4 and 15.4 ± 2.7, respectively, considerably lower than averages of 368.1 ± 110.7 and 25.1 ± 6.3 for these ratios in southern Lee County. This indicates significant P enrichment on the beaches of Sanibel and Captiva, which may result from the direct influence of the P-rich discharges from the Peace River.

Table 4. Percent carbon, nitrogen and phosphorus; C:N, C:P and N:P molar ratios, and $\delta^{15}N$ values in macroalgae collected August 3, 2005 from beaches in Lee County, FL, USA.

Date	Location	Species	¶15N (ä, n=2)	Carbon (% ± 1 SD)	Nitrogen (% ± 1 SD)	Phosphorus (% ± 1 SD)	C:N Ratio (M)	C:P Ratio (M)	N:P Ratio (M)
8/3/05	Lynhall's	*Gracilaria tikvahiae*	6.96 ± 0.11	28.6	2.8	0.32	12.0	233	19.5
		Halymenia floresia	5.71 ± 0.10	15.2	1.5	0.19	11.5	201	17.7
		Hypnea spinella	9.96 ± 0.10	28.2	2.1	0.15	15.5	468	30.5
		Ulva lactuca	9.40 ± 0.08	25.1	2.7	0.16	10.9	414	38.1
		Lynhall's Mean	8.01 ± 1.86	24.3 ± 6.2	2.3 ± 0.6	0.20 ± 0.08	12.5 ± 2.0	329 ± 132	26.5 ± 9.6
	Big Carlos Pass	*Gracilaria tikvahiae*	9.87 ± 0.08	24.8	1.5	0.14	18.8	441	23.6
		Acanthophora spicifera	8.42 ± 0.19	18.2	1.6	0.14	13.5	330	24.7
		Big Carlos Mean	9.14 ± 0.85	21.5 ± 4.7	1.6 ± 0.0	0.14 ± 0.00	16.2 ± 3.8	385 ± 78	24.1 ± 0.8
	Lover's Key	*Agardhiella subulata*	9.84 ± 0.04	19.3	1.4	0.09	16.4	530	32.6
		Gracilaria tikvahiae	9.22 ± 0.01	21.6	1.9	0.18	13.3	314	23.8
		Hypnea spinella	9.63 ± 0.17	18.4	1.7	0.22	12.6	212	17.0
		Lover's Key Mean	9.56 ± 0.29	19.8 ± 1.7	1.7 ± 0.3	0.16 ± 0.07	14.1 ± 2.0	352 ± 162	24.5 ± 7.8
	Doc's	*Agardhiella subulata*	9.92 ± 0.35	26.0	1.8	0.14	16.9	469	28.0
		Hypnea spinella	10.71 ± 0.16	21.2	1.6	0.15	15.7	359	23.0
		Enteromorpha intestinalis	10.29 ± 0.14	16.8	1.0	0.10	19.9	446	22.5
		Doc's Mean	10.31 ± 0.40	21.3 ± 4.6	1.4 ± 0.4	0.13 ± 0.03	17.5 ± 2.2	424 ± 58	24.5 ± 3.0
	Redfish Pass	*Ceramium nitens*	9.34 ± 0.00	16.9	1.7	0.23	11.3	186	16.6
		Dictyota sp.	8.84 ± 0.33	15.1	1.3	0.20	13.6	195	14.4
		Acanthophora spicifera	9.03 ± 0.17	16.0	1.7	0.19	10.9	216	20.1
		Redfish Pass Mean	9.07 ± 0.28	16.0 ± 0.9	1.6 ± 0.3	0.21 ± 0.02	11.9 ± 1.5	199 ± 16	17.0 ± 2.8
	Sanibel	*Cladophora* sp.	9.15 ± 0.00	15.2	0.8	0.14	22.1	287	13.1
		Halymenia floresia	8.81 ± 0.14	21.2	1.3	0.22	19.6	248	12.8
		Hypnea spinella	10.28 ± 0.35	22.9	1.6	0.24	16.4	247	15.1
		Sanibel Mean	9.41 ± 0.71	19.8 ± 4.0	1.2 ± 0.4	0.20 ± 0.05	19.4 ± 2.8	261 ± 23	13.7 ± 1.3
	Captiva	*Halymenia floresia*	5.34 ± 0.16	22.4	1.4	0.24	19.3	243	12.7
		Grand Mean	8.98 ± 1.47	20.7 ± 4.4	1.6 ± 0.5	0.18 ± 0.05	15.3 ± 3.5	318 ± 112	21.4 ± 7.2

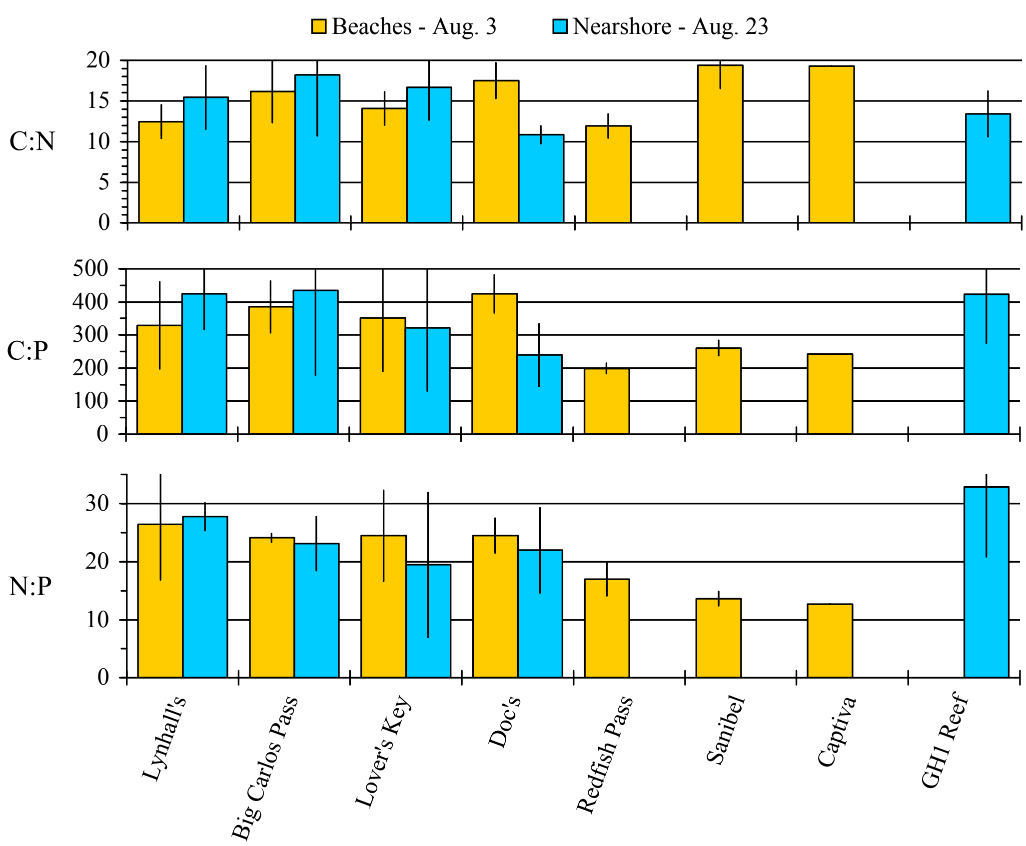

Figure 10. Clustered column graphs of macroalgal C:N, C:P, and N:P ratios in macroalgae collected at beach sites on August 3, 2005 and at nearshore sites on August 23, 2005 in Lee County, FL, USA.

In comparison, there was no significant spatial trend in C:N ratios, which averaged 15.3 ± 3.5 in the macroalgae at the seven beach sites.

The $\delta^{15}N$ value of macroalgae from the seven beach sites averaged + 8.98 ± 1.47 ‰, a relatively high value, well within the range reported for sewage nitrogen. The lowest $\delta^{15}N$ value (+ 5.34 ‰) occurred at Captiva; the highest was occurred at Doc's (+ 10.31 ± 0.40; Table 4). Macroalgae, collected from nearshore waters off four beaches and from an offshore artificial reef (GH1 Reef) in Lee County on August 23rd, again showed relatively low C:N:P values, indicating a high degree of nutrient enrichment (Table 5, Fig. 11).

Table 5. Tissue percent carbon, nitrogen and phosphorus; C:N, C:P and N:P molar ratios; and $\delta^{15}N$ in macroalgae collected from nearshore sites in Lee County, FL, USA, on August 23 and September 8, 2005.

Date	Location	Species	¶15N (ä, n=2)	Carbon (% ± 1 SD)	Nitrogen (% ± 1 SD)	Phosphorus (% ± 1 SD)	C:N Ratio (M)	C:P Ratio (M)	N:P Ratio (M)
8/23/05	Fort Myers	*Microcytis aeruginosa*	12.28 ± 0.04						
		Microcytis aeruginosa	10.72 ± 0.43						
		Fort Myers Mean	11.50 ± 0.93	-	-	-	-	-	-
	Lynhall's	*Gracilaria tikvahiae*	9.66 ± 0.13	25.0	2.0	0.18	14.4	352	24.6
		Halymenia floresia	7.92 ± 0.00	23.2	1.3	0.10	21.2	586	27.8
		Hypnea spinella	9.30 ± 0.10	25.3	2.2	0.18	13.3	370	28.2
		Ulva lactuca	8.48 ± 0.01	20.2	1.8	0.13	12.9	390	30.5
		Lynhall's Mean	8.84 ± 0.73	23.4 ± 2.3	1.83 ± 0.4	0.15 ± 0.04	15.4 ± 3.9	424 ± 108	27.8 ± 2.4
	Estero Bay	*Acanthophora spicifera*	6.22 ± 0.16	17.3	1.6	0.17	12.9	254	19.8
		Gracilaria tikvahiae	7.36 ± 0.06	24.7	1.2	0.10	23.5	617	26.4
		Estero Bay Mean	6.79 ± 0.67	21.0 ± 5.2	1.39 ± 0.2	0.14 ± 0.05	18.2 ± 7.5	435 ± 257	23.1 ± 4.6
	Lover's Key	*Acanthophora spicifera*	7.48 ± 0.20	14.8	1.2	0.20	13.9	186	13.5
		Caulerpa sertularioides	7.40 ± 0.00	30.3	2.5	0.13	14.4	580	40.6
		Agardhiella subulata	8.58 ± 0.28	18.9	1.0	0.11	22.9	449	19.8
		Gracilaria tikvahiae	8.70 ± 0.01	18.3	1.1	0.17	18.7	278	15.0
		Hypnea spinella	8.16 ± 0.13	12.6	1.1	0.28	13.6	115	8.5
		Lover's Key Mean	8.06 ± 0.58	19.0 ± 6.8	1.37 ± 0.6	0.18 ± 0.07	16.7 ± 4.0	321 ± 191	19.5 ± 12.5
	Doc's	*Agardhiella subulata*	7.62 ± 0.16	18.9	1.9	0.14	11.5	348	30.4
		Gracilaria tikvahiae	9.07 ± 0.07	18.2	1.9	0.23	11.4	202	17.8
		Hypnea spinella	7.14 ± 0.25	14.6	1.8	0.22	9.6	169	17.8
		Doc's Mean	7.94 ± 0.91	17.2 ± 2.3	1.84 ± 0.1	0.20 ± 0.05	10.8 ± 1.1	239 ± 95.1	22.0 ± 7.3
	GH1 Reef	*Bryopsis pennata*	7.82 ± 0.06	20.4	2.3	0.16	10.2	324	31.9
		Caulerpa mexicana	7.18 ± 0.11	30.6	2.6	0.13	13.5	613	45.8
		Codium carolinianum	7.95 ± 0.04	17.1	1.5	0.09	12.9	467	36.4
		Sargassum hystrix	7.03 ± 0.33	22.3	1.5	0.20	17.1	290	17.1
		GH1 Reef Mean	7.49 ± 0.44	22.6 ± 5.7	2.01 ± 0.6	0.15 ± 0.04	13.4 ± 2.8	423 ± 148	32.8 ± 12.0
		8/23/06 Mean	8.30 ± 1.40	20.7 ± 5.1	1.70 ± 0.5	0.16 ± 0.05	14.9 ± 4.1	366 ± 158	25.1 ± 9.9
9/8/05	Sanibel Red Tide	*Karenia brevis*	7.22 ± 0.64						
		Karenia brevis	8.24 ± 0.91						
		Karenia brevis	8.04 ± 2.04						
		Sanibel Red Tide Mean	7.83 ± 1.14	-	-	-	-	-	-

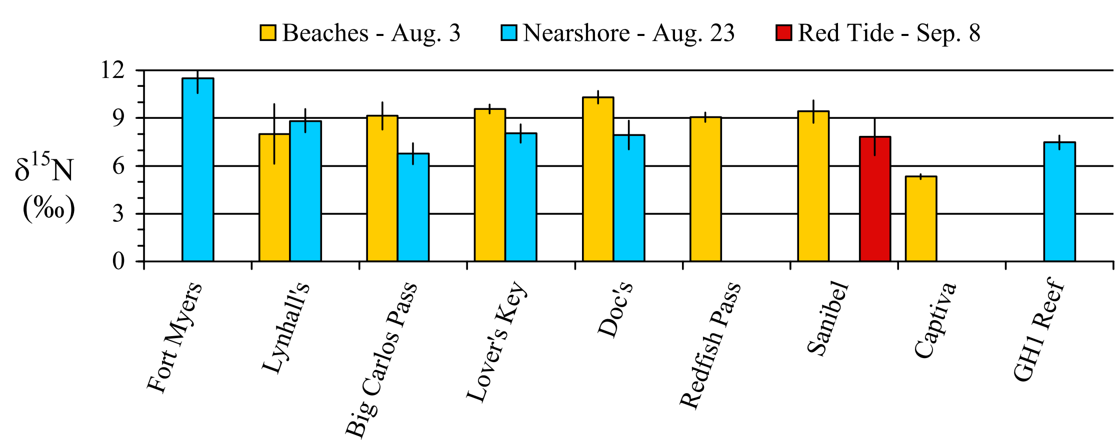

Figure 11. $\delta^{15}N$ values in macroalgae collected from beach and nearshore sites in Lee County, FL, USA,

The expansive blooms of *Microcystis aeruginosa* that occurred in the Caloosahatchee estuary at Ft. Myers in August 2005 had very high $\delta^{15}N$ values averaging + 11.50 ± 0.93 ‰ (Fig. 11, Table 5). These high $\delta^{15}N$ values are typical of sewage effluent and closely match the $\delta^{15}N$ values of macroalgae sampled in the Caloosahatchee River at the Franklin Lock in 2004 (Lapointe and Bedford, in press). The dense red tide sampled on September 8th off southern Sanibel Island had an average $\delta^{15}N$ value of + 7.83 ± 1.14 ‰, which is also within the range of sewage nitrogen. These high $\delta^{15}N$ values in *Karenia brevis* and *Microcystis aeruginosa* provide strong evidence that these blooms were supported by land-based nitrogen discharges, particularly the massive releases from Lake Okeechobee and the Caloosahatchee River that occurred in 2004/2005 following hurricanes Charley, Frances, and Jeanne. These $\delta^{15}N$ data do not support the hypothesis that the red tide off Sanibel Island was supported by nitrogen fixation from the cyanobacterium *Trichodesmium* as suggested by Lenes et al. (2001), which would have produced much lower $\delta^{15}N$ values of ~ 0 ‰ to +1 ‰ (Heaton 1986; Owens 1987).

Literature Cited

Brand, L. and Compton, A. 2006. Long-term increase in *Karenia brevis* abundance along the southwest Florida coast. Harmful Algae, in press.

Glibert, P. M., S. Seitzinger, C. A. Heil, J. M. Burkeholder, M. W. Parrow, L. A. Codispoti, and V. Kelly. 2005. The role of eutrophication in the global proliferation of Harmful Algal Blooms. Oceanography 18:198-209.

Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. Chem. Geol. (Isotope Geoscience Section) 59, 87-102.

Ketchum, B.H. and Keen, J. 1947. Unusual phosphorus concentrations in the Florida "Red Tide" sea water. J. Mar. Res. 7(1), 17-21.

Lapointe, B. E. and Bedford, B. J. 2006. Drift rhodophyte blooms emerge in Lee County, FL: Evidence of escalating coastal eutrophication. Harmful Algae, in press.

Lenes, J. M., Darrow, B. P., Cattrall, C., Heil, C. A., Callahan, M., Vargo, G. A., Byrne, R. H., Prospero, J. M., Bates, D. E., Fanning, K. A., and Walsh, J. J. 2001. Iron fertlization and the *Trichodesmium* response on the West Florida Shelf. Limnol. Oceangr. 46 (6): 1261- 1277.

Owens, N. P. J., 1987. Natural variations in ^{15}N in the marine environment. Adv. Mar. Biol. 24, 389-451.

Slobodkin, L. B. 1953. A possible initial condition for red tides on the west coast of Florida. J. Mar. Res. 12 (1),148-155.