

NOAA-SFERPM: QUESTION #3; ALGAL BLOOMS
“*Chemotaxonomic Assessment of Phytoplankton and Epiphyte Succession in the Rankin Bight - Whipray Basin Areas of North – Central Florida Bay.*”

Dr. J. William Louda
Faculty / Scientist
Organic Geochemistry Group
Department of Chemistry and Biochemistry
and Environmental Sciences Program
Florida Atlantic University
777 Glades Road
Boca Raton, Florida 33431
Phones: Office (561) 297-3309
Lab (561) 297-0010
FAX: (561) 297-2759
email: blouda@fau.edu
B.S., Biology: Wright State University
M.S., Biology: Florida Atlantic University
Ph.D., Marine Science: University of South Florida

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ABSTRACT: These investigations are exploring pigment-based chemotaxonomy for the study of recurrent phytoplankton blooms in north central Florida Bay, concentrating on the Snake-Rankin Bights through Whipray Basin and Flamingo to Sandy Key Basin / Cape Sable regions. Additionally, we have extended study to the epiphyte productivity and community structure in the same area.

The promise of *chemotaxonomy* derives from the fact that the various taxa of photosynthetic organisms evolved slightly different accessory pigments and dissection of natural pigment arrays should allow one to discern the presence, absence and relative abundance of the contributory groups (*cf. Millie et al.*, 1993). In addition to phytoplankton *per se*, epiphytes are being analyzed and bulk taxonomic changes in both populations will be related to fluctuating environmental conditions in order to address causal relationships. Taken together, the free drifting micro-algae (phytoplankton) and the attached micro-algae plus photosynthetic bacteria (epiphytes, periphyton) form a community of rather short lived ‘plants’ which should respond more quickly, relative to macrophytes, to changes in their immediate environment. Therefore, this study addresses the relatively rapid analytical methodology of HPLC-PDA for the generation of chemotaxonomic descriptions of community structures and succession.

Samples include water and periphyton collected monthly from a “T” or “Y” shaped transect (15-18 ± sites) of Snake-Rankin Bights and extending through Whipray Basin, areas shown repeatedly (NOAA chlorophyll maps: <http://www.aoml.noaa.gov/ocd/sferpm/chlmaps.html>) to experience ongoing annual bloom sequences. Samples

are also being collected west of this area (e.g. Sandy Key Basin, Cape Sable, Lake Ingraham) in order to address Gulf of Mexico waters and their influence upon entering the bay.

Recent literature reports of the linkage of anoxic microzones of epiphytic communities with the methylation of mercury (Cleckner *et al.*, 1999) directs this study to include a search for purple-S bacterial markers (bacteriochlorophyll-a, spirilloxanthin) as indicating such micro-niches.

All HPLC/PDA data are being correlated with conventional chlorophyll / pheopigment estimates (spectrophotometric / fluorometric). HPLC-derived Chemo-taxonomic estimates are being refined using data from unialgal cultures, natural blooms, and selected samples are being out sourced for microscopic examination.

Resultant (chemo-)taxonomic descriptions will be placed into the context of environmental parameter changes (N, P, salinity, *etc.*), especially alterations in water quality, quantity and timing in conjunction with all aspects of the Everglades restoration (aka "RESTUDY" = Central and South Florida Project Comprehensive Review Study).

FACILITIES: Our laboratory (Lab # 127) is located on the first floor of the Science and Engineering building of the Charles E. Schmidt College of Science on the main campus of Florida Atlantic University, 777 Glades Road, Boca Raton, Florida 33431.

The laboratory is 650 square feet and allows for graduate student office space, wet lab processing and clean areas for high performance liquid chromatography (HPLC), spectroscopy (UV/Vis [Perkin-Elmer Lambda-2 and -4], Fluorescence [Perkin-Elmer LS-3 and LS-50B]), and gas chromatography (GC[Perkin-Elmer Galaxy]). Presently we operate 4 HPLCs, 1 GPC and 1 low pressure LC system. The main instruments currently used on this project are two identical ThermoSeparations Mdl. 4100 quaternary HPLCs with a Waters-990 photodiode array detectors and a Thermo-Separations quaternary HPLC which includes an Peltier device chilled autosampler.

Departmental facilities, available to all University faculty and staff, include a Varian 500MHz NMR with microprobe and low temperature accessories, an updated GE-300 MHz NMR, a ThermoQuest LCMS, A Finnigan TSQ-MS, a JASCO spectropolarimeter (circular dichroism &c.), numerous FTIRs along with the 'normal' complement of minor instrumentation found at any major university.

Sampling in the bay was performed on the PI's personal boat, a 16 ft. Suncoast with a 50 hp Johnson and is fully Coast Guard equipped (PFDs, VHF-radio, GPS, flares, anchor, fire extinguisher, *etc.*).

PROGRESS: At this writing (09/05/02), the project extends back to September 2000. Samples have been collected on September 29-30, November 31-December 1(2000), February 1-2 (2001), March 29-30, April 18-19, May 23-24, June 13-14, July 11-12, August 23, September 20-21, October 19-20, November 15-16, December 17-18

(2001), January 10-11, February 12-13, March 14-15, April 10-11, May 9-10, June 25-26, July 16-17 and August 12 (2002). Thus, from September 2000 to February 2001 sampling was bi-monthly and has been monthly since then.

EPIPHYTES: Samples of a variety of host macrophytes (*Thalassia testudinum*, *Halodule wrightii*) and macroalgal (*Caulerpa lanuginosa*, *Penicillus capitatus*, *Laurencia sp [intricata-?]*, *Sargassum platycarpum*) have been collected and the pigments of the epiphytes and the epiphyte-free host have been analyzed.

Marcophytes and their epiphytes: Turtle (*T. testudinum*) and shoal (*H. wrightii*) grasses were freed of epiphytes by scrapping with a vinyl gloved hand, rinsing the algal material into a beaker of filtered seawater. The microalgal material is next collected onto Whatman GF/F filters and frozen in liquid nitrogen, as described for phytoplankton.

The pigments of these marine macrophytes were as expected, those of a higher green plant. Their molar distributions (*T. testudinum* / *H. Wrightii*) were: neoxanthin (5.8 / 3.3%), violaxanthin (3.0 / 4.1%), antheraxanthin (2.3 / 0.5%), lutein (19.8 / 9.6%), CHL_b (12.8 / 23.5%), CHL_a (53.1 / 56.4%), and β-carotene (3.1 / 2.8%), very similar to any higher plant (e.g. *Spinacea oleracea*). The xanthophyll cycle pigments (NEO / VIOLA / ANTHERA) are subject to significant fluctuation in accord with light, nutrient and growth histories (Demmig-Adams and Adams, 1992). Trace amounts of a few unknown carotenoids (?-lutein epoxides) and a *cis* (7-?) zeaxanthin were noticed but not pursued. As we reported elsewhere (Louda *et al.* 1999, 2000), turtle grass was found to have a rather active chlorophyllase system and extracts of fresh samples which were not first frozen to deactivate this enzyme (Holden, 1976) yielded much chlorophyllide-*a* and pyrochlorophyllide-*a*. Even with freezing, significant amounts (11 & 16% of the total CHL_a, respectively) of these phytol-free (free carboxylic acid) pigments were found. Conversely, *Halodule wrightii* did not form detectable chlorophyllides-*a*. Both species were found to contain (artifact-?) about 1% of their CHL-*a* and -*b* as the 13²-epimer.

Upon death, turtle grass leaves rapidly loses their pigments and turns brown. Some of this 'brown' color is due to diatoms which use the dead leaf as substrate. The pigments of a 'typical' dead turtle grass blade drifting about on the bottom of Florida Bay indicated (chemotaxonomically) an 'epiphytic' plus 'inquillant' population of cyanobacteria, green algae (or residual turtle grass-?) and diatoms in a ratio of about 6:14:80 (Louda *et al.*, 1999, 2000).

From the beginning of this study, it became increasingly clearer that representative sampling formed the largest obstacle to attaining data from which defendable conclusions could be drawn. Differences have been found, as will be discussed here, but uncertainties as to whether a sample set is "representative" or not, do exist. Therefore, "epiphytometers", allowing for colonization over 1 and 2 month periods prior to 'harvest', have been deployed. This was done in order to provide a more unbiased sampling technique. This, however, is being ran in parallel with natural host sampling in order to test substrate differences, if any.

Table 1 contains selections of the preliminary chemotaxonomic estimates for certain of the macrophytes and macroalgae examined to date.

Shoal (*H. wrightii*) and turtle (*T. testudinum*) grass collected in Snake Bight on 29 September 2000 were found to be dominated by diatoms and dinoflagellates. Turtle grass epiphytes also yielded zeaxanthin and CHL-*b* plus lutein, indicating the presence of cyanobacteria and green algae, respectively. Given the fact that the host is a green plant, a very minor contamination of the epiphyte assemblage with a bit of grass blade could easily explain the “green algal” signal. This possibility was dismissed through experimentation with artificial seagrass wherein chlorophyte microalgae were indeed detected.

Examining Table 1, one may notice a decline in the dinoflagellates on Snake Bight turtle grass between September and November of 2000. Whether this is significant or simply a reflection of sampling bias remains to be tested. That is, it now appears that diatoms plus their ExoPolymeric Substances (EPS) form the epiphytic ‘groundmass’ within which dinoflagellates, cryptophytes and (flagellated?) green algae may be considered as ‘visitors’. There may be certain periods (‘tourist season’) during which the visitor population increases.

Horizontal heterogeneity, within a general sampling locale, has been tested and may be a shortfall of this approach. These data are currently being analyzed and will be reported elsewhere. The use of ‘epiphytometers’, as mentioned above, appears to be an excellent way to ‘normalize’ sampling of epiphytes, especially as to productivity since t_0 can be well defined.

Besides the temporal variability mentioned above, spatial variability can be examined. In one such test case, Table 1 also contains the chemotaxonomic estimates for the epiphytes on *Thalassia* collected on September 29, 2000 in Snake Bight (SNK) and in Whipray Basin (WRB). Here, we found that the WRB samples were much more dominated by diatoms and lacked cyanobacteria and green algae. Aside from the several miles of separation between these sites, there may also be a depth related variable. That is, the Snake Bight samples came from less than one-half meter of water while the Whipray basin sample was from about 2 meters. Thus, the effect of UVA and UVB may play a part in determining the ultimate epiphytic community. All of these variables are amenable to analysis and attempts were made to negate inter- and intra-sample differences such that changes in these communities can be reasonably well coupled to water quality (nutrients, light, TDS, salinity, etc.).

As can be surmised from a quick examination of Table 1, diatoms are indeed the major epiphyte taxon in north central Florida Bay. Sporadically, we find a *Ceramium*-like red alga attached and, when present, a single thallus can form the majority of the biomass. However, as the reds appear to be lightly spread amongst the epiphyte communities in this portion of the bay, we restrict our chemotaxonomic estimates to the non-rhodophyte population. To that end, examination of the macroalgal hosts with an eye-loupe and physical removal of any reds, noting their occurrence, was routine.

TABLE 1: CHEMOTAXONOMIC ESTIMATIONS OF EPIPHYTIC COMMUNITIES.

MACRO-ALGA/-PHYTE	SITE	mo/dd/yr	EPIPHYTES PERCENTAGES.				CHL-a	%EST
			CYAN/GRN/DIAT/DINO/CRYP	$\mu\text{g}/\text{cm}^2$	HPLC			
<i>Caulerpa lanuginosa</i>	WRB	09/29/00	0 / 0 / 93 / 7 / 0					
<i>Penicillus capitatus</i>	WRB	09/29/00	<1 / 0 / 93 / 7 / 0					
<i>Laurencia sp</i>	N.WRB	02/01/01	0 / 0 / 100 / 0 / 0					
<i>Halodule wrightii</i>	SNK	09/29/00	0 / 0 / 94 / 6 / 0					
	N.WRB	04/18/01	3 / 0 / 91 / 6 / 0				99%	
<i>Thalassia testudinum</i>	SNK	09/29/00	2 / 8 / 75 / 15 / 0					
	SNK	11/30/00	12 / 9 / 79 / 0 / 0					
"	WRB	09/29/00	0 / 0 / 94 / 6 / 0					
"	WRB	02/01/01	<1 / 0 / 96 / <4 / 0					
"	N.WRB	02/01/01	8 / 3 / 86 / 3 / 0					
"	N.WRB	04/18/01	5 / 0 / 87 / 8 / 0				105%	
"	WRB	05/23/01	2 / 0 / 94 / 5 / 0	0.76			90%	
"	WRB(8)	06/13/01	4 / 0 / 92 / 2 / 2					

SITES: WRB = Whipray Basin (25°03.977'N x 80°46.179'W)

N.WRB = Northern WRB (25°08.019'N x 80°45.390'W)

SNK = Snake Bight (25°07.991'N x 80°50.855'W)

%EST/HPLC = Percent CHLa estimated from regression relationships of biomarker pigments to CHLa over Σ CHLa found by HPLC.

'EPIPHYTOMETER STUDIES': The idea of using 'fake seagrass' or, as I will call this method, 'epiphytometry', came from a conversation with Dr. Tom Frankovich at the last Florida Bay and Adjacent Marine Systems Science Conference (see Frankovich and Zieman, 2001). Basically, these investigators place Mylar™ strips buoyed by styrofoam and anchored to a weighted support in areas of seagrass growth in order to measure epiphyte loading and epiphytic light attenuation. Their studies, in more southern and south central portions of the bay, reveal colonization by benthic diatoms, coralline red algae, and filamentous red and brown algae.

Classically, 'periphytometers', often microscope slides, have been deployed in a horizontal or vertically with the long axis parallel to the surface. These are rigid collectors which stay still in any water motion. The use of Mylar strips (e.g. <1 cm x 25 cm), anchored to a structure (frame) on the bottom and floated upright in the water column, allows the artificial collection site to move to-and-fro with water motion. The flexible upright nature of the Mylar substrate moving in the water column appears to present an excellent mimic for natural seagrass, notably turtle grass. Colonization density and community similarities also support this conclusion, as described below.

Beginning in May 2001, a few of these 'epiphytometers' were constructed and deployed in Whipray Basin. The 'epiphytometer' or fake seagrass 'mini-meadows' were constructed of 1 inch schedule 40 PVC tubing. The size and shape is about 40 x 40 cm

square with an “+” in the center. Thus, construction required four “L”, four “T” and one “+” connections, plus twelve 15-18 cm pieces of 1 inch tubing. The tubing is filled with cement and joined using PVC sealants rated (non-leeching) for use in potable water supplies. Mylar (e.g. overhead transparency) is cut into ~1cm x 25cm strips and affixed to the ‘top’ of the weighted PVC framework as follows: a slit is cut into the PVC using a corundum cutting wheel in a Dremmel™ tool, the Mylar™ strip is inserted and sealed in place using a water resistant sealant such as ‘Marine Goop™’. Attachment of a small (~0.5 x 0.5 x 0.5cm) piece of closed cell neoprene sponge using the same ‘Marine Goop™’ gives the attached fake grass blade the requisite flotation into the vertical aspect.

At each site where the ‘epiphytometers’ are to be deployed, a total of 3 units is required. That is, deployments are being made for a total of 2 months only. The 60-65 day limit is being imposed based on an average 65 day lifespan for *Thalassia* long shoots (T. Frankovich, pers. commun. 2001). Growth and chemotaxonomic estimates will be made for 1 and 2 month deployment. Thus, 2 units will be at each site after the first month deployments. The third unit (t₀) is for exchange with the 2 month unit being terminated and cleaned.

Beginning in the Summer of 2001, a graduate student, Ms. Alaya Singh, began her master’s thesis research on the epiphytes of natural and ‘fake’ *Thalassia testudinum*. Hopefully, this project will provide a readily quantifiable method by which to assess epiphyte productivity and community structure. These parameters will then be linked to changes in water quality (S_{psu}, N, P, light transmission, etc.) as a monitoring tool potentially available for adaptive assessment of perturbations within the system.

Initial results are extremely encouraging in that microscopic examination of the ‘epiphytometer’ growth revealed an abundance of diatoms with occasional dinoflagellates moving through a microcarbonate laden matrix of apparently diatom derived exopolymeric substances (EPS, polysaccharides-?: cf. Louda *et al.* 2001 this website and Louda *et al.*, 2002-submitted). HPLC data match the visual assessment and more quantitative comparisons will be made.

Following are the initial data collected from the ‘epiphytometers’ as deployed for 1 month, and a comparison with the epiphytes removed from ‘real’ *Thalassia* at the same site, in this case- Station #8 (Table 3), just east of the Dump Keys.

SAMPLE	AREA(cm ²)	CHLa/c (µg/cm ²)				
		YIELD	CYAN	GRN	DIAT	DINO/CRYPTO
‘Real’	15.72	1.34 / 0.15	4	0	92	2 / 2
‘Mylar-A1’	24.19	0.44 / 0.08	1	0	91	5 / 3
‘Mylar-A2’	13.71	0.45 / 0.06	2	0	85	4 / 9
‘Mylar-A3’	12.10	0.59 / 0.09	2	0	90	4 / 4

Each 'epiphytometer' sample (A1-A3) consisted of a section cut from the 'middle' of a separate blade of Mylar™. The epiphytes from the 'real' *Thalassia testudinum* are of unknown age, but likely less than 2-1/2 months, based on the shoot life span estimates made by T. Frankovich (pers. commun. 2001). Within experimental parameters, the pigment-based chemotaxonomic estimate of community structures match for all of these samples. The higher (9%) amount of cryptophytes on sample "A2" is noted.

The pigment-based evaluation also matches my initial microscopic survey, as mentioned above. That is, a mass of diatoms embedded in an EPS matrix with an occasional dinoflagellate whirling through the observed field. Cyanobacteria could not be observed under the magnifications utilized (100-400X). Epifluorescence and higher power is planned as 'ground truthing' by contracted microscopic taxonomic evaluations are underway.

The concentration of epiphyte chlorophyll-*a*, a proxy for biomass, on native *Thalassia* is about 3X that of the 1-month growth on the Mylar strips. Data from continued sampling has revealed that the 'epiphytometer' epiphyte growth adequately mimics nature in both quality and quantity, as colonization times are extended. This, as well as deviations from direct trends, is discussed below.

Epiphyte productivity / biomass monitoring and comparison to changing water quality / quantity conditions is suggested as a viable use of these methods. These parameters should certainly exhibit changes in concert with system perturbations and feedback for 'adaptive management' decisions is quite feasible.

Figure E1 is given below and contains selected plots of epiphyte growth as monitored on "epiphytometers". Values obtained for 'real' seagrass, namely *Thalassia testudinum*, are included for comparison. In essence, all three of these growth scenarios match that predicted in the hypothesis. That is, growth can be monitored with these "epiphytometers". The dates given are the sampling date. For example, the 1 and 2 month points for the JUL01 (=July 2001) sampling are for epiphytometers placed in the field (t_0) in June and May 2001, respectively. That 2 sample sets have 2 month yields lower than 'real' seagrass taken at the same time may reflect a bit longer growth time on the real blades or it may equally as well indicate better settling characteristics for the real blades. Experiments to test selectivity (green vs. clear fake blades, epicuticular waxes, if any, and the-like) are planned. However, the October sample set for the Roscoe Key site reveals that growth to a load mimicking that found on real seagrass can also occur within a single month.

Table 2: Epiphyte data for “real” (*Thalassia testudinum*) and “fake” (mylar strips, aka ‘epiphytometers’) substrates with 1 and 2 month growths.

MONTH-SITE	(CHLa [$\mu\text{g}/\text{cm}^2$] Cyano/Chloro/Diato/Dinos/Cryptos (% EST/HPLC))		
	REAL	1 MONTH	2 MONTH
2001			
JUNE-DERELICT	(1.40) 2/0/95/4/0 (94%)	(0.30) 1/0/96/3/0 (101%)	XXX
ROSCOE	(6.59) 1/1/94/4/0 (93%)	(2.00) <1/0/95/4+/0 (87%)	XXX
DUMP	XXX	(0.49) 2/0/87/4/5 (144%)*1	XXX
JULY-DERELICT	(0.93) 2/0/94/4/0 (58%)	(0.32) 2/0/98/0/0 (97%)	(0.68) 1/1/94/4/0 (54%)
ROSCOE	(1.47) 1/0/95/3/1 (60%)	XXX	(1.78) 2/0/92/6/0 (54%)
DUMP	(1.43) 0/0/88/12/0 (39%) *2	XXX	(1.43) 2/0/92/6/0 (58%)
AUG-DERELICT	(3.02) 1/0/93/7/0 (104%)	(5.11) 1/0/92/6/0 (81%)	(6.66) 2/0/92/6/0 (101%)
ROSCOE	XXX	(1.34) 2/0/87/11/0 (117%)	(1.01) 0/0/93/7/0 (81%)
DUMP	XXX	(2.12) 1/0/96/3/0 (106%)	XXX
SEPT-DERELICT	(3.50) 0.7/0/98/1.3/0 (62%)	(0.85) 0.3/0/95.4/4.4/0 (65%)	(0.31) 0/0/96/4/0 (77%)
ROSCOE	(0.92) 0/7/83/6/3 (87%)	(0.39) 0/0/93/7/0 (74%)	(0.50) 0/0/89/7/3 (82%)
DUMP	(0.19) 0/0/100/0/0 (85%)	(0.47) 0.5/0/97.5/2/0 (80%)	(0.48) 0/0/100/0/0 (64%)
OCT-DERELICT	(0.20) 0.3/0/99.7/0/0 (90%)	(0.23) 0/0/99/1/0 (71%)	(0.28) 0/0/100/0/0 (79%)
ROSCOE	XXX	XXX	XXX
DUMP	XXX	XXX	XXX
SNAKE	(0.36) 0/28/65/6/0 (100%)	(0.30) 0/0/96/1.5/2.5 (89%)	XXX
NOV-2001+	being processed and will be reported elsewhere.		

Many additional experiments have occurred. Replicate samplings within a single ‘epiphytometer’, at a single site and within a site ‘area’ (5 epiphytometers about 100m. apart) have been conducted in order to give the statistical spread of data (=error bars for sampling). All of these results will follow with Ms.Alya Singh’s thesis. It is hoped that we will be able to offer “epiphytometers” as “hard-and-fast” monitoring / adaptive assessment tools.

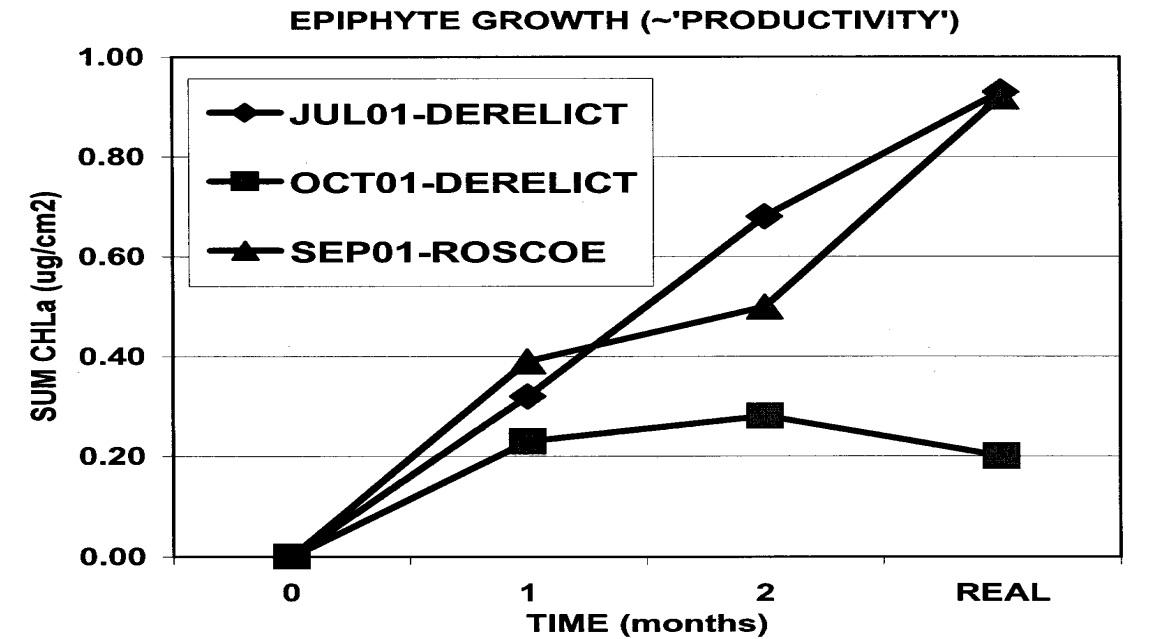


Figure E1: Plot of “epiphytometer” monitored epiphyte growth.

Macroalgae and their epiphytes: A variety of macroalgae also exists in Florida Bay and the epiphytes associated with these were also examined, though in a more cursory manner when compared to the seagrasses. Additionally, the macroalgae *per se* are being analyzed as potential sources of known pigments.

Caulerpa lanuginosa(?), *Penicillus capitatus*, *Laurencia sp [intricata-?]*, and *Sargassum platycarpum* have been collected and the pigments of the epiphytes and the epiphyte-free host have been analyzed.

The chlorophytes, *C. lanuginosa*(?) and *P. capitatus* were found to contain siphonoin or siphonaxanthin, respectively, in addition to the standard green algal/plant pigments (CHLs-*a*/*b*, lutein, neoxanthin, antheraxanthin, violaxanthin, β -carotene). Both of these algae are sessile benthic forms with many cavities to collect detritus. Thus, a separation of trapped detrital material, including resuspended sediments / surficial floc (see Louda *et al.*, 1998, 2000), from ‘true’ epiphytes is essentially impossible. However, in all cases, the ‘epiphytic’ communities were found to be vastly dominated by diatoms, according to pigment data. *Caulerpa lanuginosa* from Whipray Basin in September 2000 was estimated as having an epiphytic community comprised of diatoms, dinoflagellates and cyanobacteria in a ratio of 93.0 / 6.7 / 0.3. Additionally, there was a small but well defined presence of bacteriochlorophyll-*a*, indicating purple-sulfur bacteria, in the ‘epiphytes’ removed from *Caulerpa lanuginosa*. Using protein biomass to CHL-*a*, or

BCHL-a, ratios of 34.5:1, or 13.5:1 respectively (Steenbergen and Korthals, 1982; vanGermerden, 1980), it was estimated that the oxygenic to anoxygenic phototroph split of this community was 115:1. Given that fresh water periphyton microniches of anoxia have been implicated in the methylation and mobilization of mercury (Cleckner *et al.*, 1999) , and potentially other metals / metalloids, the existence of purple-S bacteria in a periphyton community of Florida Bay becomes significant. However, the possibility that the bacteriochlorophyll-a signal found in the epiphytes of *Caulerpa* derives from trapped resuspended surficial sediments, reported to have significant amounts of purple-S bacteria (Louda *et al.*, 2000), strongly exists.

Studies on macroalgae and their epiphytes continued on an opportunistic basis. That is, no on going sampling and/or monitoring of macroalgal epiphytes was planned. As stated, epiphyte studies were focused on *Thalassia testudinum* and its 'mimic' ("fake seagrass" or 'epiphytometers'), with minor emphasis on *Halodule wrightii*.

PHYTOPLANKTON:

Initial 'survey' samples of several sites in north-central to western Florida Bay were collected during September and November of 2000. Beginning February 1-2, 2001 thirteen main stations were established and are being visited monthly for water (phytoplankton) sampling. Table 3 is a list of these sites.

Table 3: Site descriptions, Florida Bay sampling.

<u>SITE#</u>	<u>DESCRIPTION</u>	<u>Latitude (N) x Longitude(W)</u>
1	MKRS 9/10, Flamingo	25-07.393' x 80-55.792'
2	E. "Flamingo Trough"	25-07.803' x 80-54.121'
3	Mid. Snake Bight 'Basin'	25-07.584' x 80-51.649'
4	Mid.Rankin-Camp Basin	25-07.322' x 80.49.017'
5	SE of Derelict Key,N-WRB	25-08.019' x 80.45.390'
8	E. off Dump Keys, WRB	25-06.819' x 80-46.075'
6	Central Whipray Basin	25-05.170' x 80-46.278'
7	Southern Whipray Basin	25-03.977' x 80-46.179'
9	Mkr. #5; Middle Grounds	25-05.783' x 81-01.823'
10	Sandy Key Basin	25-05.073' x 80.59.855'
11	Mkr.#1A; East Cape Sable	25-06.551 x 81.05.216'
14	E. Cape Sable; ENP bndry	25-07.854 x 81-08.289'
12	M.Cape Sable; ENP bndry	25-09.297' x 81-09.657'
OS-1	Snake Bight "Basin"(3)	25-07.991' x 80-50.855'
OS-2	Snake Bight "Basin"(4)	25-09.032' x 80-49.990'
OS-3	MKR. #8 ; W. of Flamingo	25-06.878' x 80-57.326'
OS-4	Snake Bight Chnl(AA)	25-07.357'x80-50.890'

OS-5	Nearshore trough, NE of #9	25-07.104'x81-00.890'
OS-6	E. Lk. Ingram	25-08.510'x81-05.252'
OS-7	Mouth, E. Cape Canal	25-07.480'x81-03.930'

Table 4 contains the HPLC-PDA derived yields of total chlorophyll-*a* and accessory pigment derived Chemotaxonomic estimates of the phytoplankton communities.

Table 4: HPLC Derived phytoplankton data: Total chlorophyll, Chemotaxonomic community estimates and percent measured over chemotaxonomically estimated chlorophyll-*a*.

SITE	([CHLa] μg/L) Cyano/Green/Diats/Dinos/Cryptos (%EST/HPLC-CHLa)			
	SEPT.29.2000	NOV.30/DEC.1.2000	FEB.1-2.2001	MAR.29-30.2001
1	(4.57) 0/0/100/0/0 (88%)	(1.50) 0/0/100/0/0 (67%)	(2.13) 0/15/66/0/19 (88%)	(5.61) 0/0/87/0/13 (100%)
2	xxx	xxx	(1.02) 0/24/61/0/15 (98%)	(2.02) 0/0/100/0/0 (93%)*
3	(2.01) 0/0/89/11/0 (100%)	(1.78) 0/0/100/0/0 (74%)	(0.33) 0/0/100/0/0 (65%)	(1.59) 0/0/100/0/0 (115%)*
4	xxx	xxx	(0.38) 0/0/100/0/0 (70%)	(0.61) 0/0/100/0/0 (62%)
5	xxx	(1.09) 51/0/31/19/0 (114%)	(0.26) 0/0/100/0/0 (30%)	(0.52) 0/0/100/0/0 (59%)
8	xxx	(0.90) 0/0/100/0/0 (80%)	(0.25) 0/0/100/0/0 (53%)	(0.39) 0/0/100/0/0 (81%)
6	xxx	(15.45) 92/0/8/0/0 (105%)	(0.36) 0/0/100/0/0 (64%)	(0.27) 0/0/100/0/0 (94%)
7	(1.79) 0/0/80/20/0 (81%)	xxx	(0.28) 0/0/64/36/0/0 (93%)	(0.14) 0/0/100/0/0 (??)
9	xxx	xxx	(1.17) 0/42/38/0/20 (103%)	(2.24) 0/19/61/0/20 (112%)
10	xxx	xxx	(1.38) 0/43/31/4/22 (119%)	Xxx
11	xxx	xxx	(2.76) 0/31/51/0/18 (99%)	(3.20) 0/19/64/0/17 (101%)
14	xxx	xxx	(0.90) 0/65/35/0/ (103%)	xxx
12	xxx	xxx	(0.88) 0/74/22/0/4 (109%)	xxx
OS-1	(2.40) 31/0/5/19/0 (80%)	xxx	xxx	xxx
OS-2	(8.52) 63/0/29/7/0 (91%)	xxx	xxx	xxx
OS-3	(5.57) 0/0/100/0/0 (68%)	xxx	xxx	xxx
OS-4	Xxx	xxx	xxx	(0.53) 0/0/100/0/0 (117%)

Examination of total chlorophyll and phytoplankton communities across the north-central and north-western Bay reveals several patterns and reinforces our hypothesis that chemotaxonomy can be utilized to follow microalgal dynamics. A few 'broad brush' preliminary conclusions follow. Do note that the accuracy of these estimates are still being assessed. However, I do feel that the relative order and the approximate magnitude of each taxon's contribution to the total chlorophyll-*a* pool is correct.

September 29-30, 2000. Total CHLa increased (4.6 \Rightarrow 5.6 μ g/L) westerly from Flamingo (Site#1 \Rightarrow #OS-3: Tables 3-4) and was due solely to diatoms (*viz.* Chrysophyta, likely Bacillariophyceae). Diatoms and dinoflagellates dominated Whipray Basin (Site # 7; 75:25%) and mid Snake Bight 'basin'(Site#3; 82:18%) and gave total CHLa values of 1.8 and 2.0 μ g/L, respectively. Within the Snake Bight to Shark Point

area (Sites #3 to #OS-1 to #OS-2) total CHLa increased ($2.0 \Rightarrow 2.4 \Rightarrow 8.6 \mu\text{g/L}$) in concert with increases in the amount of cyanobacteria (cyano/diat/dino = $0/82/18 \Rightarrow 20/53/27 \Rightarrow 50/38/12$). Thus, it appears that a cyanobacterial bloom existed near Garfield Bight and its effects spread, at least, southwestward.

November 30-December 1, 2000. Relative to 29-30 September 2000, total CHLa at sites #1 and #2, the eastern portions of what I call the 'Flamingo trough', decreased considerably ($\sim 5 \Rightarrow 1.5/1.8 \mu\text{g/L}$) and remained essentially diatom in pigment signal. A strong cyanobacterial bloom, reaching levels of 11.9 to 15.4 $\mu\text{g/L}$ as CHLa was present in Whipray Basin. That 2 sites in Whipray Basin proper gave significantly different total CHLa values, though each was quite high, signals patchiness at least on a 'meso' scale. These 2 sites were about 3-4000 meters apart. It is therefore suggested that bloom patchiness needs to be addressed when assessing overall productivity patterns and CHLa isopleths. In Whipray Basin from the area of the Dump Keys (Site #8) to around Derelict Key (Site #5), CHLa values dropped dramatically to about $1 \mu\text{g/L}$ and was comprised of 100 % diatoms at the Dump Keys or a nearly equal (35/36/29) mixture of cyanobacteria / diatoms / dinoflagellates at the Derelict Key site. The low CHLa signal comprised only of diatoms could be associated with the incoming tide at the time of collection. That is, this could be a reflection of waters coming through the (W-E) Snake Bight / Rankin-Camp Basin boat channel (Sites #1 \Rightarrow 2 \Rightarrow 4 \Rightarrow 8).

February 1-2, 2001. February 1-2, 2001 began the monthly sampling regime at the 13 established sites (Table 4).

The north-central bay (Sites #3 through #8:Table 4) was found to be rather depleted in phytoplankton and gave but $0.3 - 0.4 \mu\text{g/L}$ as total CHLa. The only detectable accessory pigments were CHLs- c_1/c_2 , fucoxanthin and traces of diadinoxanthin. Thus, an 100% diatom population is concluded for all sites #3-8, except #7. Site #7 (Table 4) also yielded peridinin, in addition to fucoxanthin, and a diatom / dinoflagellates community in a 69:31 ratio was estimated.

The north-western bay waters contained a diverse phytoplankton community, as signaled by a complex array of photosynthetic and photoprotectorant pigments. Total chlorophyll-*a* yields were only moderate ($1.0 - 2.8 \mu\text{g/L}$) but the presence of chlorophyll-*b*/lutein, fucoxanthin/diadinoxanthin, peridinin and alloxanthin/ α -carotene revealed the presence of chlorophytes, diatoms, dinoflagellates and cryptophytes. Diatoms varied from 53-76% of the communities across this area (Sites 1-2, 9-14: Table #4). 2 (3-?) main divisions of the north-western bay are suggested by the distribution of pigments. Cryptophytes (alloxanthin plus α -carotene) were in highest abundance in those sites closer to shore between East Cape Sable and Flamingo (Sites # 11, 9, 1, 2: Table 4). Chlorophytes (CHL*b* plus lutein &c.) were most apparent in the sites from Middle Cape Sable to Sandy Key Basin (Sites 12-14-11-9-10: Table 4). As with Whipray Basin (Site #7), only the southernmost site (#10, Sandy Key Basin) gave a quantifiable dinoflagellate signal. In this case, 6 % of the total population. It can be noted that, even though the total concentration of CHLa ranged from $1.0 - 2.8 \mu\text{g/L}$, the waters in the north-western bay and out into the Gulf waters off from middle Cape Sable were

extremely “grass-green” to the eye. This contrasted quite well with the more olive-green color of the waters of Whipray Basin during the strong (12-15 µg/L) cyanobacterial bloom in November.

March 29-30, 2001: As in February, the samples from sites in Snake Bight, Rankin/Camp Basin and Whipray Basin yielded only diatom pigments. Total CHLa decreased from Snake Bight (Site #3: 1.8 µg/L) into Whipray Basin. Within Whipray Basin, CHLa decreased steadily from near Derelict Key (Site #5: 0.58 µg/L) southward (Sites #8⇒#6⇒#7: 0.49 ⇒ 0.30 ⇒ 0.15 µg/L).

Highest CHLa values were found in the “Flamingo Trough” (Sites 1 & 2: 6.3 & 2.2 µg/L, respectively) , the middle grounds (Site #9: 2.5 µg/L) and East Cape Sable (Site #11: 3.5 µg/L). Rough seas prevented visiting sites 10, 12 and 14.

Sites near Flamingo contained diatoms and cryptophytes (~90:10, Site #1) or diatoms alone (Site #2). The finding of higher productivity (*viz.* standing crop) at Site # 1, relative to sites on either side (# 2 and #9), could be a reflection of nutrient influx from anthropogenic sources at Flamingo and/or one of many other possibilities (e.g. Redfield Ratio, subterranean P-sources, Rookery activity, *etc.*).

Chlorophytes (13-14%) and cryptophytes (12-14%) accompanied diatoms (72-75%) in the phytoplankton of the northwestern Bay (Sites #9 and 11).

April 18-19, 2001: Samples were collected from all sites (Table 4) except # 12 and 14, again due to windy weather / rough seas. An additional site, #OS-5, was collected between # 9 and #1 and much closer to shore (25° 07.104’N x 81° 00.890’W) in order to follow up on the observation from February and March data that the cryptophytes appeared to be more prevalent closer to shore.

Continuing the trends found in previous months, highest total chlorophyll-a (3-6.5 µg/L) was found in the western bay and derived mainly from diatoms (70-91%). Cryptophytes, as evidenced by the marker pigment alloxanthin, were found to be highest through the Flamingo trough (sites 1, 2, 9, 11). Chlorophytes, evidenced by chlorophyll-*b**, were most abundant (5-12%) in the westernmost (Sites 9, 11, OS5) part of the bay. As in March, sites in the northern central bay (3-8) were of lower overall CHLa (0.1-1.7 µg/L) and were strongly diatom dominated. Dinoflagellates, as signaled by peridinin, were found to be nearly co-dominant (22-53%) in Rankin Lake (site #4) and throughout Whipray Basin (Sites 5-8).

Table 4 cont. [CHLa] (µg/L) Cyano/Chloro/Diats/Dinos/Cryptos (%Est/HPLC-CHLa)

<u>SITE</u>	<u>APR.18-19,2001</u>	<u>MAY 23-24, 2001</u>	<u>JUNE 13-14, 2001</u>	<u>JULY 11-12, 2001</u>
1	(3.13) 3/0/86/0/12 (107%)	(6.85) 4/0/83/0/14 (96%)	(3.70) 14/0/72/5/8 (107%)	(3.22) 12/3/66/2/18 (103%) S= 43
2	(1.78) 0/0/82/0/18 (112%)	(4.10) 0/0/99/0/1 (89%)	(1.40) 24/0/56/0/20 (113%)	(2.35) 12/0/70/0/18 (95%)
3	(0.81) 0/0/100/0/0 (88%)	(1.37) 3/8/62/6/20 (95%)	(0.80) 14/0/32/12/43 (144%)	(1.13) 9/8/30/26/27 (116%)

4	(1.71) 0/0/62/38/0 (122%)	(0.22) 0/0/56/0/44 (84%)	(0.70) 22/0/40/38/0 (121%)	(1.18) 10/0/28/33/29 (119%)
5	(0.59) 0/0/63/37/0 (64%)	(0.33) 0/0/100/0/0 (31%)	(0.70) 5/0/25/29/41 (130%)	(0.66) 6/0/28/34/31 (99%)
8	(0.24) 0/0/51/49/0 (130%)	(0.28) 30/0/70/0/0 (50%)	(0.73) 8/0/42/20/30 (121%)	(0.74) 25/0/32/18/25 (119%) S= 51
6	(0.51) 0/0/74/26/0 (112%)	(0.13) 0/0/100/0/0 (71%)	(0.52) 0/0/23/39/38 (131%)	(0.56) 0/0/52/48/ 0 (84%)
7	(0.11) 0/0/41/59/0 (199%)	XXX	(0.56) 8/0/43/21/28 (139%)	(0.93) 16/0/21/51/11 (74%) S=49
9	(2.62)9/10/65/0/16(122%)	(2.08) 16/0/45/0/38 (49%)	(6.18) 2/0/96/3/0 (84%)	(3.25) 5/9/67/9/10 (99%)
10	XXX	(2.07) 9/0/87/0/4 (73%)	(9.64) 3/0/97/0/0 (87%)	(5.39) 2/4/80/4/9 (95%) S= 42
11	(6.47) 2/8/77/0/14(104%)	(5.48) 3/0/88/0/9 (84%)	(6.44) 5/0/85/4/6 (94%)	(5.50) 2/0/81/2/14 (94%)
12	XXX	(2.53) 6/0/66/8/16 (119%)	(0.65) 33/12/37/8/10 (132%)	XXX
12Mid	XXX	XXX	(0.60) 38/14/31/7/10 (113%)	XXX
12Bot	XXX	XXX	(3.39) 12/0/48/15/25 (251%)	XXX
OS-1	XXX	XXX	XXX	XXX
OS-2	XXX	XXX	XXX	XXX
OS-3	XXX	XXX	XXX	XXX
OS-4	XXX	XXX	XXX	XXX
OS-5	(3.23)8/16/59/0/17 (117%)	(7.34) 3/0/87/0/10 (93%)	(8.72) 1/0/95/0/4 (82%)	(6.56) 7/0/79/0/14 (78%)
OS-6	XXX	XXX	(4.60) 9/0/77/0/14 (102%)	(2.22) 12/0/60/0/28 (123%)
OS-7	XXX	XXX	(9.77) 2/0/92/0/6 (85%)	(3.22) 4/0/83/0/13 (123%)

May 23-24, 2001: The chlorophytes (CHL**b**) which were detected in samples collected during February, March and April, were below detectable limits in May.

The western bay held with good production of CHL**a** (2-7.3 $\mu\text{g/L}$) from diatoms. Cryptophytes remained prevalent (3-30%) in the western Flamingo trough with the highest relative abundance (30%) found at the Middle Grounds (Site #9).

Standing crop of CHL**a** continued to be low (0.13 – 0.33 $\mu\text{g/L}$) along the Whipray Basin transect (Sites 5-8). An relative increase in cyanobacterial signal, as zeaxanthin, was noted near the Dump Keys (Site #8).

A signal from chlorophytes (~10% of total CHL**a**) was detected in the Snake Bight channel (Site #3). However, macerated seagrass due to high boat traffic in this area cannot be discounted even though every effort is made to visually examine filters. As this is the only sample in the May 2001 suite which had CHL**b**, the author suspects it as spurious(?). This was later shown not to be the case and Chlorophyta is concluded.

Cryptophytes were found to contribute about 18 – 32% of the CHL**a** in the phytoplankton from the Snake Bight channel (Site #3) and Rankin Lake (Site #4) areas.

June 2, 2001; Extra Sampling: Liaison with SFWMD and the NOAA overflight of northern Florida Bay, during which new microwave technology for the remote measurement of salinity and temperature technology was utilized, will allow us to compare HPLC-based quantitation and chemotaxonomy with a wide variety of *in situ* data (SFWMD: on-board real-time flow-through fluorescence, turbidity *etc.*) as well as the remote sensing data (NOAA). Twelve samples were collected by Dr. Chris Madden of the SFWMD. Sites included most of the northern eastern and central bay (Buttonwood Sound, Maderia Bay, Whipray Basin, Rankin Lake, Terrapin Bay and Monroe Lake). A separate report (site = ?), will give the site locations, selected physical data, pigment data and discussion for these samples.

June 13 –14, 2001: CHLa levels at the east end of the ‘Flamingo Trough’ (Sites 1-2) were found to be about ½ those found the previous month and therefore about equal to those in April of 2001. CHLa derived from diatoms and cyanobacteria / cryptophytes, in that order. Within the Snake Bight channel (Site #3) a significant signal was found from chlorophytes (~10% CHLa) and dinoflagellates (~6%). Finding CHLb for a second month in a row at this site tends to refute the notion of macerated seagrasses and indicates a true presence of chlorophyte phytoplankton.

The Rankin Lake (#4) and Whipray Basin (#5-8) sites all reveal a two to four-fold increase in total CHLa (0.13-0.33 to 0.52-0.73 µg/L) accompanied by significant increases in the relative amount of CHLa contributed by dinoflagellates and cryptophytes.

The western ‘Flamingo Trough’ (Sites 9 & 11) and Sandy Key Basin (Site #10) underwent a three-fold increase in total CHLa, relative to May 2001, apparently due to increases in the amount of diatom-derived CHLa.

Site #OS5, close to shore in the ‘Flamingo Trough’, exhibited a slight increase in total CHLa (7.3 to 8.7 µg/L), relative to the previous month. The CHLa contributions of the various taxa remained essentially unchanged (diatoms >> cryptophytes > cyanobacteria).

Samples collected in Lake Ingram (#OS6) and at the mouth of the East Cape Canal (#OS7) contained high levels of total CHLa (4.6 & 9.8 µg/L, respectively) with contributions coming from taxonomic distributions identical to those in #OS5. Elevated CHLa at the mouth of the East Cape Canal may (note: “may”) indicate the mixing of waters from Lake Ingram and more interior portions (N-rich ??) with P-rich gulf waters. This needs to be followed up with Organic-N, TN, TIN, TP, SRP *etc.* analyses, which are beyond the scope of the present project.

Waters off of Middle Cape Sable at the Park Boundary (Site #12) reflected a four-fold decrease in total CHLa (2.5 to 0.6 µg/L), relative to May 2001. The contribution of CHLa by the various taxa revealed an order of diatom > cyanobacteria > chlorophytes > dinoflagellates ≈ cryptophytes.

A benthic sample (resuspended surficial 'floc') at Site #12 was found to have greatly increased *pheopigments* including pheophytin-*a*, pheophytin-*a*-epimer, pyropheophytin-*a*, pheophorbide-*a* and pyropheophorbide-*a*. Overall data on the pheopigments in the phytoplankton samples is being processed and will be reported separately in the near future.

July 11-12, 2001: Discussion here includes mention of taxon distributions and are given in the order" cyanobacteria / chlorophytes / diatoms / dinoflagellates / cryptophytes. Data discussed is within Table 4.

Sites 2, 1 & 9, an E-W transect of the "Flamingo Trough", had CHLa values (2.4 – 3.2 µg/L) and a taxonomic spread (5-10 / 3-9 / 66-67 / 2-9 / 10-18) not very different from the June values.

Sites 3 and 4, Snake Bight and Rankin Lake, revealed 40 and 68% increases in total CHLa. This may be due to a combined absolute increase in dinoflagellates plus cryptophytes while total cyanobacteria plus diatoms were relatively stable, thus changing relative abundances slightly.

The Whipray Basin sites 5, 8, 6 and 7, forming a N-S transect, had moderate CHLa (0.6-0.9 µg/L). Total CHLa for these sites from February 1 through April 18, inclusive, was but 0.1-0.6 µg/L, with most values only at 0.1 – 0.3 µg/L. Throughout this period diatoms and dinoflagellates plus cryptophytes have dominated. The present suite gave taxonomic estimates of 9-10 / 0-8 / 28-30 / 26-33 / 27-29. Thus far, only one sample (Central Whipray Basin, Site #6, February 1, 2001) has exemplified the "cyanobacterial bloom" (CHLa > 15 µg/L and cyanos > 90%) of this basin.

The highest CHLa concentrations were found in the western province, here made up of sites 10, 11 and OS5. This is the Sandy Key Basin to East Cape Sable and along shore to the east from the cape to OS5 which is located at the eastern edge of a small Cul du sac of "deeper" (z > 2 m) water. Diatoms are the dominant (79-80%) taxon, with cryptophytes a distant (9-14%) second.

August 22-23, 2001: The eastern "Flamingo Trough" (sites 1 – 2), extending into Snake Bight (Site #3) had moderate CHLa (1.6-1.7 µg/L) and was comprised mainly of a diatom plus cryptophyte assemblage (8 / 4-15 / 43-60 / 2-9 / 25-26) and (11 / 11 / 33 / 12 / 18). The Snake Bight site having a more even spread of taxa, as shown.

Rankin Lake (#4) had elevated CHLa (2.9 µg/L) presumably due to an increase in the cyanobacterial population (63 / 0 / 8 / 12 / 18), relative to July.

The Whipray Basin N-S transect (5->8->6->7) revealed increasing CHLa (0.4-> 1.2 –1.4 µg/L) to site #6 (Central Whipray), followed by a decrease (0.8 µg/L) in the southern basin (#7). The highest values, sites #8 and #6, corresponded with population dominance by cyanobacteria plus either dinoflagellates or diatoms, respectively.

Sites # 9, #11 and OS5 had a strong CHLa signal (3.5-4.7 µg/L) and were dominated by diatoms plus cryptophytes.

Table 3 cont. [CHLa] (µg/L) Cyano/Chloro/Diats/Dinos/Cryptos (%Est/HPLC-CHLa)

<u>SITE</u>	<u>AUG. 22-23, 2001</u>	<u>SEPT. 20-21, 2001</u>	<u>OCT. 18-19, 2001</u>
1	(1.72) 8/4/60/2/25 (114%) S= 37	(9.09) 1/12/77/3/8 (115%) S=35	(4.62) 2/5/79/6/9 (116%) S=34
2	(1.57) 8/15/43/9/26/ (154%) S=37	(5.32) 0/0/93/7/0 (93%) S=34	(3.52) 3/12/68/5/12 (153%) S=34
3	(1.58) 11/11/33/12/34 (143%) S= 37	(0.47) 1/18/44/15/22 (142%) S=34	(6.47) 80/0/10/4/6 (92%) S=29
4	(2.89) 63/0/8/12/18 (109%) S= 33	(1.25) 0/9/47/20/23 (122%) S=37	(4.17) 18/5/64/10/4 (102%) S=30
5	(0.45) 10/25/16/31/18 (162%) S= 36	(3.13) 66/1/11/13/9 (81%) S=29	XXX
6	(1.40) 31/7/35/11/16 (103%) S= 39	(0.95) 5/1/24/51/20 (91%) S=37	(4.63) 10/0/79/7/5 (81%) S=34
7	(0.85) 15/27/17/16/26 (135%) S= 39	(0.85) 3/0/26/49/23 (95%) S=37	(0.44) 12/0/62/25/1 (90%) S=30
8	(1.23) 41/17/15/20/7 (109%) S= 34	(1.31) 32/1/22/25/20 (115%) S=35	(0.72) 11/0/87/2/0 (78%) S= 30
9	(3.49) 13/6/64/4/13 (104%) S= 40	(5.53) 2/1/83/6/9 (96%) S=37	(3.94) 4/6/76/6/8 (116%) S=35
10	(1.20) 14/14/29/11/32 (133%) S= 39.5	(6.39) 1/5/82/5/7 (96%) S=37	(2.54) 3/9/69/6/13 (113%) S=35
11	(4.52) 3/9/66/4/17 (103%) S= 37	(6.78) 2/4/79/6/10 (115%) S=37	(3.54) 3/9/69/3/15 (138%) S=35
12	XXX	(2.41) 8/10/70/4/7 (114%) S=38	(1.52) 8/10/52/10/19 (121%) S=34
12-mid	XXX	(1.85) 6/6/75/6/6 (118%) S=37	XXX
12-bot	XXX	(>6) 4/0/83/5/9 (151%) S=38	XXX
OS-5	(4.71) 8/17/44/5/26 (106%) S= 37	(9.94) 1/4/82/3/10 (101%) S=36	(3.81) 3/7/70/7/14 (119%) S=34
OS-6	XXX	(5.00) 8/11/62/4/16 (113%) S=34	(2.53) 12/11/61/0/16 (106%) S=30
OS-7	XXX	(4.90) 7/11/66/3/12 (114%) S=35	(3.38) 3/10/76/2/9 (103%) S=32
Sant-A	<u>25°09.002'N x 80°45.292'W</u>	(0.33) 9/0/40/51/0 (93%) S=31	(0.80) 53/0/21/0/26 (86%) S=25
Sant-B	<u>25°08.533'N x 80°44.599'W</u>	(3.65) 71/0/9/0/11 (98%) S=29	(1.31) 45/0/30/13/12 (86%) S=26

S = Salinity (ppt or psu or ?)

Sandy Key Basin (Site #10) was found to have the lowest CHLa (1.2 µg/L) value since February 1 (1.3µg/L). Taxonomically, the strong diatom dominance for the majority of the year except February, within Sandy Key Basin was not observed on August 23, 2001.

September 20-21, 2001: Salinities were very high (40-51 psu) in the June-July period and with rains began to drop through August (~37 psu), where the bay and the Gulf were equal, and the north-central bay began trending towards more estuarine-like salinities (29-31 psu). Fresher waters (29 psu) were found entering the extreme north of Whipray Basin (Site #5) and the newly added site (Sant-B) in eastern Santini Bight near Crocodile Dragover (Table 4). The pattern found on September 20-21, 2001 was that open Gulf salinity (37psu) was present in the open Gulf (sites 12 & 11), basins attached in an open (site 10) manner and those connected *via* Conchie Channel and the basins to the north and east of Dildo Key Bank (*viz.* Sites 4, 6 & 7). These latter sites are southern Rankin Lake and central through southern Whipray Basin. Sites to the north and east of this pattern exhibited various degrees of lowered salinity. Snake Bight was at 34 psu and influenced sites #2 and #1 (35 and 36 psu, respectively) on the outgoing tide. Two locations of lower salinity included north-central Santini Bight (site "Sant-A) at

31 psu and extreme easterly Santini Bight / northeastern Whipray Basin (site "Sant-B) at 29 psu. This 29 psu water extended out into Whipray basin and could be found at site #5.

The present study is not about salinity *per se*. However, the inflow of at least some fresh(er) waters from the Seven Palm, Middle and Monroe Lakes, through Terrapin Bay is bringing with it increased cyanobacteria and potentially higher supplies of organic nitrogen. The water at Santini A appears diluted by rain fall and water from the mangrove fringe / mainland which is "filtered" along its path rather than allowed to flow directly, as the "Santini-B" and #5 sites have received. Santini Basin proper was at 31 psu and had only 0.33 $\mu\text{g/L}$ CHLa and co-dominated by diatoms and dinoflagellates ("D&D"). The more easterly Santini site (Sant-B) and Whipray Site #5 had elevated CHLa values (3.65 and 3.13 $\mu\text{g/L}$, respectively) and were greatly dominated by cyanobacteria (71 and 66 %, respectively). The influence of this fresh-cyanobacterial water was seen throughout Whipray Basin as CHLa values declined, in conjunction with increasing salinity, from site #8 (eastern Dump Key Pass: 1.31 $\mu\text{g/L}$, 32% cyano) to sites 6 and 7 in central (0.95 $\mu\text{g/L}$; 5% cyano) and southern (0.85 $\mu\text{g/L}$: 3 % cyano) Whipray Basin. The dominance of a diatom / dinoflagellate / cryptophyte ("DDC") community increased in accord with decreasing cyanobacterial influence.

Samples from fresher waters behind the coast line, such as from Paurotis Pond, routinely exhibit high CHLa values and yielded aphanizopyll (=4-hydroxymyxoxanthophyll), typical of certain nitrogen-fixing filamentous cyanobacteria (*cf.* Swain, 1985), such as *Aphanizomenon*, from which it is named. It is suggested here that these fresh(er) bays and lakes, often quite heavily utilized as rookeries, may have higher nutrients, notably P from ichthyotrophic bird guano. Thus, *in situ* generation of organic nitrogen by diazotrophic cyanobacteria could then add a fresh supply of N_{org} quite close to the bay. Evaluation of this suggestion is beyond the scope of the present study but does intrigue enough for future investigation. Snake Bight, having a salinity of 31 psu indicating 'dilution' as well, had very low CHLa (0.47 $\mu\text{g/L}$) and was dominated by the "DDC" type community.

The eastern "Flamingo Trough" nearer shore sites (#2->#1->OS5) had quite high CHLa (5.3->9.1->9.9 $\mu\text{g/L}$, respectively) and were diatom dominated (77-93%). Other sites in the western Bay and into the Gulf also were diatom dominated with only slightly lower (5.5-6.8 $\mu\text{g/L}$) total CHLa values. Lake Ingram drainage to the south (Sites OS6 and OS7) had moderately high amounts (5.0 $\mu\text{g/L}$) of CHLa dominated by diatoms (62-82%) plus about 11% 'chlorophytes'. Regarding Lake Ingram, the northern outflow, between Middle and North Cape Sable, was sampled in September. The tide was strongly receding. Here, total CHLa was a bit higher (6.2 $\mu\text{g/L}$) than the lake's southern drainage and taxonomically (3 / 3/ 83 / 0/ 11) was more diatom dominated.

Site #12, in the Gulf of Mexico at the park boundary off middle Cape Sable had 2.4 $\mu\text{g/L}$ total CHLa and, while still dominated (70%) by diatoms, had a significant amount (10%) of 'chlorophytes'.

Overall, CHLa values, except for Snake Bight, appear to be on the rise from a relative low in August, throughout the sampling area. As noted many times before, this is due mainly to diatoms in the western bay and Gulf and to cyanobacteria in the north-central bay. In the north-central bay when CHLa values are at 1.0 µg/L and below, a diatom / dinoflagellate / cryptophyte mixed community dominates. In the Gulf and western Bay, diatoms are usually greatly dominant, though 'chlorophytes', dinoflagellates and cryptophytes all contribute in more or less significant to co-dominant patterns.

October 18-19, 2001: Sites in what I term the “Flamingo Trough” (#1,2,9, & 11) and site #10 (Sandy Key Basin), all exhibited an approximate halving of their total chlorophyll-a values, relative to September values. That is a drop from around 5-9 µg/L in September to about 2-4 µg/L in October. At these sites diatoms remained dominant and noticeable increases in the percentage of cryptophyte contributed pigments were found.

Sites in Snake Bight (#3: 6.47µg/L), Rankin Lake (#4: 4.17µg/L) and mid-Whipray Basin (WRB #6: 4.63µg/L) all revealed moderate bloom conditions. However, only the Snake Bight site was cyanobacterial dominated (~80%). Rankin Lake and central Whipray Basin had only moderate amounts of cyanobacterial chlorophyll-a (10-18%) and were dominated by diatoms (64-79%). As sites to the immediate north (#8: Dump Keys east) and south (#7: south WRB) had much lower total CHLa values (0.4 – 0.7µg/L), it appears that the central bay diatom bloom might be contiguous across the bank between the Dump and Roscoe Keys.

The extreme northern reaches of Whipray Basin, communicating with Santini Bight over to Crocodile point (sites ESA and ESB: Table 3), had about 1µg/L total CHLa split amongst cyanobacteria, diatoms and cryptophytes.

Table 3 cont. [CHLa] (µg/L) Cyano/Chloro/Diats/Dinos/Cryptos (%Est/HPLC-CHLa)

<u>SITE</u>	<u>NOV. 15-16, 2001</u>	<u>DEC. 17-18, 2001</u>	<u>JAN. 10-11, 2002</u>
1	(2.25) 9/20/44/2/26 (115%) S=27	(5.48) 3/3/88/2/5 (108%) S=29	(0.71) 1/55/15/8/21 (104%) S=32
2	(2.18) 8/17/57/0/18 (132%) S=27	(6.44) 50/0/48/2/0 (105%) S=28	(0.63) 0/34/27/0/39 (126%) S=32
3	(0.95) 14/30/32/0/24 (112%) S=28	(13.29) 95/0/5/0/0 (126%) S=24	(0.52) 2/29/30/0/38 (89%) S=32
4	(0.98) 11/9/45/11/25 (104%) S=28	(1.67) 93/0/7/0/0 (96%) S=22	(2.88) 89/0/11/0/0 (112%) S=30
5	(0.64) 0/0/42/33/25 (106%) S=20	(0.50) 57/0/21/22/0 (93%) S=22	XXX
6	(0.38) 4/0/49/21/26 (85%) S=22	(0.51) 29/0/32/23/16 (77%) S=25	(6.88) 95/0/5/0/0 (121%) S=26
7	(0.32) 0/0/69/0/31 (120%) S=24	(0.35) 25/0/58/17/0 (66%) S=26	(5.49) 93/0/7/0/0 (113%) S=28
8	(0.67) 13/0/42/15/29 (114%) S=22	(0.58) 9/0/66/22/3 (85%) S=24	(1.59) 77/0/13/10/0 (116%) S=25
9	(2.23) 8/10/63/3/17 (139%) S=28	(3.52) 5/9/81/0/5 (100%) S=30	(2.52) 0/15/59/0/26 (92%) S=32
10	(2.27) 4/14/72/0/9 (118%) S=30	(2.54) 1/4/86/0/8 (114%) S=30	(2.14) 2/19/44/3/33 (96%) S=32
11	(5.19) 6/7/69/5/13 (103%) S=27	(5.03) 4/7/84/0/5 (106%) S=29	(3.70) 1/15/58/4/21 (115%) S=30
12	XXX	(1.47) 21/20/44/0/15 (101%) S=32	(3.97) 2/11/62/5/20 (112%) S=31

12-mid	XXX	(1.52) 19/13/53/2/14 (117%) S=32	XXX
12-bot	XXX	(>3) 12/6/71/0/12 (184%) S=32	XXX
OS-5	(4.16) 4/12/70/0/14 (104%) S=25	(4.47) 2/6/82/1/9 (119%) S=29	(1.38) 2/22/44/0/32 (108%) S=30
OS-6	(1.99) 14/20/47/0/19 (110%) S=26	(8.41) 5/4/83/0/8 (119%) S=27	XXX
OS-7	(1.03) 18/24/32/0/26 (111%) S=25	XXX	(4.77) 54/10/16/0/20 (93%) S=27
Sant-A	(0.31) 0/0/71/26/3 (129%) S=21	(1.06) 52/0/17/11/20 (123%) S=20	(0.26) 27/0/73/0/0 (51%) S=25
Sant-B	(0.85) 4/0/43/38/15 (105%) S=21	(0.41) 58/0/29/13/0 (65%) S=20	(2.48) 92/0/8/0/0 (114%) S=25
TERRAPIN BAY (12/01) →		(21.2) 100/0/0/0/0 (91%) S = 9	XXX

November 15-16, 2001: Again, discussion is relative to the previous month.

Within the E-W transect of the “Flamingo Trough” (E→W: Sites #2, 1, 9, 11), total CHLa decreased moderately (3.5-4.6 → 2.2µg/L) except in the western Florida Bay / Gulf of Mexico transition site (#11) where values were about double the previous month. Weather precluding getting to the offshore mid-Cape Sable site so correlation between sites #11 and #12 are not possible. However, elevated CHLa at site #5, a nearshore “mini-basin” (possibly the “sawfish hole” of early Flamingo settlers lore), suggests a zone of higher productivity wrapping around East Cape Sable and eastward a bit. sandy Key Basin (#10) fit well with the CHLa values and Chemotaxonomic spread of the “Flamingo Trough” sites.

The Snake Bight (#3) cyanobacterial bloom was found to have decreased from 6.5 → 1 µg/L and shifted to a diatom plus cryptophyte dominance. Likewise, the Rankin Lake (#4) site revealed decreased CHLa (4.2 →1µg/L) but with little change in (chemo)taxonomic makeup.

The Whipray Basin transect sites (N→S: #5, 8, 6, 7) were found with about 0.6µg/L CHLa to the north (#5,8) with a near co-dominance of diatoms, dinoflagellates and cryptophytes. Mid-Whipray Basin had noticeably lower total CHLa (~0.38) but with a similar phytoplankton distribution. The most southern WRB site (# 7) had a bit lower CHLa (0.32µg/L) but totally lacked a detectable dinoflagellates (=peridinin) signal.

The nearshore sites at the northern reaches of WRB, namely the eastern Santini Bight (ESA: 0.8→0.3µg/L) and crocodile point (ESB: 1.3 →0.8µg/L) sites exhibited lower CHLa, as indicated, a loss of cyanobacterial signal and an increase in the importance of dinoflagellates. Salinities at these sites have been decreasing from September (29-31 →25-26 → 21 psu).

December 17-18, 2001: Relative to November, sites in the “Flamingo Trough” closer to Snake Bight (#1,2) exhibited a nearly threefold increase in CHLa. Site #2, closest to Snake Bight was co-dominated by cyanobacteria and diatoms while the site farther west (#1), having a bit lower (5.5 µg/L) CHLa than site #2 (6.4 µg/L), reflected the diatom dominance pattern of the western bay. Snake Bight proper (#3) was found to have a strong cyanobacterial (95%cyano/5%diat) bloom with total chlorophyll-a levels at 13.3

µg/L. This cyanobacterial influence could be found in Rankin Lake and Whipray Basin but total chlorophyll-a levels were an order of magnitude lower. Samples from eastern Santini Bight to Crocodile Point had about 1 µg CHLa/L and were about 50% cyanobacteria with more minor contributions of diatoms, dinoflagellates and cryptophytes.

A sample taken from Terrapin Bay, not a routine site, revealed a tremendous quantity of cyanobacteria (21.2 µg CHLa/L and 100% cyano).

The productivity of cyanobacteria in nearshore to behind shore waters (S = 20 -> 9 psu) hints at an influence of these populations on the upper bay. That is, as many cyanobacteria can fix N₂ into organic matter, the potential for the influx of so-called “new nitrogen” is real. P may be supplied to behind shore waters *via* ichthyotrophic avian populations. That is, rookeries may spur productivity and direct N-fixation by affecting the Redfield ratio in those waters. These are speculations but appear worthy of investigation.

The western bay had total CHLa values from 2.5 – 8.4 µg/L and consistent diatom dominance. Greens (prochlorophytes?) plus cyanobacteria were found to be co-dominant with the diatoms in the waters offshore of middle Cape Sable (#12).

Samples of HPLC chromatogram and UV/Vis spectra generated by the PDA are given below.

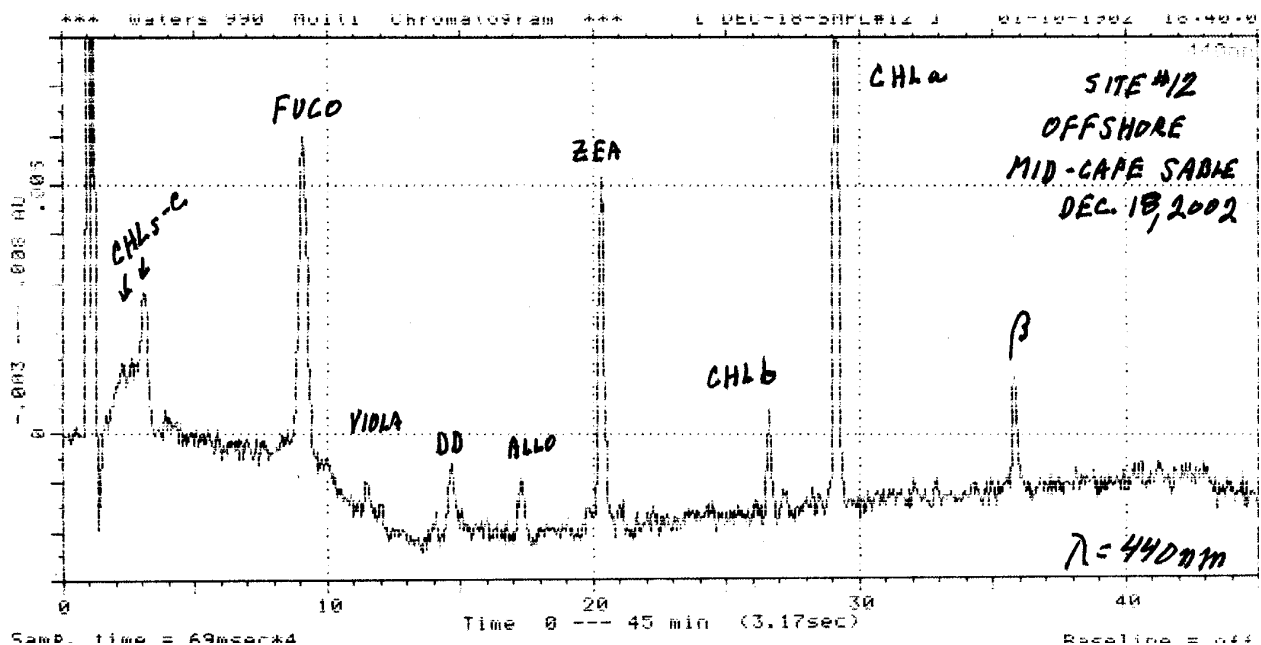


Figure 1: HPLC Chromatogram of the extract of phytoplankton recovered at site #12, offshore mid-Cape Sable, on December 18, 2001. Total CHLa = 1.47 µg /L.

Chemotaxonomic estimate (cyano/green/diat/dino/crypto) = 21 / 20 / 44 / 0 / 15.

Figure 1 is the chromatogram of a mixed phytoplankton community recovered from about 2 miles offshore from mid-Cape Sable. The resolution of the chlorophylls-c (CHLsc) is not as good as usual on this system. That is, some unknown highly polar materials tend to “smear” this grouping. Runs with knowns and samples from upland fresh waters (e.g. Lake Okeechobee) yield excellent resolution of CHLidea, and CHLs-c1/-c2. Manual integration with PDA spectral analysis is routinely used to adjust for this problem. Baseline drift, such as the drop at about 10 minutes, is due to viscosity changes during the gradient run and is left uncorrected on purpose. Several very small peaks are also visible here. The little peak just before diadinoxanthin (DD) is cis-fucoxanthin ($\lambda = 330, 444$). There is a small peak on either side of zeaxanthin. The preceding peak is lutein and the following peak is (7-) cis zeaxanthin, likely formed by the reduction of the acetylenic (=triple) bond in diatoxanthin. These peaks appear extremely small yet interpretable spectra are obtainable. Important to the phytoplankton community in the western bay is chlorophyll-b, the PDA generated UV/Vis spectrum of which is given below.

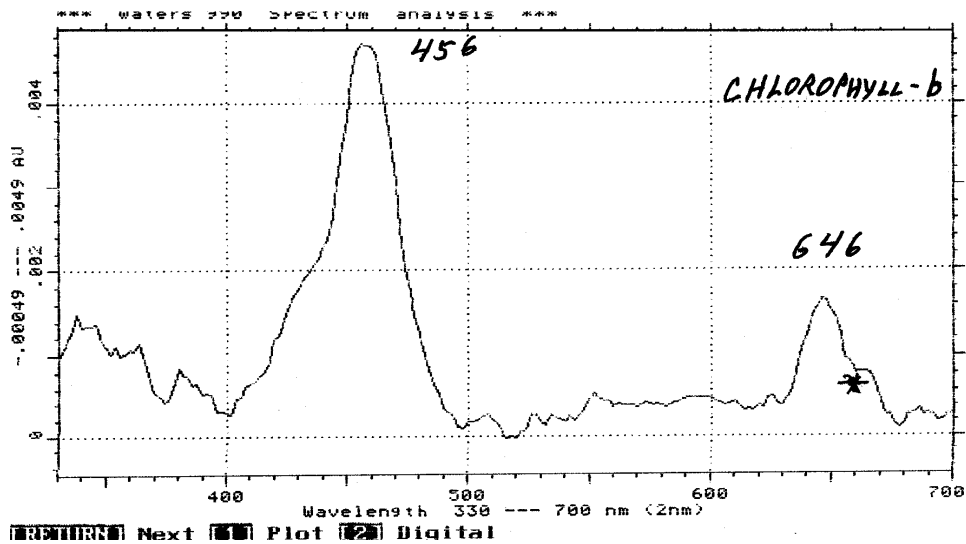


Figure 2: UV/Vis spectrum (PDA) of chlorophyll-b. *Asterisk indicates a ‘glitch’ in background correction due to the intense energy at 656.1 nm in the emission spectrum of the deuterium lamp.

In the north-central bay, cyanobacterial blooms are often prevalent. In these cases, a mixed phytoplankton community is lacking. An example of such a population is given in Figure 3, an HPLC chromatogram for the waters of Snake Bight.

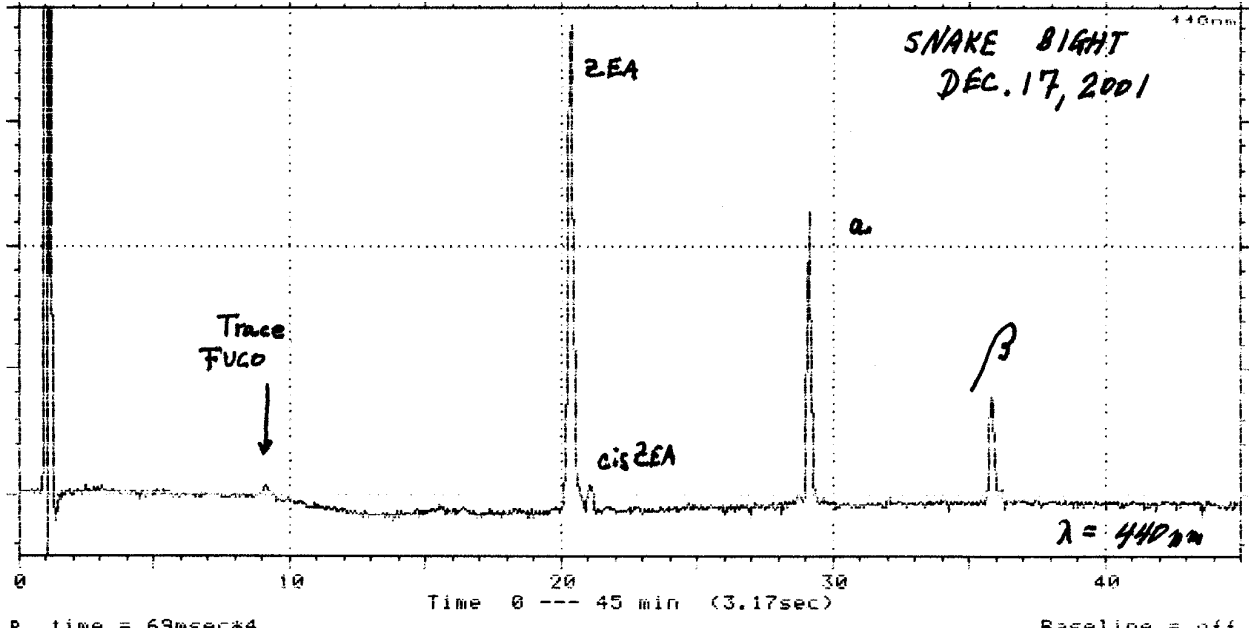


Figure 3: HPLC Chromatogram of the phytoplankton recovered from site #3, Snake Bight, on December 18, 2001. Total CHLa = 13.3 $\mu\text{g/L}$. Chemotaxonomic estimate (cyano/green/diat/dino/crypto)= 95 / 0 / 5 / 0 / 0.

The tremendous dominance of cyanobacterial pigments is easily discerned from this chromatogram. Allowing for a minor amount of the total CHLa deriving from diatoms, the CHLa / ZEA ratio comes in at about 0.87. That means that for every mole of zeaxanthin, we “should” have 0.87 moles of cyanobacterial CHLa. This ratio (CHLa/ZEA) is found to vary around unity (0.85 – 1.2) in north-central Florida Bay cyanobacterial blooms and matches exactly that found in the fresher (S = 9 – 20psu) waters of the mangrove fringe and behind shore lakes (aka the Transition Zone).

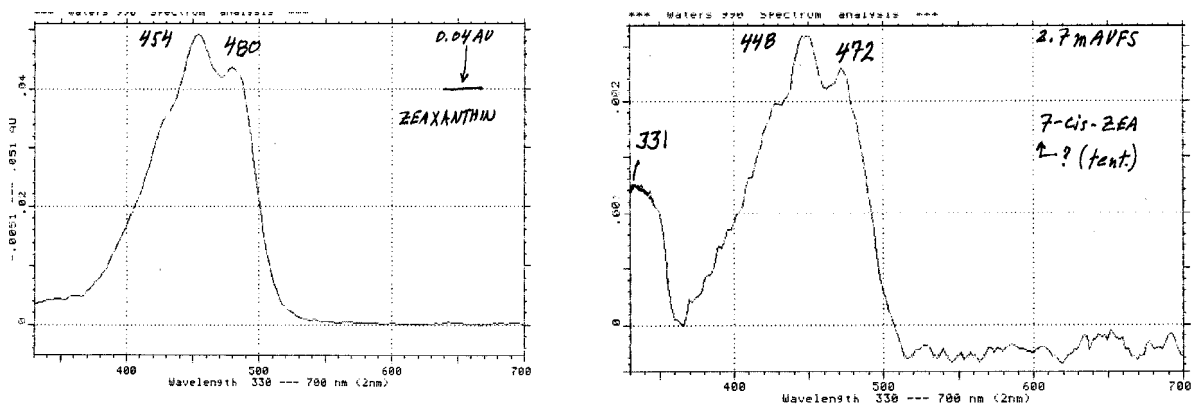


Figure 4: UV/vis (PDA) spectra of zeaxanthin (ZEA; left) and (-) cis-zeaxanthin (cisZEA: right). Scales are 0.045 and 0.0027 AUFS.

The potential influx of cyanobacteria and so-called 'new nitrogen' from the mangrove fringe and the waters behind shore (Transition Zone) and the spread of these blooms out into the bay proper are being actively pursued.

January 10-11, 2002: During the period from mid-December 2001 to mid-January 2002, the standing crops, as reflected by total CHLa values, and community structures changed significantly in most areas.

In the eastern "Flamingo Trough" (sites #1-2), total CHLa dropped precipitously from 5.5-6.5 to 0.6-0.7 $\mu\text{g/L}$. December diatom dominance was replaced by co-dominance of greens and cryptophytes. Site 2, closer to Snake Bight, also lost all of its previous cyanobacterial signal.

Further west in the middle grounds (#9), Sandy Key Basin (#10) and east Cape Sable (#11) areas, total CHLa changed a bit from December values. However, in December, diatoms were dominant (81-84%) whereas, in January, diatoms were diminished somewhat (44-59%) as cryptophytes (21-33%) increased from their December lows (5-6%).

The north-central and central bay was found to be affected by cyanobacterial population changes. Recall the strong cyanobacterial bloom within Snake Bight in December. In January, total CHLa values collapsed from the December high of 13.3 $\mu\text{g/L}$ to only 0.52 $\mu\text{g/L}$. Additionally the 95/5 split of CYANO/DIATS in December was replaced by a community of greens/diat/cryptos (29/30/38%). A moderate cyanobacterial (93-95%) bloom (CHLa 5.5-6.9 $\mu\text{g/L}$) was recorded in Whipray Basin (Sites 6-7). Interestingly, the 5-7% diatom contribution still gives about 0.34-0.38 $\mu\text{g/L}$ as diatom CHLa. This is 'interesting' as that is what I have found as a background level for diatoms in Whipray on a year-round basis. Thus, the cyanobacteria appear to supplement rather than supplant the diatom population. Strong diatom dominance was also found in Rankin Lake (89%, 2.9 $\mu\text{g CHLa/L}$) and at Crocodile Point (92%, 2.5 $\mu\text{g CHLa/L}$), though not in the 'bloom' abundance found for Snake Bight in December nor Whipray Basin in January.

Without closer temporal spacing it is impossible to adequately describe the December-January cyanobacterial bloom dynamics. However, it appears that the Snake Bight bloom of December 'may' have moved through southern Rankin Lake and into Whipray Basin. At the same time, cyanobacterial populations were increasing in the extreme NE section of Whipray Basin (*viz.* Crocodile Point).

During January, samples were collected from West Lake, a fresher (S= 13 psu) area well back from the coast but communicating with Garfield Bight (NE Snake Bight) via a series of creeks through smaller lakes called Long Lake and "The Lungs". The HPLC chromatogram of the phytoplankton from West Lake (Figure 5) reveals the total dominance of cyanobacteria. The CHLa to ZEA ratio was found to be unity (1.0:1) in this case.

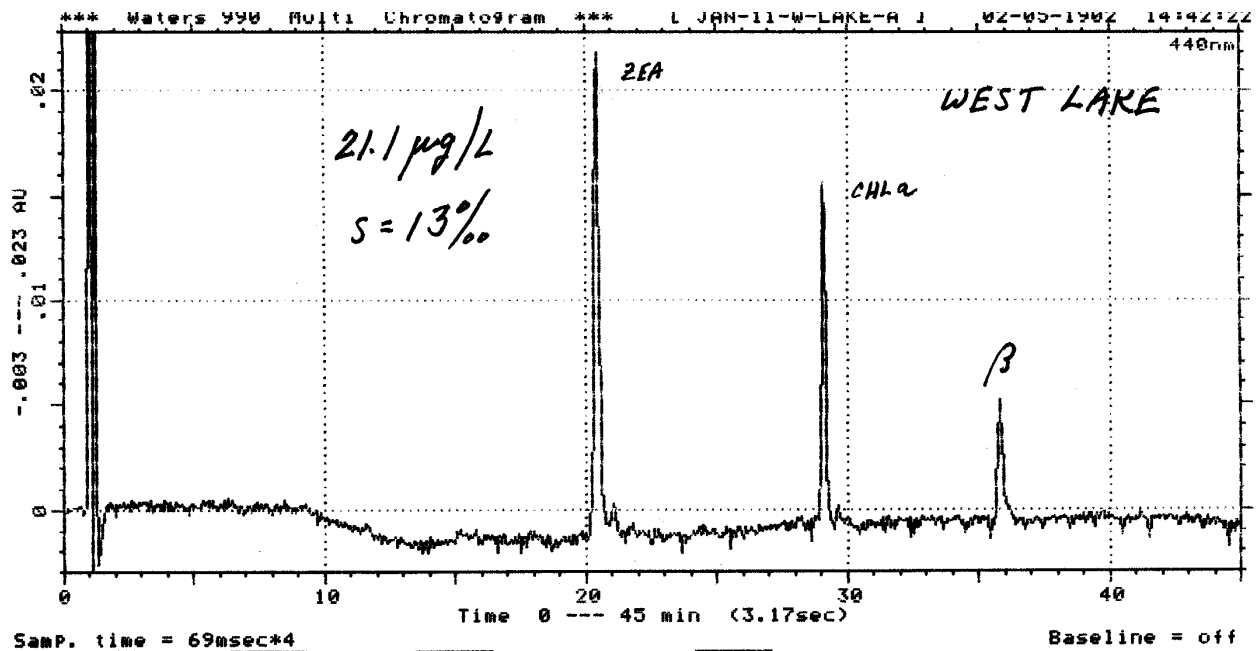


Figure 5: HPLC chromatogram ($\lambda = 440 \text{ nm}$) of the phytoplankton recovered from West Lake on January 11, 2002. Total CHLa = 21.06 $\mu\text{g/L}$.

Table 3 cont. [CHLa] ($\mu\text{g/L}$) Cyano/Chloro/Diats/Dinos/Cryptos (%Est/HPLC-CHLa)

<u>SITE</u>	<u>FEB. 12-13, 2002</u>	<u>MAR. 14-15, 2002</u>	<u>APR. 10, 2002</u>
1	(5.12) 4/22/48/4/23 (111%) S=31.5	(2.86) 3/16/56/0/25 (110%) S=33	(15.78) 1/0/88/0/12 (100%) S=37
2	(1.01) 0/31/41/0/28 (94%) S=32	(1.13) 1/21/49/0/28 (122%) S=33	(1.80) 66/0/18/9/7 (125%) S=40
3	(0.41) 4/27/30/9/30 (132%) S=31	(0.53) 0/18/18/13/51 (172%) S=33	(0.53) 11/0/66/23/0 (105%) S=37
4	(4.36) 94/0/6/0/0 (110%) S=32	(11.90) 97/0/3/0/0 (116%) S=35	(1.04) 26/0/60/0/14 (98%) S=35
5	(0.38) 9/0/62/0/29 (62%) S=26	XXX	XXX
6	(1.09) 40/0/30/7/23 (99%) S=28	(0.51) 13/0/22/33/32 (106%) S=32	(1.61) 22/0/78/0/0 (83%) S=37
7	(0.27) 10/0/63/27/0 (47%) S=30	(0.55) 6/0/40/30/24(90%) S=32	XXX
8	(0.66) 22/0/45/12/22 (119%) S=29	(1.40) 68/0/16/17/0 (103%) S=30	(2.35) 4/0/96/0/0 (83%) S=35
9	(2.42) 6/8/66/5/15 (121%) S=33	(4.20) 2/7/78/0/13 (130%) S=34	XXX
10	(1.61) 7/13/53/7/20 (113%) S=33	(2.22) 3/11/69/0/17 (133%) S=34	XXX
11	(2.97) 6/16/54/3/21 (123%) S=34	(4.46) 1/9/79/0/11 (138%) S=34	XXX
12	(1.53) 18/16/34/5/26 (106%) S=34	(1.47) 21/20/44/0/15 (101%) S=33	XXX
OS-5	(3.17) 2/18/56/2/23 (112%) S=32	(4.28) 0/6/79/0/14 (118%) S=33	XXX
OS-7	(1.70) 5/22/42/0/31 (136%) S=32	(2.46) 8/16/33/6/33 (140%) S=33	XXX
Sant-A	XXX	XXX	XXX
Sant-B	(0.31) 51/0/49/0/0 (42%) S=26	(0.47) 26/0/55/0/19 (80%) S=29	XXX

February 12-13, 2002: Comparisons are made to the previous month and overall trends, as usual. Site #1, at the end of the Flamingo channel (Mkrs. 9/10) had an elevated CHLa value ($>5 \mu\text{g/L}$) and was not immediately related to sites to the east (#2) nor west (#9-11), by neither total CHLa nor taxonomic distribution. A zone of higher productivity *may* have occurred along the southern side of the Flamingo to East Cape Sable land mass as Site # OS5 exhibited a similar phytoplankton community (diat>crypto \geq chloro) and also had an elevated ($>3 \mu\text{g/L}$) CHLa, relative to surrounding yet more offshore sites (#9, 11). All of the western Bay (Sites 1, 9, 10, 11, and OS5) had the diat>crypto \geq green community. Site # 12, offshore mid Cape Sable, exhibited a bit high (18%) cyanobacteria than the remaining west bay sites. CHLa values, except for sites #1 and OS5 as mentioned, ran between 1.5 – 3.0 $\mu\text{g/L}$.

The central Bay (sites 5, 6, 7, 8, and ESB) were of low CHLa ($\leq 1 \mu\text{g/L}$) and were somewhat variable in taxonomic makeup with diatoms being most prevalent or at least co-dominant. Site # 7, in southern Whipray Basin, exhibited elevated (~27%) an elevated dinoflagellate signal. Southern Whipray Basin (#7) has often been found to exhibit higher dinoflagellate signal, relative to the more northern reaches of the central bay (sites # 6,8, 5, 4, 3, ESA, ESB).

An extra sample (“N.Rankin/Garfield”), which is now added rather routinely, was collected at the junction between northern Rankin Lake and Garfield Bight (ca. 25°08.800’N x 80°48.509’W; Z ~ 0.6 m.: farther north when more water is present). This sample had elevated CHLa (5.75 $\mu\text{g/L}$) and a (chemo-)taxonomic makeup of 90/2/2/0/6. Linkage of this site, and its proposed influence from Alligator Creek (The Lungs thru West Lake drainage), to site #4 in southern Rankin Lake is easy made. This further supports the author’s proposal that the cyanobacterial ‘blooms’ emanate from the fresher waters of the northern (mangrove) transition zone.

March 14-15, 2002: Sites through the “Flamingo Trough” (# 11,9, OS5) were quite uniform in both CHLa yield (4.2-4.4 $\mu\text{g/L}$) and (chemo-) taxonomic makeup (78-79%diat, 11-14% crypto, 6-9% greens, 0-2% cyano and no detectable dinoflagellate signal). Proceeding east to sites #1 and #2, this pattern exhibits straightforward dilution with the lower productive waters of Snake Bight (#3: 0.5 $\mu\text{g/L}$, crypto dominated and even spread of other divisions except no cyanos). Site #10, Sandy Key Basin, was similar to the “Flamingo Trough” communities but at about half their standing crop (~ 2.2 $\mu\text{g/L}$). The westernmost site, offshore mid-Cape Sable (#12) had still lower standing crop (~1.5 $\mu\text{g/L}$) and a high cyano plus green signal.

Rankin Lake (site #4) once again was characterized by a large standing crop (CHLa~12 $\mu\text{g/L}$) of phytoplankton which was overwhelmingly dominated by cyanobacteria (97%) plus a minor amount (3%) of diatoms. Samples taken from West Lake; at the ‘headwaters of the “West, Long, Cuthbert Lakes, Lungs and Alligator Creek system; were found to contain 97-100% cyanobacteria with minor amounts (0-3%) of diatoms and to have total CHLa values of 27-28 $\mu\text{g/L}$. Again, input of these waters through Alligator Creek into Garfield Bight and either to Snake Bight or, in this case, to Rankin Lake, depending upon wind and tidal forcing, is suggested.

The Whipray Basin sites (#6-7) revealed but 0.5 μg CHLa/L and diat/dino/crypto>cyano communities. Site #8, just to the east of the Dump Keys revealed the input and mixing of the Rankin Lake cyanobacterial charged waters. That is, CHLa was 1.4 μg /L and the community had a 63% cyanobacterial pigment signal. The extreme northeast portion of the Whipray Basin environs (site #ESB), just off Crocodile Point, had low CHLa (0.5 μg /L) but still revealed 25% cyanobacterial signal, with diatoms (55%) and cryptophytes (19%) accounting for the remained of phytoplankton pigments.

April 10, 2002: Sampling during April 2002 was severely hampered by windy conditions. The dates chosen, ostensibly between fronts, were still windy (>20-25knts) and many sampling sites could not be reached in the author's skiff. Extra samplings were performed behind and within the fresher waters behind the mangrove transition zone. Salinities in April began to reflect the summer trend towards hypersalinity.

The only site actually in or connected directly to the western bay sampled was site #1, markers 9/10 out of Flamingo. The highest CHLa (15.8 μg /L) recorded by the author in the western bay was this sample. Diatoms accounted for 87% of this signal, with cryptophytes (12%) and cyanobacteria (~1%) contributing the remainder. It is tempting to and actually is suggested that this highly elevated diatom signal; about 1.5- 2.0 times the maxima recorded for diatom bloom events in June, September and December of 2001; is related to the "blackwater event" recently reported for the Gulf (see <http://www.floridamarine.org> and http://www.mote.org/~emuleller/MEERA/monthly_reports.html). According to Dr. Peter Ortner (April 11, 2002:pers. Commun.), this event was likely a *Rhizosolenia* bloom. Thus, remnants of that bloom, as core eddies, might well be expected to be scattered throughout the western sections of Florida Bay and passes through the Keys. Examination of the HPLC/PDA data for this sample revealed no indication of peridinin nor gyroxanthin, as would have been the case were *Karenia brevis*, or other dinoflagellates, present in significant biomass.

The Garfield Bight / N. Rankin Lake sample again had a cyanobacterial (100%) bloom (16.4 μg /L) which had apparently spread, with obvious dilution, to both Snake Bight (site 3, CHLa= 1.8 μg /L but with 66% cyano) and south Rankin Lake (site #4: CHLa = 1.0 μg /L with 26% cyano). Site #3, in the lower middle of Snake Bight, had only 11% cyanobacterial signal and very low (0.5 μg /L) CHLa. Strong winds out of the east during this period could easily be the explanation for waters from Garfield Bight being 'blown' across the top of Snake Bight. The spread of cyanobacteria from the outlets of the northern mangrove transition zone is suggested as a topic for further research. This could involve physical oceanographers and stable isotope geochemists as well as investigations such as being reported herein.

Whipray Basin (sites 6,8: too rough to go to #7) had moderate CHLa levels (1.6-2.4 μg /L) and were dominated by diatoms. Site #8 had lower CHLa and higher cyanobacterial signal (22%) possibly indicating the mixing of Whipray Basin diatom communities with the southern Rankin Lake waters.

Samples from West Lake again revealed strong cyanobacterial bloom conditions (CHLa = 20µg/L, 100% cyano). Again, the cyanobacterial source is continual.

In April, samples of ponds isolated from Florida Bay were also collected. A sample from Mrazek Pond, an isolated pond just east of Coot Bay Pond and having 9 psu salinity, was found to have the enormous CHLa load of 441 µg/L. Chemotaxonomically this was contributed 85% by diatoms, 12 % by cryptophytes and only 3% by cyanobacteria. However, these values should be taken cautiously as 3 unknown chlorophylls-a were present. Based on their polarity, eluting successively about 1.5 minutes prior to CHLa (UNKN#1), and then 1.5 minutes from each other (UNKNS#2 and #3), these *may* be CHLa with incomplete phytol chains. That is, if these 3 were esterified with C15, C10 and C5 precursors to phytol (“geranylgeranyl, farnesyl, isopentenyl”), then an elution pattern as found might be expected. The UV/Vis of these 3 unknowns exactly matches CHLa *per se* and does not match any known bacterial chlorophyll, either via spectrum or HPLC behavior. These pigments may be novel and are worthy of future (re-) investigation with HPLC-MS and other organic spectrometric methods. A sample from Paurotis Pond, with a salinity of 5 psu and communicating somewhat with the extreme eastern portion (Hell’s Bay etc.) of the Shark River system, was found to have moderate (8 µg/L) CHLa being contributed by chlorophytes (55%), cyanobacteria (38%) and some (7%) diatoms.

Table 3 cont. [CHLa] (µg/L) Cyano/Chloro/Diats/Dinos/Cryptos (%Est/HPLC-CHLa)

<u>SITE</u>	<u>MAY 13-14, 2002</u>	<u>JUN. 25-26, 2002</u>	<u>JUL.16-17, 2002</u>
1	(2.28) 8/0/81/0/11 (115%) S=41.5	(4.05) 21/21/38/4/16 (102%) S=32	(1.18) 23/11/30/5/31 (129%) S=30
2	(1.18) 3/0/73/0/24 (109%) S=41.5	(2.73) 11/19/50/7/12 (119%) S=31	(1.17) 6/0/37/10/46 (125%) S=29
3	(0.75) 9/0/40/37/14 (114%) S=41	(1.71) 0/0/64/20/16 (89%) S=30	(0.84) 6/0/21/6/68/0 (152%) S=28
4	(0.45) 10/12/36/24/19 (110%) S=37.5	(0.98) 14/0/64/22/0 (88%) S=29	(14.7) 93/4/3/0/0 (97%) S=22
5	(0.50) 6/0/85/9/0 (93%) S=35	(5.70) 75/6/10/9/0 (91%) S=27	(7.32) 73/8/5/8/6 (115%) S=21
6	(0.54) 13/0/68/19/0 (99%) S=39	(2.30) 9/3/68/20/0 (120%) S=31	(7.10) 90/4/2/4/0 (96%) S= 25
7	(1.31) 11/0/74/10/5 (47%) S=39	(2.70) 13/4/68/15/0 (102%) S=32	(3.91) 80/0/5/15/0 (99%) S=27
8	(1.11) 3/0/85/13/0 (66%) S=35.5	(1.03) 19/5/48/29/0 (79%) S=30	(20.8) 92/3/5/0/0 (90%) S= 20
9	(0.87) 35/10/29/0/25 (140%) S=40	(4.18) 10/5/69/4/11 (101%) S=35	(1.88) 20/3/46/8/23 (109%) S=35
10	(1.49) 33/12/50/5/0 (106%) S=40.5	(2.71) 8/8/63/8/13 (124%) S=35	(5.50) 6/3/82/4/5 (89%) S=35
11	(4.57) 10/11/65/0/14 (100%) S=39	(2.70) 21/7/48/9/15 (105%) S=34	(4.34) 6/3/82/3/6 (87%) S=35
12	XXX	(2.43) 16/9/66/0/9 (94%) S=36	(1.06) 28/12/32/5/23 (118%) S=37
OS-5	(4.08) 15/11/60/0/14 (105%) S=40.5	(3.26) 19/14/53/3/11 (110%) S=32	(2.13) 31/8/54/0/7 (103%) S=29
OS-6	XXX	(12.62) 56/8/19/11/6 (85%) S=24	(3.47) 22/15/22/0/41 (111%) S=25
OS-7	XXX	XXX	XXX
Sant-A	XXX	XXX	XXX
Sant-B	(0.17) 0/0/100/0/0 (75%) S=26	XXX	XXX
N.Rank	XXX	(7.17) 63/10/18/9/0 (97%) S=25	XXX
W-Lk	XXX	XXX	(19.5) 79/17/4/0/0 (50%) S=11

MAY 13-14, 2002: Strong cyanobacterial signals have dissipated by May and salinities are still rising. Most of the bay is above 37 psu and salinities of 40 psu were noted for the western bay.

The north-central regions of Whipray Basin, Rankin Lake and Snake Bight had mixed phytoplankton communities (3-13/0-12/36-85/9-37/0-19), mainly dominated by diatoms.

Cryptophytes and dinoflagellates were co-dominant with diatoms in Rankin Lake and Snake Bight. Water sampled in Terrapin Bay at Crocodile Point (~ESB) had the lowest CHLa recorded (0.17 µg/L) and had only fucoxanthin (diatom) as a detectable biomarker pigment.

Samples in the western bay revealed a moderate amount of diatom dominated community which apparently became diluted (4.57→4.08→2.28→1.18µg/L) as one sampled eastward from East Cape Sable (#11→#OS5→#1→#2) along the northern part of the "Flamingo Trough". Cryptophytes and cyanobacteria were co-second level dominant taxa. Seas were too high to sample site #12 for the second month in a row.

JUNE 25-26, 2002: This was the last funded sampling on this project.

The western bay (sites 12,11,10) had 2.4 → 2.7 µg-CHLa/L and diat>crypto~cyano>green~dinos as a mixed community.

The "Flamingo Trough" (sites 9→ 1→ 2 → 3) had decreasing CHLa (4.2 → 1.7µg/L) with diat>cyno~crypto~green>dino from site 9 → 2 and then diat>dino>~crypto at site #3 in Snake Bight.

Southern Rankin Lake (site#4) had only about 1 µg-CHLa/L with diats>dinos>cyanos. Fresher waters (S = 25psu) entering northern Rankin Lake had elevated CHLa (~ 7.2µg/L) with a greatly increased presence (63%) of cyanobacteria.

The northern reaches of Whipray Basin (site #5, off Derelict Key) also reflected the input of fresh water (S =27 psu) from the mangrove transition zone, elevated CHLa (5.7 µg/L) and a high percentage (75%) of cyanobacteria. Going south in Whipray Basin (sites 5 → 8 → 6 → 7) one notices a lens of low productivity water at site 8, relative to site #5 to the north and sites 6 and 7 to the south. Ostensibly this reflects southern Rankin Lake water flowing through the Dump Keys. Sites 6 & 7 had about 2.3 → 2.7 µg-CHLa/L and a dominance of diatoms over dinoflagellates and cyanobacteria.

Thus, towards the end of July, fresher waters were just beginning to introduce cyanobacteria into the north central bay. Examination of the data for July and August reveal the spread of these bloom conditions.

JULY 16-17, 2002: This project officially terminated on 06/31/02. Sampling during July and August 2002 was at the author's expense and was performed in order to provide a full 2 year time line to the study.

In the 'Flamingo Trough', a cryptophyte 'event' (bloom-?) appears to have been present and was centered around the "Sawfish Hole" (site OS5). Here, CHLa values were about 3.5 µg/L and pigment (alloxanthin) data indicated a community of cryptophytes > diatoms > dinos +cyanos. Cryptophytes were calculated to be just under 79% of the total (CHLa) community. The dilution of this small cryptophyte 'bloom' can be followed eastward through the 'Flamingo Trough'. That is, from station OS-5 →#9 → #1 → #2 → #3, total CHLa decreases from ~ 3.5 → 1.9 → 1.7 → 1.8 → 0.8 µg/L along with a

consistent drop in the percentage of (alloxanthin) cryptophyte derived CHLa. At station #3, located in Snake Bight at the extreme eastern edge of the influence of the Flamingo Trough, cryptophytes were undetectable and dinoflagellates were greater than diatoms (chrysophytes). Cryptophytes laden were detected offshore of mid Cape Sable and made up about 1/3 of the community, though the yield of CHLa (~ 1µg/L) was but low.

To the south of Cape Sable, along the western edge of Florida Bay (sites # 10 + 11), a diatom dominated (82%) community with moderate levels (4.3 – 5.5 µg/L) of total CHLa were revealed.

The north-central bay, including Rankin Lake and Whipray Basin, was found to be experiencing a cyanobacterial bloom with CHLa levels of 7 –21 µg/L. The 'heart' of the bloom appears to have been located in southern Rankin Lake (site #4) through the Dump Keys and into the upper reaches of Whipray Basin (site#8). Sites to the north (site #5) and south (site #6) of the Dump Keys (site #8) had ~ 7µg/L total CHLa. All of these were greatly dominated by cyanobacteria, as evidenced by the large zeaxanthin signal. Southern Whipray Basin had lower (3.9 µg/L) total CHLa and an increased presence (~ 15%) of diatom signal. Whether the main influx of fresher waters was from the West Lake / Long Lake / Lungs complex through Alligator Creek into northern Rankin Lake or from Monroe lake / Terrapin Bay into the extreme northeast reaches of Whipray Basin, or both, is unable to be told from this sampling density. Unfortunately samples at my stations in northern Rankin Lake or eastern Santini Bight over to Crocodile Point were not collected. It is becoming increasingly clear however, that the north-central Florida Bay cyanobacterial blooms are directly linked to the input of fresher cyanobacterial laden waters from the mangrove transition zone and its hinterlands.

AUGUST 12, 2002: Sites #1 and 2, the eastern reaches of the "Flamingo Trough", had moderate CHLa (1.8 µg/L) and a dominance of diatoms over other taxa in a well mixed community.

Site #3, just off 'Tin Can Channel' in Snake Bight, had bloom conditions with CHLa ~ 14.7µg/L and an equal spread of cyanobacteria, diatoms and cryptophytes. This, together with salinity intermediate (32 psu) to the western bay (34-35 psu) and Rankin Lake (27 psu), may well represent the mixing of P-rich western Bay waters with N-rich ("new N_{org}") cyanobacterial laden waters from the Rankin Lake / mangrove transition zone. That is, diatoms and cryptophytes, given sufficient N sources, appear to have bloomed along with the cyanobacterial population.

The Whipray Basin transect (sites 5 → 8 → 6) also revealed the input of fresher waters with salinities of 23 → 26 →30 psu, respectively. Chlorophyll-a values, vastly dominated by cyanobacterial contributions, went from 12.7 → 8.8 µg/L along this transect.

Though not sampled, these cyanobacterial laden waters could be visually followed well below the Whipray Keys and only began to take on the olive color of diatom dominated clearer waters as the Pollack Keys were approached.

Table 3 cont. [CHLa] (µg/L) Cyano/Chloro/Diats/Dinos/Cryptos (%Est/HPLC-CHLa)

<u>SITE</u>	<u>AUG 12, 2002</u>
1	(1.84) 14/11/54/9/11 (127%) S=35
2	(1.80) 6/9/64/12/8 (148%) S=34
3	(14.74) 36/0/30/0/34 (104%) S=32
4	(9.86) 94/0/3/3/0 (101%) S=27
5	(12.70) 88/12/0/0/0 (95%) S=23
6	(8.83) 79/6/6/9/0 (98%) S=30
8	(12.99) 94/6/0/0/0 (87%) S=26

Cyanobacteria in North-Central Florida Bay: Figures 6 and 7 serve to compare total chlorophyll-a values and the percentage which cyanobacteria form as a portion of the total phytoplankton community at several north-central Florida Bay sites.

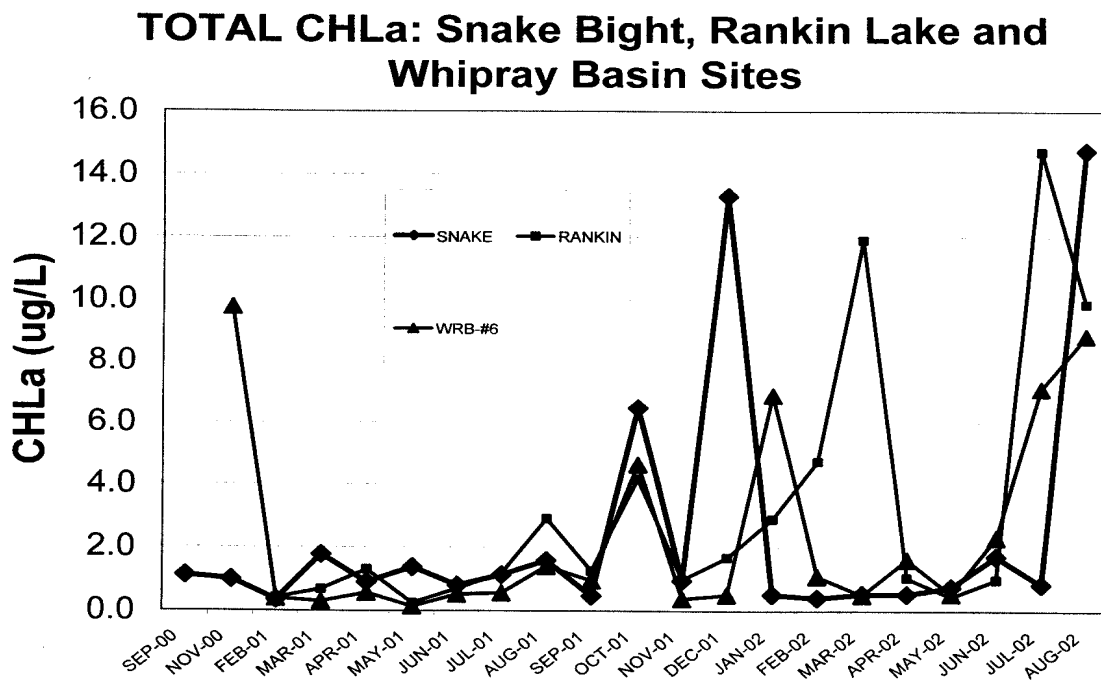


Figure 6: Total chlorophyll-a at north-central Florida Bay sites.

% CYANOBACTERIA : Snake Bight, Rankin Lake and Whipray Basin Sites

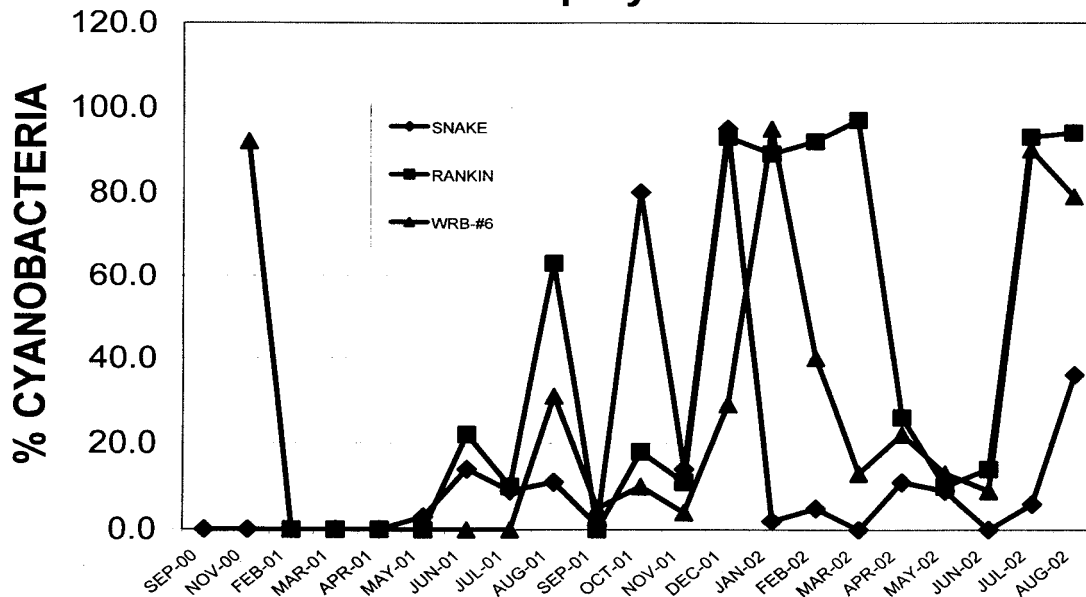


Figure 7: Cyanobacteria as a percentage of the total phytoplankton at north-central Florida Bay sites.

These figures (Figs. 6-7) then together serve as a graphic representation of the importance of cyanobacteria in driving the highs in phytoplankton productivity in the northern and central bay.

This database is only 24 months. However it appears that the timing of at least the Fall-Winter cyanobacterial bloom in mid-Whipray basin is not a strict temporal phenomenon but rather one linked directly to the inflow of fresher cyanobacteria laden waters from the mangrove transition zone. That is, as might be expected, parameters such as water influx (S_{psu}) and nutrient (N, P, Fe, Si, etc.) supply likely play a more important role than the calendar *per se*. The only parameter which is intimately linked to the calendar will be photoperiod, all others (T, rainfall etc.) vary. Thus, the cyanobacterial bloom recorded in November of 2000 either occurred in September of 2001, and reached only about 1/3 the intensity, or was totally absent. Likewise, in 2002, the largest bloom occurred during July-August in the north-central Bay.

Elevated CHLa levels ($>4\mu\text{g/L}$) in Rankin Lake (#4) and mid-Whipray Basin (#6) during October 2001 (Figure 6) were in fact due mainly to diatoms (Figure 7) rather than cyanobacteria, which accounted for only about 10-20% of these communities.

The strongest cyanobacterial events recorded here include sporadic ‘blooms’ in Snake Bight. That is, from a summer mean of about $1\mu\text{g CHLa/L}$, the phytoplankton CHLa in Snake Bight increased from $1 \rightarrow 2 \rightarrow 1 \rightarrow 6 \rightarrow 1 \rightarrow 13 \rightarrow <1 \rightarrow <1 \rightarrow <1 \rightarrow <1 \rightarrow <1 \mu\text{g/L}$ over the period of July 2001 through May 2002. Thus, these ‘productivity’ events,

characterized by diatoms in August 2001 and by cyanobacteria in both October and December 2001, were each followed by a low in standing stock ('crash'). Given that sampling is only monthly, it cannot be told if we are sampling the 'highest-highs' and the 'lowest-lows' nor what the actual temporal spacing of these events is. However, the yearly cycle is far from Gaussian in its onset, persistence and demise. During April 2002, the spread of cyanobacteria from eastern Garfield bight, just south of the outflow (Alligator Creek) from the West, Long, Cuthbert Lakes and the Lungs, was 'observed'. That is, a sample of opportunity was taken close to Alligator Creek in Garfield Bight and it was found to be ~100% cyanobacteria with a CHLa level of 16.4 µg /L. Regular sites (3, 4, 5, 6) to the south, east and southwest of that site did not reveal either elevated CHLa values nor higher percentages of cyanobacteria than "usual" (10-20%). However, site # 2, at the extreme western edge of Snake Bight had CHLa values about 3x those of the rest of south Snake Bight and was about 2/3 cyanobacteria. Thus, given the extremely strong winds on April 10, 2002 (Easterly, >20-25knts), it appears that the cyanobacterial inoculum from the fresher waters emanating from Alligator Creek was literally blown west across the top of Snake Bight and then were deflected south by land in the area of Gibby Point south to Site #2 were these waters mixed with the incoming tide.

Mid-Whipray Basin (#6) and Rankin Lake (#4) both exhibited cyanobacterial dominance January and again in July-August of 2002. South Whipray Basin (#7) had somewhat elevated CHLa and cyanobacterial dominance as well. Sites farther north in Whipray Basin (#8, #5) as well as along the transition zone (ESB), also revealed elevations of CHLa and cyanobacterial dominances (77-92%). Thus, the strongest spread of cyanobacterial populations 'appears' to be from the Garfield-Snake Bight areas through mid-to-southern Rankin Lake and out into mid-Whipray basin. This is likely augmented by a secondary, yet less intense, spread from the Terrapin Bay outflow by Crocodile Point. Santini Bight, with no direct inflow other than directly off land, appears to become isolated from Terrapin Bay outflow and rarely reveals any elevated cyanobacterial signal.

It must be pointed out that the above speculations are just that-speculative. Additional data and a much longer and more closely spaced temporal records would be needed to verify or refute these muses. Data sets which may add insight to these phenomena would include fresh water outflow volumes from Alligator Creek and Terrapin Bay plus wind forcing (direction, velocity) data.

'CHLOROPHYTES': The presence of 'chlorophytes' in these waters was first noted using HPLC-PDA based chemotaxonomy during the February 2, 2001 sampling off of Cape Sable. As stated, this was an extremely calm day following an extended period of calm. The waters were grass green to the eye. Based on the abundance of chlorophyll-*b*, 'chlorophytes' were estimated to form from 31 to 74 % of the community in sites extending from the middle grounds (#9) / Sandy Key (#10) area to the ENP marker off of middle Cape Sable. This signal was still present, though indicating a decreased contribution (19%) at sites 9 and 11 the following month (March, 2001). In April, this contribution was again lower (8-10%) and during May and June, the 'chlorophytes' were

absent at sites #9-11, with 12% of the total population being due to CHLb containing organisms in June. During July, August and September these plankton contributed about 4-9, 6-14 and 1-5% of the community, respectively.

Prior to August 2001, the 'chlorophyte' signal was either very low or absent in the Whipray Basin, Rankin Lake, Snake Bight areas. During August and extending into September, this changed and about 11-17% of the communities in August were 'chlorophytes'. This contribution waned to about 1-9%, except for 18% in Snake Bight, during September.

The reader may have noted the title 'chlorophytes' being in semi-quotes. This was done in order to draw attention to the, at present, tentative nature of this conclusion. That is, there is a chance that the "chlorophyll-b" identified is divinyl chlorophyll-*b* (DV-CHL*b*) or that it is CHL*b* but accompanying divinyl chlorophyll-*a* (DV-CHL*a*). In either case, it would be indicative of **prochlorophytes** rather than chlorophytes (eukaryotic) *per se*. Complicating this identification is the sporadic lack of detectable lutein. Prochlorophytes would contribute zeaxanthin rather than lutein.

Repeated trials to 'dissect' the CHL*a* peak in a search for DV-CHL*a* (Soret at 442 vs. 430 nm for CHL*a*) have proved fruitless. To date, this 'CHL*b*' (Soret at 456 vs. 478nm for DV-*b*) does appear to be a chlorophyte rather than a prochlorophyte pigment.

CRYPTOPHYTES: Cryptophytes, rarely mentioned in the literature on Florida Bay phytoplankton, are most assuredly an important part of these communities. That is, the presence of alloxanthin, with confirmation coming from elevated α -carotene levels, verifies their presence. However, a dearth of CHL*a* to alloxanthin data on the cryptophytes in general and those in Florida Bay specifically does make the estimates given herein a bit tenuous. That is, recalling those caveats, we are using "CHL*a* = 3.8 x allo" as the estimator of cryptophyte contributed CHL*a*. This may or may not be reality for Florida Bay. However, in defense of this estimator, calculations using this value are more often at the $\sim 100 \pm 10\%$ ($CHL_{a_{found}} / CHL_{a_{est}}$) level when cryptophytes are a 'significant' portion of the community.

NOTE: Extinction coefficients ($E^{1\%}_{1cm}$) for peridinin and fucoxanthin were updated (see Jeffrey *et al.*, 1997: Appendix E) for the data herein. This lowered the relative abundances of both pigments a minor amount, relative to previous calculations (Louda *et al.*, 1998, 2000). This had the distinct advantage of bringing the "estimated CHL*a*-to-found CHL*a*" values closer to 100% for both phytoplankton and epiphyte samples.

"GROUND TRUTHING": Several samples have been sent to 2 different laboratories for microscopic examination. To date, neither the independent microscopic exams nor either micro-exam and the chemotaxonomy agree very well. These conundra are being studied. In defense of the *objective* HPLC pigment-based (chemo) taxonomy, only the

exacting percentages can be argumentative. That is, if alloxanthin is present then cryptophytes must be present, *et cetera*. However, all methods agree when there is strong dominance by either cyanobacteria or diatoms. Pigment analyses sometimes miss extremely minor (<1%) contributions noted by microscopy and microscopy appears to miss or severely underestimate cryptophytes (=alloxanthin plus α -carotene) and chlorophytes(=CHLb plus lutein). Study on these discrepancies will continue and results (conclusions ?) will be reported elsewhere.

Latest correlations show a strong, though hardly linear, relationship between the percentage of 'pheopigments' in a sample and the amount by which estimated-over-found CHLa deviate from unity. This points to pigment degradation as a complicating factor in the accuracy of pigment-based chemotaxonomy in shallow well-mixed environments. Sediment resuspension would certainly complicate the process.

CONCLUSIONS:

Epiphytic CHLa on the major seagrasses (*Thalassia testudinum*, *Halodule wrightii*) is vastly dominated by diatom contributions, save an occasional large biomass contributed by epiphytic (macro) rhodophytes. Dinoflagellates and cyanobacteria do sporadically occur on the seagrasses but no trends are yet apparent. Sampling standardization was needed and "epiphytometers", have been deployed at several sites. The use of 'epiphytometers' has been shown herein to be an excellent tool for monitoring (mimicking-?) epiphyte productivity on SAV, notably *Thalassia testudinum*.

Epiphytes on the macroalgae, especially 'rougher' forms such as *Caulerpa lanuginosa* and *Penicillus capitatus*, appear to be a combination of 'true' epiphytes plus trapped resuspended sediments. The minor occurrence of purple-S bacteria in the material released from the surface of *Caulerpa (?)lanuginosa* could reflect the presence of an anoxic micro-niche or, just as easily, the presence of resuspended microphytobenthos.

Data clearly reveal a mixed phytoplankton community in the northwestern bay. The communities found include either a chlorophyte > diatom > cryptophyte or a diatom > chlorophyte > cryptophyte more to the west (offshore Cape Sable: Sites 10-11-14-12) and a diatom > chlorophyte > cryptophyte in an East-West pattern through the "Flamingo trough" (Sites 9-1-2). This mixed community has total CHLa over 1 μ g/L to generally less than 5 μ g/L but up to about 10 μ g/L. This contrasts with the Snake-Rankin through Whipray areas which, when found to have low total (0.15 – 0.5 μ g/L) CHLa, is dominated by diatoms with a sporadic appearance of dinoflagellates. When CHLa levels elevate past 1-2 μ g/L in this area, that increase is directly due to cyanobacterial blooms, such as the 15 \pm μ g/L found in Whipray Basin (Site #6) in February 2001 and (>3 μ g/L) in the northern portion of Whipray Basin / eastern Santini Bight. Dinoflagellates, as discerned by the peridinin signal, appear more prevalent in the southernmost sites of Whipray Basin (#7) and the western bay (Sandy Key Basin. Site #10). Whether this indicates communication of the Sandy Key Basin / 'Flamingo Trough' area with Whipray Basin through Conchie Channel and across the 1 meter expanse

north and east of Dildo Key Bank or the entrance of (unsampled) Gulf waters from the south and west of Whipray, remains for others (physical oceanographers) to tell.

Regarding “pheopigments”: Pheopigments are generally absent or well below detection limits when CHLa values are below 1 µg/L. Over 2-3 µg/L, enough CHLa is present and being “processed” that pheopigments become quite amenable to analysis. At the diatom dominated sites of the western Bay and nearshore Gulf of Mexico off Cape Sable, the pheopigments generally follow the (molar) abundance order: pyropheophorbide-a (pPBIDa) > pheophorbide-a ≥ pheophytin-a > pyropheophytin-a. At site #12, off middle Cape Sable, the abundance of pPBIDa increases with depth in the water column and is in excess of CHLa at the sediment water interface. Pheopigment abundances are highest in the western bay during the Winter months. A full description of pheopigment dynamics will be reported elsewhere (Louda *et al.*, 2003-in preparation).

Along with the HPLC analyses, we are collecting classic spectrophotometric data sets on all of these samples. Fluorimetry will soon be added as well. Alternate chemotaxonomic estimations will be attempted using the CHEMTAX model of the CSIRO (Mackey *et al.*, 1997) when that program becomes operational in our hands.

CLOSING NOTES ('caveats'): Remember, for the present, these data give estimates of the relative amount of chlorophyll-a contributed by each taxon. These estimations relate much more closely to biomass than to cell numbers ! Stated another way, how many nano-/pico-cyanobacteria fit into a diatom ? Additionally, no allowances for taxonomic differences in the amount of CHLa per unit C-biomass were made.

Post-study data dissemination:

Presently, one manuscript (Louda and Monghkrongsri, 2002) on Chlorophyll-a and accessory pigment analyses is being submitted to the *Bulletin of Marine Science*. A manuscript on the degradation of pigments in Florida Bay and adjacent waters is in preparation for submittal to the 21st. International Meeting on Organic Geochemistry (Krakow, Poland: Sept. 8 – 12, 2003) with publication(Louda *et al.*, 2003) in the Elsevier journal *Organic Geochemistry*. Three additional publications, one on the linkage of pigment-based chemotaxonomy to microscopic community evaluation, one on the 2 year (9/2000 – 9/2002) trends in the phytoplankton of north-central and western Florida Bay, and one on the epiphyte study are planned.

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