
Final Report: Technical Report
(Deliverable 10)

Bioavailability and Sources of Nutrients and
the Linkages to Nuisance Drift Algae

Prepared for

The City of Sanibel in Partnership with Lee County

800 Dunlop Road
Sanibel, Florida 33957

¹Ai Ning Loh, ²Larry E. Brand, ¹David W. Ceilley, ³Matthew Charette, ⁴Loren Coen, ¹Edwin M. Everham III, ¹David C. Fugate, ⁵Raymond E. Grizzle, ⁴Eric C. Milbrandt, ⁶Bernhard M. Riegl, ⁶Greg Foster, ⁴Keleigh Provost, ¹Leslie L. Tomasello, ³Paul Henderson, ³Crystal Breier, ³Qian Liu, ¹Taylor Watson, and ¹Michael L. Parsons

¹College of Arts and Sciences, Florida Gulf Coast University, Fort Myers, Florida

²Miami, Florida

³Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

⁴Marine Laboratory, Sanibel-Captiva Conservation Foundation, Sanibel, Florida

⁵Jackson Estuarine Laboratory, University of New Hampshire, Durham, New Hampshire

⁶National Coral Reef Institute, Nova Southeastern University, Dania, Florida

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Foreword

This document is the Technical Report for the Final Report (Deliverable 10) prepared for the City of Sanibel and Lee County. An accompanying document (the Executive Report) is designed as a stand-alone document for those individuals interested more in the findings of the study, rather than all of the technical aspects utilized to reach the findings. This Technical Report contains all of the information presented in the Executive Report, but also contains all of the methodological details, data presentation, and analysis of this study. The Technical Report should therefore be referred to when the reader wishes to learn more information on how the data were gathered and interpreted to obtain the presented results and findings.

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Technical Summary

The sources of available nutrients and their role in producing large-scale macroalgae blooms on Sanibel Island and the waters of Lee County, Florida are the focus of this study, which integrates hydroacoustic surveys with bioassays, macroalgae surveys, stable isotope analyses, and a hydrodynamic model. Sampling was conducted from the Caloosahatchee River downstream of Lake Okeechobee, down to the lower tidal Caloosahatchee Estuary, San Carlos Bay and the nearshore coastal waters from Redfish Pass to Wiggins Pass. This broad geographic scope represents a hypothesized gradient of nutrients and algal biomass downstream and away from the mouth of the Caloosahatchee Estuary, and includes hypothetical areas of algal growth and accumulation at inshore and nearshore locations. This study was funded by a partnership between the City of Sanibel, Lee County, the Tourist Development Council and the West Coast Inland Navigation District. This two-year study began in May 2008 and has completed two full years of field sampling. This is the final report for the study.

Over-fertilization of estuaries with nutrients from urban and agricultural sources is both a local problem for the Caloosahatchee Estuary and a problem for most estuaries worldwide (Bach and Josselyn, 1978; Maze et al., 1993; Valiela et al., 1997). Beginning in the winter of 2003-2004, unusually large masses of drift red macroalgae accumulated on Sanibel Island, Bonita Springs and Fort Myers Beach. Several subsequent drift macroalgae events have been a nuisance to area beaches since then, prompting the City of Sanibel, Lee County, the Tourist Development Council and WCIND to collect additional information specifically targeting the sources and possible causes of drift algae blooms in SW Florida. Earlier work suggested that enriched nutrient concentrations (approximately 10 fold) were found in 2005 associated with large freshwater discharge events (Lapointe and Bedford, 2007). Concentrations at offshore reefs, however, were only 2-3 times enriched, suggesting that there was substantial absorption and cycling of nutrients en route to offshore locations (Lapointe and Bedford, 2007). Additional information and research was needed to determine the sources and fate of nutrients in the coastal zone along with several additional objectives listed below which were identified by the study team with City of Sanibel and Lee County officials.

The first three objectives of this study set out to study the importance of surface water (Lake Okeechobee versus basin sources; Objective 1), sediment-regenerated (Objective 2), and submarine groundwater discharge (Objective 3) inputs of nutrients in the overall nutrient budget of the Caloosahatchee River and Estuary (Objective 8), and subsequent macroalgal bloom events. Other factors (besides nutrients) must also be considered when discussing the build-up of macroalgal biomass and subsequent deposition on area beaches. There must be suitable substrate upon which macroalgae can grow and/or attach (Objective 4). Light, temperature, and salinity also play a role in algal physiology and growth (Objective 5). Hydrologic processes have to detach and transport the algae to the shoreline (Objective 7). Grazing activities may

keep algal growth in check, preventing a significant build-up of biomass (Objective 9). The overall goal of this study was to determine the conditions leading to massive algal stranding events on our local beaches. Any knowledge gained on this front can therefore be incorporated into current and future management practices to control, or respond to, future events.

Water discharges from S-79 and salinity along the Caloosahatchee Estuary during this study showed typical seasonal patterns of high flows and low salinity during the wet season (August – October) and low to no flow and high salinity during the dry season (May – July and November – June). Surface and bottom salinities along the Caloosahatchee Estuary were higher during S-79 low flow periods and were lower during S-79 high-flow periods. Synoptic nutrient and phytoplankton surveys (Objective 1) were conducted to determine whether Lake Okeechobee or basin nutrient sources were more important to nuisance algal growth and to provide information on cumulative inputs of nutrients from Lake Okeechobee and the East Caloosahatchee Basin into the Caloosahatchee Estuary and Gulf of Mexico. Monthly sampling along the Caloosahatchee River and Estuary began in May 2008 while bi-monthly sampling of coastal Gulf of Mexico stations began in June 2008. All field work continued for a full 24 months and ended in June 2010. Samples from the Caloosahatchee River were collected upstream of the control structures (S-77, S-78 and S-79), while samples in the Caloosahatchee Estuary were collected at four stations along the salinity gradient from S-79 to San Carlos Bay. Gulf of Mexico samples were collected from 12 stations and overlapped with nutrient microcosm experiments and sampling of macroalgae (see below).

Dissolved inorganic nitrogen and phosphorus (DIN and DIP, respectively) concentrations along the Caloosahatchee River (upstream of S-79) increased during the wet season and decreased during the dry season, corresponding with water flows and were similar at all three stations (S-77, S-78 and S-79). Dissolved organic carbon (DOC) concentrations also showed the same seasonality and station-to-station differences, although the November 2008 – May 2009 dry season saw higher DOC concentrations than the wet season preceding it. Dissolved organic nitrogen (DON) concentrations were fairly constant during the study period and made up of ~80% of the total dissolved nitrogen pool for the Caloosahatchee River. Dissolved organic phosphorus (DOP) concentrations showed similar trends as DIP concentrations and is comprised of ~30% of the total dissolved phosphorus pool. Concentrations of DON and DOP were also fairly similar between all three stations. The highest abundance of microalgae was upstream near Lake Okeechobee, suggesting that this is the major source of nutrients generating algal blooms in the system. Cyanobacteria were also most abundant upstream near Lake Okeechobee.

Within the Caloosahatchee Estuary (downstream of S-79), dissolved organic matter (as DOC, DON and DOP) and dissolved inorganic nutrients (as DIN and DIP) concentrations were generally higher at the upstream site (near Beautiful Island) compared to the San Carlos Bay site. Concentrations of DOC, DON, DOP, DIN and DIP did not differ between surface and bottom water samples and showed similar seasonal trends as for salinity at these sites (higher

concentrations during wet season, lower concentrations during dry season). Dissolved organic N again made up the majority of the total dissolved nitrogen pool. There was an increase in chromophyte algae (probably diatoms) as river water flowed into the estuary (near Beautiful Island) and the increasing salinity changed the species composition of the algal community. In general, microalgal abundance showed dilution with increasing salinity in the estuary, indicating that freshwater is a much greater source of nutrients than seawater in this area. Abundance of benthic microalgae on the sediment surface is highest in the Caloosahatchee Estuary and declines going offshore, indicating the major source of nutrients to benthic microalgae is derived upstream from the Caloosahatchee River.

In coastal Gulf of Mexico, dissolved organic matter and dissolved inorganic nutrient concentrations were lower than those found within the Caloosahatchee Estuary. Concentrations of DOC, DON, DOP, DIN and DIP were fairly similar between sites and season. Dissolved organic N again made up the majority of the total dissolved nitrogen pool while DOP and DIP comprised similar fractions of the total dissolved phosphorus pool. Phytoplankton concentrations were significantly more abundant offshore during the wet season than during the dry season.

Bioassays indicate that nitrogen is the limiting nutrient throughout the system. Nitrogen bioassays show the highest amount of bioavailable nitrogen upstream near Lake Okeechobee, suggesting that this is the major source of nitrogen generating algal blooms in the system. A comparison of these data with nutrient data indicates that not only inorganic nitrogen but much of the organic nitrogen is available to algae. Bioavailable nitrogen shows dilution with increasing salinity in the estuary indicating that freshwater is a much greater source of nitrogen than seawater in this area.

Daily dissolved inorganic nutrient (NO_2^- , NO_3^- and NH_4^+ and DIP) fluxes from sediment microcosm experiments (Objective 2) were calculated for June 2008 – 2010 for three long-term stations; GOM04, GOM06 and GOM16. For NO_2^- , NO_3^- and DIP, fluxes being regenerated from the sediment were generally balanced by fluxes of nutrients into the sediments during the course of the year regardless site. The magnitude of NO_2^- , however, was very small compared to NO_3^- and DIP fluxes. The magnitude of the exchange of DIP fluxes measured was greater at GOM06 and GOM16 compared with GOM04 while the opposite was true of NO_3^- fluxes. There were no differences in the magnitude of NO_2^- between sites. Ammonium (NH_4^+) fluxes however, were mainly positive and were highest at GOM06. However, there were two significant negative flux events at GOM04, which occurred during the two driest sampling period of the study (May 2009 and May 2010). Ammonium fluxes were also the highest of the four nutrient measured, and the predominant nitrogen species.

Groundwater discharge (Objective 3) was quantified using the naturally occurring radioisotopes radon and radium. These tracers are useful in this regard due to their natural enrichment in groundwater (100-1000 times greater than surface water) relative to other sources of freshwater

to coastal systems (e.g. runoff and rainfall). Groundwater radium concentrations are among the highest we have observed in over 10 years studying submarine groundwater discharge, influenced by deposits of phosphorite, a naturally occurring phosphate-bearing mineral that also contains appreciable quantities of uranium and its decay products. It is also notable that radium was enriched in groundwater irrespective of salinity and wet vs. dry season. Concentrations of four isotopes of radium (223, 224, 226, and 228) from the Gulf of Mexico, through the estuary salinity gradient and along the river to its origin at Lake Okeechobee were only a factor of 2-5 times lower than groundwater, which suggests significant groundwater-surface water exchange.

Box model results yielded radium-derived SGD rates of $3.3 \times 10^6 \text{ m}^3/\text{d}$ (dry) and $1.3 \times 10^6 \text{ m}^3/\text{d}$ (wet) for the Caloosahatchee Estuary and compared well with water and salt balance checks for the system. Using nutrient concentrations from groundwater sampling wells located as close to the location of discharge as possible, i.e., at the estuarine land-water interface and not at inland wells, groundwater nutrient fluxes were estimated. For TDN, the groundwater flux to the estuary during the dry (3410 kg/d) and wet (1400 kg/d) seasons were of the same order of magnitude as the flux through the Franklin Lock. However, the flux of DIN, a significantly more bioavailable form of nitrogen, was almost 7-times higher for groundwater than from the river. Like TDN, groundwater phosphate fluxes were of the same order of magnitude as the river. These results show that the groundwater is highly enriched in nitrogen and phosphate, making groundwater a likely important component of the local nutrient demand of bloom-forming algae.

Areas within San Carlos Bay and offshore of Sanibel Island were surveyed using hydroacoustics and towed-video methodologies (Objective 4). The overall objective was addressed in three phases from 2008 through 2010. These included an initial effort in October 2008 (Phase I) and a second effort in April-May, 2009 (Phase II). Finally, an additional towed-video survey (Phase III) was conducted in May 2010 to assess (“ground-truthing”) Phases I and II hydroacoustic classifications and to add an additional biogenic layer (e.g., worm tubes, pen shells, etc.) that was relevant to potential macroalgal attachment and growth given that during the effort no major macroalgal events were observed. The hydroacoustic data were acquired with a BioSonics DT-X echosounder and a multiplexed single-beam digital transducers operating at 38 and 418 kHz. Eleven acoustic parameters derived from the 38 and 418 kHz signals were submitted to a novel multi-pass Discriminant Function classification scheme to refine the training dataset into end-member structural and biological elements.

The hydroacoustic surveys ranged from nearshore depths of 2 m to as far as 11 km offshore. The towed-video surveys included sub-meter depths within San Carlos Bay and extended as far as 24 km offshore (approximately 15 m depth). The hydroacoustic and towed-video data were classified into one of five (total of 5) visually-apparent categories of seabed roughness, reflecting the variable potential of the seabed to act as a macroalgae attachment site. Classes 1 and 2 consisted primarily of unconsolidated mud and sand sediments, and are least suitable for macroalgal attachment and growth. Class 3 is a marginal substrate for a macroalgal “bloom”,

consisting of packed sand and large, intact shell debris. Classes 4 and 5 offered the best conditions for macroalgal attachment and growth. Class 4 consisted of either unconsolidated shell hash or exposed rocky bottoms. Class 5 consisted primarily of submerged aquatic vegetation (SAV), mainly seagrasses.

The majority (approximately 80%) of acoustic classifications were of soft bottom sediments (Classes 1-2), but there were two significant expanses of rough seabed thought to be suitable for macroalgae attachment. These two areas covered a total of 19 km², within which approximately 56% of the hydroacoustic ‘records’ were classified as “rough” (Classes 3, 4 and 5). The first was a large area of seagrass beds and “live hard-bottom” in the mouth of San Carlos Bay, where large amounts of macroalgae were variably present during the April-May 2009 surveys. The second was offshore of Lighthouse Point, near the mouth of San Carlos Bay. This area is located near a large sand bar that extended from the beach to approximately 6 km offshore. Along the west side of this sandy area was substantial acreage of moderate to high bottom “roughness”, mostly in the form of unconsolidated, shelly hash. The average depths of these two acoustically-rough areas were only 5.0 and 4.0 m, so sufficient irradiance to initiate a macroalgal ‘bloom’ would be likely much of the year. These textured and shallow areas on or near the mouth of San Carlos Bay are presumably potential source areas for the initiation of macroalgal biomass (attachment and growth). Under the appropriate conditions algae could be readily transported onto the areas’ beaches, especially given the close proximity to the islands beaches.

In contrast, the areas further offshore in the Gulf of Mexico were classified predominantly as soft sediments with low bottom “roughness” based on both the hydroacoustic and video surveys. The area offshore of Redfish Pass had a moderate (approximately 22%) proportion of “rough” acoustic classifications out to 5 km offshore, but from 5-10 km offshore the bottom was classified as >95% soft sediments. The other two Gulf of Mexico areas of focus were acoustically classified as >95% soft sediments from nearshore to 11 km offshore. The towed-video transects over a larger area of focus indicated there were relatively small areas that harbored large concentrations of shelly and/or “live hard-bottom” occurring sporadically at distances greater than 10 km offshore. Further assessments of these survey data in the context of available nutrients and ambient light levels will be needed to fully assess the bloom potential for these offshore sites, but it would appear that the open Gulf of Mexico waters around Sanibel-Captiva are probably not a major source of drift macroalgae.

Thirteen stations were established in June 2008 to conduct quantitative sampling of macroalgal communities (Objective 5), to collect sediment cores (Objectives 1 and 2), and to collect water samples for nutrient analyses (Objective 1). Stations were visited bimonthly for a total of 12 sampling events concluding in June 2010. The study area included the area North from Captiva Pass to the southern boundary of Wiggins Pass. Stations were established inshore and offshore, from Sanibel down to Fort Myers Beach in an effort to address gradients related to nutrients and

freshwater (decreasing away from the Caloosahatchee River) and to ensure locations near Sanibel and Fort Myers Beach were included.

During the two-year study, there were no large-scale beach stranding events equal in magnitude to the events that were photographed and described from 2003-2007. Despite the lack of catastrophic macroalgal strandings, we found several new lines of information about the spatial and temporal patterns of macroalgal populations and characterized the types of habitats where they commonly occur. When the detailed information from the 13 stations is combined with large-scale habitat mapping efforts (Objective 4), the area's most likely to be sources of macroalgae and times of year when it is expected to wash up on area beaches are better understood.

There were a total of 96 macroalgal species collected and identified during the two-year study. Most of these species are branching red algae (Division Rhodophyta), with 12 species of brown algae (Division Phaeophyta), 14 total species of green algae (Division Chlorophyta) and 1 common cyanobacteria (Division Cyanophyta). A total of 20 macroalgal species were collected and identified on area beaches since 2003. Only four of the 13 stations sampled routinely had macroalgae in moderate abundances. Inshore algal communities differed from offshore communities, in which offshore communities had higher species richness and diversity. Offshore algal species typically had well-developed discoid and rhizoid holdfasts, likely making them less susceptible to dislodgement or breakage versus inshore species.

Seasonal patterns indicated a late spring to summer growth period, maturing in late summer, and disappearing in early winter. Algal biomass tends to be highest in inshore areas during the spring, and offshore areas during the late summer. Algal physiologic parameters (quantum yields, a proxy for growth, and nutrient content) are highest in the spring, indicating that the algae are likely growing faster (inshore) at this time. The conditions most suitable for algal growth appear to be moderate temperatures (<25°C), high salinities (>35), and low/moderate light intensities (15 – 35 $\mu\text{E}/\text{m}^2/\text{s}$), conditions which are more typical of the dry season and spring (or deeper, cooler offshore waters). In order to obtain massive algal biomass, however, one requires a large nutrient pool. The results of Objective 5 suggest that the nutrient pool may be from a groundwater, sediment, or local source. The algae growing on the artificial reefs do not appear to be a source of the drift algae washing up on beaches, although the lack of a major stranding event prevents this statement from being presented strongly. Some sites are more conducive to algal growth than others, which when coupled to the results of Objective 4 indicate that there may be “hot spots” for growth that might be monitored on a regular basis.

The Volunteer Scientific Research Team (VSRT) is a not-for-profit group of divers that has assisted Lee County staff in the mapping of the structural aspects of artificial reefs following deposition and more recently in conducting fish surveys on the various reefs. For this study, the VSRT assisted in the location of natural reefs and in the collection of attached algae from three natural and three artificial reefs with training and supervision by FGCU faculty. No less than 45

individual species of attached macroalgae were identified from the reef surveys including 28 species of Rhodophyta, six species of Phaeophyta, and 11 species of Chlorophyta. The most frequently encountered taxa included *Botryocladia occidentalis*, *Euchema isiforme denudatum*, *Udotea sp.*, *Gracilaria mammillaris* and *Dictyota cervicornis*. However, the greatest biomass was observed to be *Sargassum* spp. (mostly *S. filipendula*) with little differences between the natural and artificial reefs in terms of species composition. Geographic location of reefs and season of the year was more important than reef type (artificial vs. natural) in determining the overall algal community structure. The GH artificial reef is closest to the mouth of the Caloosahatchee and consistently had lower species richness than most other sites further offshore. Anecdotally, urchins tended to occur in groups of many individuals together and were associated with a lack of macroalgae where they occurred even when the same type of substrate nearby was colonized by numerous macroalgal species. A cluster analysis using the SIMPROF test found that the beach algae communities were significantly different than all the reef samples and separated from reef clusters at approximately 7% similarity or were at least 93% dissimilar.

Toward the achievement of Objective 6, the ecological consequences of algal deposition beaches, we: 1) established 16 monitoring sites for bimonthly quantification of background levels of algal deposition; 2) responded to five deposition events, two of which, the event on Fort Myers Beach in June, 2008 and the *Sargassum* event on Sanibel in July 2009, were larger (approximately 1050 and 750 tons of wet algal biomass respectively) and 3) initiated laboratory experiments on the rate of decomposition of algae and subsequent release of nutrients.

Generally background levels of algae deposition on the beaches were low. The most commonly occurring red algae taxa, where mechanical damage and decomposition did not preclude identification, included: *Botryocladia*, *Gracilaria*, *Solieria*, and *Lomentaria*. Algal biomass was typically too fragmented from mechanical damage resulting from wave action and was usually significantly decomposed, precluding an ability to quantify relative abundance of taxa.

Beach decomposition experiments showed that approximately 30% of the biomass is lost in the first week, and approximately 40% of the biomass persists through two months. In microcosm decomposition experiments, the nutrient export approached zero after approximately two weeks. In that time the biomass was reduced on average 12% in the aquaria. A total nutrient release was calculated, and related to per unit dry biomass lost (599 mg N/Kg dry biomass decomposed) and used to scale up and estimate the total nitrogen that could be released from a deposition event (e.g. 125-130 kg for the larger events like the June 2008 deposition on Fort Myers Beach). This estimate may be too low, as the field decomposition experiments indicated approximately three times the biomass loss in the field, relative to biomass loss in the microcosms. The larger volume of beach sand and greater distance to pass through sand, may counter balance the nutrient release along a beach.

The deposition events during the study period were much less intensive and extensive than the events in 2003-2005. The events examined during this study were also relatively ephemeral,

with durations of two weeks or less. In deciding the response to a deposition event, municipalities must consider the: cost for removal, potential for high tides to remove the deposition, negative impact on the recreational use of the beach, and possible positive ecological implications of the deposition; i.e. stabilization of beach sands and enrichment of beach biotic communities.

The Regional Ocean Modeling System (ROMS) has been chosen to simulate and predict the hydrodynamics and sediment transport in the region of interest (Objective 7). ROMS is widely used and respected by the scientific community, is open code, and has a robust support group. Unlike other proprietary models, the ROMS model that has been developed for this project will be available for use and modification by the funding agencies and supported by the ROMS users group long after the completion of this specific project.

A ROMS compatible grid has been carefully constructed from high-resolution bathymetry data acquired from the South Florida Water Management District and integrated with coastline data obtained from NOAA. The major forcings of the model are wind, tide, and freshwater discharge. Winds are obtained from the Page Field General Aviation Airport located close to the Caloosahatchee River. Wind speed and direction are hourly mean values and are applied uniformly over the grid. Other forcings of secondary importance include air pressure, relative humidity, and temperature, all also obtained from Page Field. The model simulations cover a range of conditions. All of the simulations are done during the dry season or the wet season of 2008. This year and the time interval of the wet season run were chosen because it encompasses one of the more significant macroalgal stranding events which occurred in July 2008. Each simulation is run for 45 days, time to allow the model to spin up and simulate both neap and spring tides within each run. The dry season runs are from February 1 to March 16, and the wet season runs are from June 22 to August 5. Because the macroalgal stranding event did not occur during the maximum discharges, a further simulation was performed to estimate residence times during these high discharge events. This simulation ran from July 22 to September 7.

The sediment transport model, as the hydrodynamic model with which it is coupled, is from an Eulerian viewpoint, i.e., it predicts accretion, erosion and other processes within each cell and does not "know" where sediment that enters the cell originally came from, nor where sediment that leaves the cell will ultimately end up. In order to investigate where water that originates in one place, e.g. the Caloosahatchee River is transported, it is necessary to use a particle tracking model, which is from a Lagrangian viewpoint. Essentially, neutrally buoyant particles, or "drifters" are released at specified locations and followed through the simulation. Rather than being associated with specific grid cells, the drifters may move continuously within and across grid cells. The drifters provide an estimation of where neutrally buoyant material such as dissolved organics, or near-neutrally buoyant particles might be transported. It is difficult to predict the size and density of estuarine aggregates, however, very small aggregates composed of mostly organic material may behave as if they were neutrally buoyant and drifter paths give

some insight into the potential transport of these very small porous organic particles as well as the transport of dissolved material. Eight locations outside of the river where macroalgal stands were discovered became of interest. At each location, five drifters were released near the bottom to simulate possible stranding sites on land.

Wind is the primary long term (i.e. over weekly time scale) forcing factor of the water compared to tidal action or river discharge outside of the Caloosahatchee River and Estuary. An inadvertent experiment with Redfish Pass shows that the pass is important not only for flushing of Pine Island Sound, but without the pass, the retention of particles in the sound made them available for transport south under the causeway onto Fort Myers Beach and even into Estero Bay during short term wind events. The difference in wet season results with Redfish Pass closed and opened support the idea that synoptic wind events are more important physical factors to moving the water, compared to tidal and riverine forcing. When Redfish Pass was closed, water remained longer in Pine Island Sound, then during a short term wind event, was blown south towards Fort Myers Beach.

At a smaller scale, tidal propagation tends to move sediment northwards once outside of Caloosahatchee River and Estuary. This is supported by the general morphology of Sanibel and Captive islands. Sediment tends to build up on ebb deltas making them available for storm transport. When winds from the north coincide with flood currents there is significant longshore sediment transport along the south coast of Sanibel.

Residence time in the Caloosahatchee River and Estuary during the wet season ranges from less than 5 days at the lower extent and 10-20 days in the mid river section, to over 45 days in the upper river between Beautiful Island and S-79. Residence times during the dry season are longer, with less along channel mixing. This is evident by the much sharper gradient between the long and shorter residence times in the mid river region. Residence times in the Caloosahatchee are relatively unaffected by winds, rather tidal dispersion and freshwater discharge are the primary determinants.

To achieve Objective 8, we had redirected our efforts to avoid unnecessary replication of current modeling efforts in the region. Modifications to this objective includes the evaluation of simulation results from existing hydrodynamic and water quality models such as the HSPF & EFDC as well as all available watershed assessments. The existing models and watershed assessments allowed us to draw conclusions relative to the effects of high flow river run-off on the development of red tides and macroalgal growth. We also developed a list of specific resource management recommendations geared towards nutrient reduction strategies for managing drift algal blooms in the waters of Lee County.

Previous studies (CRWPP, 2009) found that while nutrient inputs from Lake Okeechobee constitute approximately 50% of the total nitrogen and phosphorus entering the lower Caloosahatchee River and Estuary, local inputs from the West Caloosahatchee and Tidal

Caloosahatchee sub-basins are also significant (approximately 40% of inputs). Non-point sources of nutrients in the West Caloosahatchee sub-basin are primarily agricultural, whereas the primary non-point sources in the Tidal Caloosahatchee include both agricultural and residential activities, with significant differences in nutrient inputs between wet and dry seasons.

These nutrient loadings did not include submarine groundwater and sediment fluxes of nutrients into the Caloosahatchee River and Estuary. When TN and TP loadings from all known sources into the Tidal Caloosahatchee are enumerated to include those quantified in this study (Objectives 2 and 3), the biggest nutrient source to the Tidal Caloosahatchee is still upstream of S-79, with 18%-27% of the nutrients coming from Lake Okeechobee. However, downstream of S-79, submarine groundwater sources of nutrients cannot be discounted, with loadings equal to those from Lake Okeechobee (25%-27%). Sediment fluxes of nutrients are low, and depending on the season may be a small sink for nutrients within the Caloosahatchee River and Estuary instead of a source (see Objective 2). Total loadings and the average nutrient concentrations for the Tidal Caloosahatchee from this study were used to calculate nutrient residence times for the dry and wet seasons. Residence times for TN and TP were higher during the dry season (19 and 15 days, respectively) compared with during the wet season (10 and 11 days, respectively) and is in agreement with modeling efforts to determine the residence time of water in the Caloosahatchee River and Estuary (see Objective 7).

The overall scope of Objective 9 was to address grazing as a top-down mechanism to check macroalgal blooms. This objective synthesized local information related to the observed blooms from 2003 to the present. It also looked at potential meso- and macrograzers in the area of study based on its and other efforts (Objectives 4 and 5 for example). Limited field and lab experiments with urchins as grazers, their survival and also an assessment of what we know from the literature on palatability, defenses, herbivore feeding capabilities were also conducted.

Interestingly and surprisingly, few meso- or macrograzers were collected in any large numbers at inshore sites in Pine Island Sound or San Carlos Bay, with the exception of a few fish such as pinfish and parrotfish. The few invertebrate grazers (e.g., 2 species of sea urchins) were collected only near passes presumably as these are areas with stable higher salinities. Few individuals were found anywhere else inshore in seagrass beds in either Pine Island Sound or San Carlos Bay. Low to variable salinities and potential for harmful algal bloom impacts may be part of the reason that urchins are rare inshore except for near passes to the Gulf of Mexico. We also found significant accumulations of drift or attached macroalgae in our trawls and in water sampling during April 2010, with the majority being *Rhodophyta* observed previously in at the two inshore sampling stations (see Objective 5).

Single species feeding trials indicates that urchins ate significantly more *Caulerpa racemosa* (offshore species), *Agardhiella subulata* (offshore/nearshore species) and *Acanthophora spicifera* (inshore/nearshore species) than they did *Gracilaria blodgettii* (offshore/nearshore), *Hypnea spinella* (inshore species) or *Spyridia filamentosa* (inshore). In addition, from

observations made every 15 minutes during the first 2 hours of trials, it seems that the urchins consumed more of what they happened to come upon first, with no significant difference between species detected.

Survivability caging experiments indicated that urchins survive in many of the salinities around the island. The only cage that experienced urchin mortality was the SCCF Shell Point RECON unit, which experienced several large fluctuations in salinity often to near 5 psu. Salinities lower than 18 psu perhaps for extended periods appear to be stressful for *Lytechinus*, with values < 5 perhaps causing 100% mortality. Nearshore stations that exhibit a significantly larger numbers of urchins per 100 m² than inshore or offshore sites, and also did not typically have large algal accumulations, were selected for exclusion cages experiments.

For the three months that cages were deployed, algae was found at only two of the eight sites. Redfish RECON site only had 1% algae growth inside the cages, with similar density of algae outside the cages. Results from GOM04 similarly illustrates that grazer control resulted in more growth inside of the cages than in unprotected area outside of cages. However, the presence of algae as well as similar species found would indicate that while reducing grazer pressure (i.e. juvenile urchins found inside cages with some algae) allowed for larger percent cover found inside the cages, other factors most likely contributed to the presence of algae at this site. While these experiments were limited, trends indicated that these areas may be more controlled by nutrient availability than by excluding grazers.

All the information from this study was synthesized in a conceptual model for algal growth. Factors such as nutrient availability or grazer pressures that influence algal growth was simultaneously assessed with when and where algae can grow, and how physical factors such as wind and currents will result in large stranding events on local beaches. Four scenarios were then developed to explain the four most likely conditions that our research findings suggest would lead to a stranding event on area beaches.

Introduction

Over-fertilization of estuaries with nutrients from urban and agricultural sources is both a local problem for the Caloosahatchee Estuary and a problem for most estuaries worldwide (Bach and Josselyn, 1978; Maze et al., 1993; Valiela et al., 1997). Beginning in the winter of 2003-2004, unusually large masses of drift red macroalgae accumulated on Sanibel Island, and then later in Bonita Springs and Fort Myers Beach, FL (Ruane, 2004). There was widespread speculation that the macroalgae were stimulated by high nutrient loads associated with significant water releases from Lake Okeechobee. Indeed, there are several examples worldwide where large accumulations of macroalgal biomass have been linked to excessive nutrient inputs, including Buzzards Bay, MA (Valiela et al., 1997), Venice Lagoon, Italy (Maze et al., 1993) and Bermuda (Bach and Josselyn, 1978). Eutrophication, defined by Nixon (1995) as an increased rate of primary production due to excessive nutrient inputs, represents the greatest single threat to coastal ecosystem health (Cloern, 2001). Researchers have studied if phosphorus (P) or nitrogen(N) based compounds contribute more to eutrophication at any specific time and/or locale (Rabalais, 2002). It is clear, however, that both P and N loadings to aquatic systems have increased several-fold since pre-industrial times due to increased inputs of phosphate- and nitrate-based fertilizers, atmospheric N deposition, and domestic/agricultural waste water run-off (Vitousek et al., 1997; Bennett et al., 2001).

The Caloosahatchee River and Estuary (CRE) is no exception. In the 1980's, the Florida Department of Natural Resources (now known as the Florida Department of Environmental Protection, FDEP) determined that the Caloosahatchee had reached its nutrient loading limits based on high chlorophyll *a* and low dissolved oxygen concentrations. Watershed development resulted in water management practices to accommodate development whereby sheet flow is interrupted and water releases are regulated through weirs. Increased fertilizer use, coupled with an ever-increasing human population is likely increasing nutrient inputs to the CRE, possibly leading to an intensification of macroalgal blooms and red tides (Brand and Compton, 2007; Lapointe and Bedford, 2007). While this cause-effect relationship is plausible, surprisingly little data are available to properly test this hypothesis. Loh (2008) have experimentally determined that N limits phytoplankton production in the Caloosahatchee Estuary during the wet season. However, it is still unknown whether N or P limits macroalgal production and how nutrient limits algal (both micro and macro) production in near coastal waters of the Gulf of Mexico.

In addition, surface waters (and associated land run-off) are not the only sources of nutrients that should be addressed. The decomposition of organic matter in the sediments and the subsequent release of nutrients up into the water column can also be an important source of nutrients to macroalgae (Tyler et al., 2001). Submarine groundwater discharges (SGD) may also be significant, as Swarzenski et al. (2007) have demonstrated that SGD accounts for over half of the total nitrogen inputs to Tampa Bay. Additionally, nutrient measurements from monitoring and research programs indicate that about 20% of the nitrogen load entering the CRE is inorganic

while the remainder (80%) of the nitrogen load is organic, mainly in the dissolved pool (Loh, 2008 and this study). The extent to which this organic fraction, whether in particulate or dissolved form, can support algal production is not known, however. In the form of suspended solids, these organic loads can settle slowly in the inshore, nearshore and offshore environments. Other studies have shown that over time these organics are converted by a variety of biological processes, both aerobic and anaerobic, to inorganic nutrients which are more efficiently used by a variety of macro- and microalgae (Carlson, 2002), which can have a detrimental effect on the estuarine and marine ecosystem when eutrophication occurs (Bach and Josselyn, 1978; Maze et al., 1993; Valiela et al., 1997). Ultimately, phytoplankton (microalgae) and macroalgae are in competition with each other for nutrients. Phytoplankton blooms can also reduce light availability to macroalgae and reduce their growth rate and/or stimulate them to detach from the bottom. While all inorganic nutrients are available to algae, only a portion of organic nutrients are bioavailable. Because of the complex nature of natural organic matter, there is no simple way of knowing which portion is bioavailable and which is not.

During 2005, enriched nutrient concentrations in CRE (approximately 10 fold higher) were associated with large freshwater discharge events from Lake Okeechobee (Lapointe and Bedford, 2007). Nutrient concentrations at offshore reefs, however, were only 2-3 times enriched, suggesting that there was substantial absorption and cycling of nutrients en route to offshore locations. Macroalgal samples from the Caloosahatchee Estuary were enriched in $\delta^{15}\text{N}$, suggesting that nitrogen came from a human or animal source (>10 ‰). However, significant enrichment of $\delta^{15}\text{N}$ was not observed on local beaches (6.0 ‰), and fell within the scale for groundwater influenced only by atmospheric deposition (McClelland et al., 1997). Clearly, more information is needed to better understand the role of these various nutrient sources in stimulating local macroalgal blooms. The first three objectives of this study set out to study the importance of surface water (Lake Okeechobee versus basin sources; Objective 1), sediment-regenerated (Objective 2), and SGD (Objective 3) inputs of nutrients in the overall nutrient budget of CRE (Objective 8), and subsequent macroalgal bloom events.

Studies of previous, local algal stranding events have revealed the following information. An examination of the algae that washed up on Sanibel during the winter 2003 – 2004 stranding event was dominated by the red algae *Chondria atropurpurea*, *Gracilaria caudata*, *Hypnea spinella*, and *Solieria filiformis*. All are common in Southwest Florida as attached or drift species (Dawes, 2004). These species have broad tolerances to light, temperature, and salinity which partly explain their ubiquity in South Florida estuaries and coastal waters. Dawes (2004) outlined a typical seasonal cycle of macroalgal growth for the Tampa Bay region that may be applicable for the coastal waters of Lee County as well. In Tampa Bay, macroalgal growth initiates in the winter months when plankton biomass and turbidity are lowest and reaches maximum biomass during the rainy season when nutrient concentrations are highest. Subsequently, water turbidity and temperatures increase during the rainy season, coinciding with an early fall die back in Tampa Bay.

This sequence of events demonstrates that other factors (besides nutrients) must also be considered when discussing the build-up of macroalgal biomass and subsequent deposition on area beaches. There must be suitable substrate upon which macroalgae can grow and/or attach (Objective 4). Light, temperature, and salinity also play a role in algal physiology and growth (Objective 5). Hydrologic processes have to detach and transport the algae to the shoreline (Objective 7). Grazing activities may keep algal growth in check, preventing a significant build-up of biomass (Objective 9). Each of these factors is introduced in more detail below.

Very little information was available at the beginning of this study regarding locations where macroalgae was likely to grow. Literature review (see Appendix 5.1) indicates that candidate habitats include hard bottom (e.g., exposed limestone), shells (e.g., pen shells) or worm tubes, and seagrass beds. In terms of shelf habitats, much of the previous research is largely descriptive with few long-term or wide-reaching investigations. Benthic studies conducted during the Hourglass expeditionary cruises (Joyce and Williams, 1969) indicated that shallow (20 ft.) stations were composed of quartz and crushed shell with living and dead mollusks (*Atrina* sp., *Butrycon* sp.). Mid-depth stations (60 ft) contained abundant limestone outcroppings with up to 3 ft. of relief colonized by sponges, alcyonarians, stony corals (*Solenastrea hyades* and *Cladocora arbuscula*). The featureless seafloor in between was typically quartz sand colonized by *Halophila decipiens* and *Caulerpa* spp. Subsequent studies have demonstrated that the SW Florida shelf is composed of sand ridges, exposed areas of limestone, and limestone covered by a veneer of sand and/or shell hash (Cutler, 1988; Hine and Locker, in press). Therefore, natural shelf habitats suitable for macroalgae growth appeared to be patchy at best.

The creation of the artificial reef program in Lee County waters has been hailed as a major success of the Marine Sciences Division. The program has enhanced near-shore sport fishing and SCUBA diving opportunities for residents and visitors alike that, in turn, stimulates the local economy. The largest of these artificial reefs is the Edison Bridge reef, which has become a renowned and popular fishing and diving destination in SW Florida. The economic investment in creating these reefs has been greatly exceeded by the economic and environmental benefits derived. However, these reefs appear to be suffering from the overgrowth of marine algae in recent years which may negatively impact fish habitat and diving aesthetics. Anecdotal information from local dive clubs indicates that various species of red algae have colonized the artificial reefs in recent years and may be having negative effects on desirable species of reef organisms. Percent cover of red algae was not quantified, but considered to be so problematic that divers have been actively “weeding” the reefs to remove attached red algae over the past two years (S. Hassett, Vice President, VSR Team, personal communication). Lapointe et al. (2006) concluded that macroalgal C:N:P ratios from material collected from G-H reef in Lee County were indicative of nutrient enriched waters. In normally oligotrophic subtropical and tropical waters, nutrient-enriched macroalgal blooms can alter food-web dynamics by increasing the abundance of grazers in seagrass (McGlathery, 1995) and coral reef ecosystems. A similar shift in food webs may be occurring on local reefs as a result of macroalgal dominance. In addition,

these reefs may be serving as important structural attachment habitat for macroalgae (and marine fauna) that might not otherwise survive on the benthos.

A commonly observed habitat supporting the growth of macroalgae is within the protected bays and estuaries. Drift macroalgae is often associated with seagrasses and is usually not attached to any substrates or is growing on the surface of the sediment (psammophytic). These mobile inhabitants include *Hypnea cervicornis* (Rhodophyta), which are valuable to amphipods and small crustaceans in Tampa Bay and have been proposed as a mechanism of transport from seagrass areas across sand ecotones. *Digenia simplex*, *Gracilaria verrucosa*, *Gracilaria folifera*, and *Laurencia poitei* (Rhodophyta) are frequently found in large clumps or windrows in protected areas throughout Florida (Hooks et al., 1976; Brown, 2001). The movement and concentration of these large masses of algae are attributed to wind and tide, but can also be trapped in areas with seagrass. Brown (2001) found 14 dominant species of drift alga common to Tarpon Springs, Cockroach Bay, and Tarpon Bay on Sanibel Island. The drift algal species were similar between sites and stable year around, with peak biomass occurring in late winter and spring. The most common taxa included species of the genera *Gracilaria*, *Hypnea*, *Chondria*, *Acanthophora*, and *Laurencia* (Rhodophyta). In addition, the genera *Ulva* (Chlorophyta), *Lyngbya majescula* (Cyanophyta), and *Sargassum filipendula* and *S. pteropleuron* (Phaeophyta) were common. The growth rates of drift seaweeds residing in the bays and estuaries are not well understood, partly because of the transport and accumulation processes. Josselyn (1977) reported a wet weight increase of 2-5% d⁻¹ during late fall and spring for the red alga *Laurencia poiteau* in Card Sound and an annual production of 21 g dry wt m⁻² y⁻¹. At Tarpon Bay, the daily growth rate for seven drift red algal species averaged 1.1% for *Spyridia filamentosa*, 2.0% for *Hypnea musciformis*, 4.5% for *Acanthophora spicifera*, and 7.7% for *Gracilaria caudata*, which was the highest rate measured. Daily growth rates indicated that at least half of the dominant drift algae could produce a high biomass within a short period.

While Dawes (2004) indicated that the drift algae species common to Lee County waters have broad tolerances to temperature, light, and salinity, suboptimal conditions can be encountered that can limit algal growth. For example, salinities can drop to zero during wet season (especially if releases from Lake Okeechobee are significant), which can negatively affect algal physiology. Additionally, waters can get too warm (>25°C) which can lead to photorespiration or photoinhibition (Rivers and Peckol, 1995). Light intensity can be too high or too low to support algal growth as well. PAM (Pulse Amplitude Modulated) fluorometry is becoming an increasingly useful tool in studies of algal physiology and ecophysiology, where measurements are made to assess photosynthetic efficiency (estimated as quantum yield). Simply stated, quantum yield estimates will vary between zero and one, as an indicator of increasing physiological health. Quantum yield measurements have been used to study physiological stress caused by temperature (Eggert et al., 2006), salinity (Theil et al., 2007), nutrient deficiencies (Masojidek et al., 2000), and light intensity (Haeder et al., 1998). One of our goals, therefore,

was to utilize quantum yield measurements to better understand the physiology of algae under changing environmental conditions typical of our coastal/estuarine ecosystems.

While abiotic factors (nutrients, light, temperature, and salinity) affect algal growth and physiology (i.e., bottom-up controls), grazing can keep rigorous growth in check (i.e., a top-down control) – as long as grazing rates are equal to or greater than algal growth rates. Several nearshore or inshore studies in the Gulf of Mexico have shown that sea urchin grazing on macroalgae can be substantial, with *Lytechinus variegatus* often creating large-scale seagrass losses in grass beds along the coasts of Florida (e.g., St. Joe Bay, Biscayne Bay, Steinhatchee, Valentine and Heck, 1991, Heck and Valentine, 1995; 2006). This urchin has been shown to create areas of low seagrass biomass in northwestern Florida, along with another sea urchin *Tripneustes ventricosus*, also a seagrasses “denuder” observed elsewhere in the subtropical and tropical western Atlantic. Predation has often been suggested to control sea urchin densities in temperate, subtidal hard substrates. However, larval supply, disease related mass-mortalities, post-metamorphic larval/juvenile survivorship, parasitism, storms and food abundance have also been implicated in controlling urchin populations (e.g., Watts et al., 2001; Heck and Valentine 1995; 2006). It is possible that grazing rates decreased in the region (possibly due to one of the above factors), which could have lead to an increase in algal biomass. This scenario is explored in Objective 9.

Macroalgae may detach and drift for a variety of reasons including growth cycles (i.e., annual life cycles), alternation of generations, or as a response to changing environmental conditions. A literature review indicates that little is known about these potential triggers of detachment, but rather the importance of wave and current energy was evident. For example, Hawes and Smith (1995) reported that thalli (plants) of the green algae, *Ulva lactuca* (a species common in our coastal waters), will detach when hydrodynamic drag reaches 0.7 N. As drag varies by thallus height, thallus area, and water velocity, the complexity not only among different size thalli, but among different algal species, is clear. Be that as it may, however, it is safe to assume that as water velocities increase, more algae will detach.

Another significant unknown factor is the role played by hydrology in drift algal events. For example, are stranding events the result of large algal biomass build-ups, or the unfortunate result of water movements, or a combination of both? Hydrodynamic models have been developed for the CRE, including the CH3D-IMS model (Sheng et al., 2003). Such models can model the distribution of nutrients and associated water movements. Particle tracking routines have also been incorporated to track the movement of sediments and neutrally-buoyant particles, which can simulate the movements of drift algae. The goal of Objective 7 was to develop and use a hydrodynamic model to stimulate hydrologic conditions during typical dry season and wet season scenarios, in addition to creating scenarios most likely to transport significant amounts of water to Sanibel and Fort Myers Beach that theoretically would lead to a stranding event (if drifting algae were present in the water).

Drift algae stranding events can have many negative impacts. Dead biomass of macroalgae can cause hypoxia, anoxia, and die-off of seagrasses and other benthic biota (Valiela et al., 1997), thereby reducing habitat for sport and commercially important fisheries. The effects of large deposits of red drift algae on benthic fauna also need to be considered since they may have trophic-level impacts that are not yet understood. Massive piles of rotting algae also have aesthetic impacts. Beach-goers do not want to be around rotting algae and local businesses suffer when people move away from such deposits, or leave the beach altogether. While algal strandings are a noxious sight, decomposition rates are relatively rapid with up to 65% of the original algal mass degrading after 25 days (Sassi et al., 1988). Grazers also play a significant role once algae are ashore, feeding directly or indirectly on the stranded material. Decomposition of algal biomass can provide an important supply of organic and inorganic nutrients that can be rapidly assimilated by other macroalgae, phytoplankton, and phytobenthos. The impacts of such nutrient releases on our local beaches are unknown and were the focus of Objective 6.

The study described below was designed to investigate the bioavailability of nutrients, the sources of those nutrients and the linkages between nutrient availability and nuisance red drift algae. We also included components to survey the benthic substrate within San Carlos Bay and Pine Island Sound, as well as surveys in nearshore shelf waters to determine which areas were most conducive for algal attachment and/or accumulation. A hydrodynamic model was developed to simulate various scenarios to explain water movement in and around Sanibel Island and Fort Myers Beach. The role of grazers in controlling macroalgal growth was examined, as was the fate of the algae once deposited on the beach. The research project encompassed an area reaching from Lake Okeechobee down to the Gulf of Mexico, offshore a distance of 25 km. This broad geographic scope represents a hypothesized gradient of nutrients and algal biomass downstream and away from the mouth of the Caloosahatchee Estuary, and includes hypothetical areas of algal growth and accumulation at inshore and nearshore locations. The overall goal of this study was to determine the conditions leading to massive algal stranding events on our local beaches. Any knowledge gained on this front can therefore be incorporated into current and future management practices to control, or respond to, future events.

Study Objectives

The sources of bioavailable nutrients and their role in producing large scale macroalgae blooms on Sanibel Island and the waters of Lee County, FL were the foci of this study. The following were the specific objectives.

1. To determine whether Lake Okeechobee or basin sources are more important to algal biomass and productivity with an emphasis on those associated with organic and benthic nutrient sources.
2. To determine if benthic nutrient flux derived from degradation of organic matter is a significant contributor to harmful algal blooms.
3. To determine the submarine groundwater flux of nutrients to coastal waters.
4. To determine the distribution of macroalgal biomass using underwater video and hydroacoustic surveys.
5. To determine the distribution and productivity of attached macroalgal biomass and the conditions that are favorable for uncontrolled growth, including the examination of substrate attachment.
6. To determine the ecological consequences of the accumulation of nuisance algal biomass by measuring the decomposition rates and nutrient recycling on area beaches.
7. To verify and calibrate a hydrodynamic model for the system to address the potential fate of nutrients that are generated and exported from the Caloosahatchee River and the fate of detached macroalgae as they enter the near shore waters around Sanibel Island and to develop a sediment transport model and implement the coupled sediment and hydrodynamic model for the same time periods as the hydrodynamic model.
8. To evaluate existing water quality models, watershed plans and/or assessments for the Caloosahatchee and Estero Bay watersheds to help guide nutrient reduction strategies for managing drift algal blooms in Lee County waters.
9. To identify the role of macroalgal grazers in determining macroalgal distribution patterns.
10. To provide critical management tools to more accurately predict the fate and transport of nuisance algal blooms on area beaches.

Materials and Methods

Work Plan implementation began soon after the execution of the contract in May 2008 and was completed in October 2010. Below are the details of the methods used for each objective.

General Description of Study Area

Southwest Florida is one of the fastest growing areas in the United States. For the Caloosahatchee River and Estuary (CRE), watershed management is typified by large freshwater releases during wet (rainy) summer months from Lake Okeechobee through the Franklin Locks (S-79) with little or no releases during dry winter months. In addition, freshwater is also released from Lake Okeechobee for flood control and agricultural uses. Water runoff and freshwater releases typically occur during summer, followed by storm water runoff depressing the salinities for extended periods (Doering and Chamberlain, 2005).

Water is released from Lake Okeechobee through the S-77 control structure in Moore Haven into the Caloosahatchee River (sometimes called the C-43 canal) which connects the lake to the estuary and on to the Gulf of Mexico (see Fig. 8.1). Downstream of S-77, along the Caloosahatchee River, there are two other control structures (S-78 and S-79) before the tidal reaches of the Caloosahatchee. The freshwater basins for the Caloosahatchee are divided into the East Basin and the West Basin. The East Basin drains into the Caloosahatchee River between S-77 and S-78 while the West Basin drains into the Caloosahatchee River between S-78 and S-79. The tidal (and estuarine) portion of the Caloosahatchee spans from S-79 down to Shell Point and flows into San Carlos Bay. The Caloosahatchee then enters the Gulf of Mexico at the Sanibel Causeway (CRWPP, 2009).

This research project encompassed an area reaching from Lake Okeechobee down to the Gulf of Mexico, offshore a distance of 25 km. More detailed descriptions of specific study sites and stations are given under each objective.

Objective 1: To determine whether Lake Okeechobee or basin sources are more important to macroalgal biomass and productivity with an emphasis on those associated with organic and benthic nutrient sources. (Loh and Brand)

Caloosahatchee River (C-43 Canal) and Estuary Synoptic Surveys

Monthly synoptic surveys for this objective began in May 2008 and continued without any interruption throughout the duration of the study. Samples from the Caloosahatchee River were collected upstream of the control structures (S-77, S-78 and S-79), while samples in the Caloosahatchee Estuary were collected by boat (Fig. 1.1). Locations for these stations are listed in Table 1.1. For the months of May and June 2010, these stations were sampled bi-weekly. These stations provided information on cumulative inputs of nutrients from Lake Okeechobee

and the East and West Caloosahatchee Basin via the Caloosahatchee River (from S-77 to S-79) and nutrients ultimately entering the estuary, San Carlos Bay and eventually the Gulf of Mexico (GOM).

Caloosahatchee River stations were sampled for surface (~0.5m) dissolved and particulate organic matter and dissolved and particulate nutrients. Samples were also collected for surface water microalgal abundance and related nutrient bioassay experiments. At the estuarine stations, samples were collected for surface (~0.5m) and bottom (~0.5m above bottom) dissolved and particulate organic matter, dissolved and particulate nutrients and sedimentary organic matter. Samples were also collected for surface and bottom water microalgal abundance and nutrient bioassays, and sediment surface microalgal abundance. In addition, measurements of dissolved oxygen concentration, temperature and salinity in the surface and bottom water at each station were taken using a YSI 600 XL multi-parameter water quality sonde. The physiochemical parameters were measured at the surface and bottom at each station.

Water samples were collected in duplicates using pre-cleaned polycarbonate bottles. Sediment samples were collected manually using small acrylic core tubes. Samples were put on ice and returned to the laboratory for immediate processing. Upon return to the laboratory, water samples for dissolved organic matter and nutrients were filtered through pre-combusted 0.7- μm Whatman GF/F glass-fiber filters. All filtered water samples and filter pads were frozen in pre-cleaned amber glass bottles and in petri dishes at -20°C until laboratory analysis.

Gulf of Mexico Synoptic Surveys

Bi-monthly sampling of offshore stations (Fig. 1.2, Table 1.1) was coordinated with Objective 5 (see below) and began in June 2008. At each station, samples were collected for surface (~0.5m) and bottom (~0.5m above bottom) dissolved and particulate organic matter, dissolved and particulate nutrients and sedimentary organic matter as described above. Samples were also collected for surface and bottom water microalgal abundance and nutrient bioassay experiments, and sediment surface microalgal abundance as described above. In addition, measurements of dissolved oxygen concentration, temperature and salinity in the surface and bottom water at each station were taken using a multi-parameter water quality sonde. The physiochemical parameters were measured at the surface and bottom at each station. All water samples collected were filtered on the day of collection and frozen until analyses as described above.

Carbon, Nitrogen and Phosphorus Analyses

Water samples were analyzed for dissolved (as nitrite, NO_2^- ; nitrate, NO_3^- ; ammonium, NH_4^+ ; total dissolved nitrogen, TDN; orthophosphate, PO_4^{3-} ; and total dissolved phosphorus, TDP) and particulate (as particulate nitrogen, PN and particulate phosphorus, PP) nutrients as well as associated dissolved and particulate organic carbon (DOC and POC). All sampling methods, processing and analyses (unless otherwise stated) are detailed in the Florida Gulf Coast

University, Coastal Watershed Institute Quality Assurance Project Plan Part V (Loh, 2005) and are summarized in Table 1.2.

Nutrient samples were thawed and analyzed for DOC, dissolved inorganic nitrogen (DIN; as NO_2^- , NO_3^- and NH_4^+), TDN, dissolved inorganic phosphorus (DIP; as PO_4^{3-}) and TDP concentrations as described in Loh and Bauer (2000). Briefly, DOC was analyzed using a Shimadzu TOC-V high-temperature combustion instrument (Benner and Strom, 1993). DOC concentrations were measured directly by non-dispersive IR absorbance of CO_2 released from HTC of organic C. TDN samples were digested to NO_3^- using the wet alkaline persulfate oxidation method as described in Koroleff (1983), followed by NO_3^- analysis by a Bran+Luebbe AutoAnalyzer 3. 10-mL aliquots of samples were sealed in 20-mL pre-combusted glass ampoules containing 1 mL of the 5% alkaline persulfate oxidizing reagent, and then boiled under pressure in an autoclave for 1 hour. The DIN species (NO_2^- , NO_3^- and NH_4^+) in unoxidized aliquots of the samples were also analyzed by autoanalyzer. Concentrations of dissolved organic nitrogen (DON) were obtained by taking the difference between the TDN concentrations in the oxidized aliquot and the DIN concentrations in the unoxidized aliquot according to:

$$[\text{DON}] = [\text{TDN}] - [\text{DIN}] \quad (\text{Eq 1.1})$$

TDP samples were also oxidized the wet alkaline persulfate oxidation method as described in Koroleff (1983), followed by quantification of the total orthophosphate produced using a Bran+Luebbe AutoAnalyzer 3. 10-mL aliquots of samples were sealed in 20-mL pre-combusted glass ampoules containing 1 mL of the 5% alkaline persulfate oxidizing reagent, and then boiled under pressure in an autoclave for 1 hour. The total orthophosphate concentration was then quantified by the autoanalyzer. The DIP concentrations in unoxidized aliquots of the samples were also analyzed by the autoanalyzer. Dissolved organic phosphorus (DOP) concentrations were obtained by taking the difference between the TDP concentrations in the oxidized aliquot and the DIP concentrations in the unoxidized aliquot according to:

$$[\text{DOP}] = [\text{TDP}] - [\text{DIP}] \quad (\text{Eq 1.2})$$

Sediment and particulate samples collected on filter pads were dried and acidified with 10% hydrochloric acid to remove any carbonates (Loh et al., 2006). The sediment and filters were then packed into small silver cups and analyzed for sedimentary and particulate organic carbon (SOC and POC) and nitrogen (SN and PN) content using a Thermo Flash 2000 Organic Elemental Analyzer. A subset of these samples was sent to the University of California, Davis Stable Isotope Facility for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes analyses using isotope-ratio mass spectrometry. Isotopic signatures and C:N ratios of the sediment and particulate samples were used to indicate the source(s) of these particles and were selected to complement the set of macroalgal samples available for isotopic analyses (see Objective 5 below).

Sedimentary and particulate phosphorus samples were extracted using 1M HCl followed by spectrophotometric analysis for orthophosphate using the phosphomolybdate blue method (Aspila et al., 1976). One fraction of the sample was oxidized using a high-temperature ashing oxidation method at 550°C for 1.5 hours followed by extraction using 1M HCl. Another fraction was not oxidized prior to extraction. Following extraction, both fractions were analyzed as for orthophosphate. The orthophosphate content of the HCl extract before and after dry ashing gives the sedimentary and particulate inorganic phosphorus (SIP and PIP) and total sedimentary and particulate phosphorus (TSP and TPP) content, respectively. The organic phosphorus content was then determined by difference according to:

$$[\text{SOP}] = [\text{TSP}] - [\text{SIP}] \quad (\text{Eq 1.3})$$

$$[\text{POP}] = [\text{TPP}] - [\text{PIP}] \quad (\text{Eq 1.4})$$

Microalgal Analyses

Replicate 100 ml water samples were filtered through GF/F glass fiber filters and the filters were frozen until extracted (within a few days). One set of filters were extracted for 60 minutes with 10 ml of dimethyl sulfoxide and then with an added 15 ml of 90% acetone at 5°C overnight and measured fluorometrically before and after acidification for the measurement of chlorophyll and phaeopigment concentrations. Fluorescence measurements were made with a Turner Designs 10-AU fluorometer equipped with an infrared-sensitive photomultiplier and calibrated using pure chlorophyll *a*. The extracts were also analyzed for chlorophylls *a*, *b*, and *c* with a SPEX Fluorolog-3 spectrofluorometer. Another set of filters were extracted overnight with a phosphate buffer (pH 6.5, with 0.01% mercaptoethanol added) at 5°C and analyzed for phycoerythrin and phycocyanin with a SPEX Fluorolog-3 spectrofluorometer.

The top 5 mm of the sediment cores were sliced off and frozen until ready for extraction. They were extracted with 90% acetone and then analyzed for chlorophyll *a* with a Turner Designs 10-AU fluorometer.

Nutrient Bioassays

Four replicate water samples were used in the nutrient bioassays. To each natural water sample was added: nothing (A); 50 μM nitrate (B); 10 μM phosphate (C); and 50 μM nitrate and 10 μM phosphate (D). After nutrients were added, the samples in 25 mm culture tubes were monitored daily for approximately a month by *in vivo* fluorescence using a Turner 10 fluorometer. A comparison of the nitrate and phosphate additions was used to determine if nitrogen or phosphorus was the limiting nutrient in the ecosystem. A nutrient bioassay index was calculated as: (B-C)/(B+C). Positive numbers indicate nitrogen limitation and negative numbers indicate phosphorus limitation. The addition of nothing (A) and both nitrogen and phosphorus (D) were used as controls.

Nitrogen limitation bioassays (B) were used to determine how much of organic nitrogen was available to algae. Potential algal abundance from nitrogen was calculated assuming 1 µg chl *a*/100 µg C and a molar ratio of 106C/16N/1P.

Objective 2: To determine if benthic nutrient flux derived from degradation of organic matter is a significant contributor to drift algal blooms. (Loh and Brand)

Microcosm Experiments

Bi-monthly sampling of three offshore stations (see Objective 1 above) for microcosm experiments to determine benthic nutrient fluxes began in June 2008 and was coordinated with Objective 5 (see below). A total of 12 such experiments were conducted during the course of this study at the FGCU Vester Marine and Environmental Field Station in Bonita Beach. With the exception of the June 2008 and November 2008 experiments, all experiments were conducted with cores collected from GOM04, GOM06 and GOM16 (Fig. 1.2). Cores for the June 2008 experiment were collected from GOM04, GOM06 and GOM11 while cores for the November 2008 experiment were collected from GOM04, GOM06 and GOM09 (Fig. 1.2).

Triplicate cores were collected using acrylic core tubes by divers one day before the experiments. On the day of the experiments, overlying water in the core tubes was carefully removed and replaced with bottom water collected from each site. Any visible fauna or flora was removed prior to the start of each experiment. The cores were then incubated in the light for 2 hours followed by in the dark for another 2 hours at ambient temperature with 30 cm of water above the sediment surface. In addition to the sediment cores, triplicate ambient water column controls from each station were also incubated. Water in each core/water column was stirred by individual Azoo micro-pumps.

Subsamples of the overlying water were collected every 45 minutes to detect changes in DOC and dissolved nutrient (as NO_2^- , NO_3^- , NH_4^+ , DON, PO_4^{3-} and DOP) concentrations due to benthic regeneration. Samples were collected using pre-cleaned polypropylene syringes and filtered through pre-combusted 0.7-µm Whatman GF/F glass-fiber filters. All filtered water samples were frozen in pre-cleaned polypropylene centrifuge tubes at -20°C until laboratory analysis. All dissolved organic matter and nutrients were analyzed as described in Objective 1 (see above). Sediment grain size for each station was analyzed using a Malvern Mastersizer particle size analyzer. In addition, dissolved oxygen concentrations and temperature for each core/water column were monitored throughout the duration of each experiment using a YSI-85 probe.

At the end of each experiment, water from each core/water column was collected for nutrient bioassay experiments conducted in the same way as for regular water samples (see Objective 1 above). These bioassays were used to determine the availability of any nutrients regenerated

from these sediment cores for algal blooms. While the bioassays were conducted with microalgae, the nutrients they utilized are also available to macroalgae and thus a good indicator for the potential to generate large blooms of macroalgae.

*Objective 3: To determine the submarine groundwater flux of nutrients to coastal waters.
(Charette)*

Measurements of Radium and Radon

The methodological approach for this objective was based on the use of radium (Ra) isotopes, a naturally-occurring tracer of SGD. Recently, radium has been shown to be a useful indicator of SGD, which we define as advective flow of groundwater into the coastal zone (Moore, 1996; Rama and Moore, 1996; Krest et al., 2000; Charette et al., 2001). Three factors make radium a useful tracer of SGD: (i) it is naturally enriched in groundwater relative to seawater by several orders of magnitude; (ii) it has four isotopes with half-lives ranging from 4 days to 1600 years (^{224}Ra , $t_{1/2} = 3.66$ d; ^{223}Ra , $t_{1/2} = 11.4$ d; ^{228}Ra , $t_{1/2} = 5.75$ y; ^{226}Ra , $t_{1/2} = 1600$ y); and, (iii) it behaves conservatively once released into marine waters.

Groundwater flux studies were carried out during April 13-16, 2009 and October 12-15, 2009. During each time period, scientists were divided into two sampling teams: estuary and groundwater. The estuary team conducted sampling of the Caloosahatchee over three days (Gulf of Mexico, lower and upper estuary; Fig. 3.1). The groundwater team sampled wells maintained by Lee County Natural Resources.

For radium isotopic analysis within the estuary, 20-100 L of surface water was filtered into a plastic container, which was then slowly pumped or gravity fed (<1 L min^{-1}) through MnO_2 -coated acrylic fiber to extract the Ra (Moore and Reid, 1976). Subsamples for nutrients and salinity were also collected at this time. Ancillary water quality parameters were recorded with a YSI 600XLM sonde.

More than 40 groundwater samples were collected from various locations along the estuary edge and from various depths within the surficial aquifer (Fig. 3.1). Lee County monitoring wells were purged with three times the well volume prior to sample collection. Estuarine groundwater samples were collected with Push Point samplers (MHE Products, Inc.). Briefly, the stainless steel piezometer was driven to the depth of interest. Samples were pumped through plastic tubing using a peristaltic pump. For Ra analysis, groundwater was pumped directly through the Mn fiber and the filtrate (10-20 L) was collected to determine the sample volume. Samples for nutrients were collected into 30 mL, acid cleaned scintillation vials using a capsule filter. Basic water properties including salinity, pH, dissolved oxygen, and redox potential were recorded on site with the YSI sonde using a flow cell attached to the well pump. The groundwater samples were collected in concert with the surface water samples.

In the laboratory, the MnO₂-fiber was rinsed and partially dried. Activities of ²²³Ra (t_{1/2} = 11.4 days) and ²²⁴Ra (t_{1/2} = 3.66 days) were measured on a delayed coincidence counter as described by Moore and Arnold (1996). The fiber was then ashed in a muffle furnace (820°C for 16 hours), ground, and homogenized before being packed in a counting vial and sealed with epoxy to prevent ²²²Rn loss (Charette et al., 2001). Once ²²²Rn had reached secular equilibrium with its parent, activities of ²²⁶Ra (t_{1/2} = 1600 years) and ²²⁸Ra (t_{1/2} = 5.75 years) were determined by γ -counting in a well detector (Canberra, model GCW4023) by the ingrowth of ²¹⁴Pb (352 keV) and ²²⁸Ac (911 keV). Calibration of the well detector was achieved by counting four ashed MnO₂ fiber standards having the same activity range and geometry of the samples. Nutrient analyses (nitrate, phosphate, ammonium, silicate) were performed using standard methods on a Lachat QuickChem 8000 Flow Injection Analyzer. Total dissolved nitrogen (TDN) was determined using wet chemical oxidation followed by nitrate analysis as above.

Box Model for Radium-derived Submarine Groundwater Discharge

The surface water and groundwater radium data were used in a box model of the estuary to derive the SGD rate for April and October (Fig. 3.2). The model is described mathematically as follows:

$$F_{SGD} = \frac{\left(\left[\frac{(A - A_{ocn})}{T_w} \right] - \left[\frac{F_{riv} A_{riv} + F_{riv} A_{desorb}}{V} \right] - \left[\phi D_s \left(\frac{\partial^2 A_{sed}}{\partial z_{sed}^2} \right) \right] \right) z_{wc}}{A_{gw}}$$

where F_{SGD} is the per unit area fluid flux via SGD ($m^3 m^{-2} d^{-1}$ or $m d^{-1}$) and A_{gw} is the groundwater endmember radium-226 activity. Other terms include A , which is the average activity ($dpm m^{-3}$) of the isotope within the study area, A_{ocn} is the isotope activity of the ocean water that exchanges with the study area (Gulf of Mexico in this case), z_{wc} is the water column or mixed layer depth (m), V is the water volume of the study area (m^3), T_w is the water residence time (days), F_{riv} is the volumetric water flux from the Caloosahatchee River as reported at Franklin Lock ($m^3 d^{-1}$), A_{riv} is the average fresh water endmember activity for the river, A_{desorb} is the suspended sediment activity available for release to the water phase (analysis of the particulate Ra phase at Franklin Lock), ϕ is the sediment porosity (unitless), D_s is the isotope (e.g. ²²⁶Ra) diffusion coefficient in pore water of sediments ($m^2 d^{-1}$), and $d^2 A_{sed}/dz^2$ is the vertical gradient of the isotope concentration in pore waters of the sediments. Given that ²²⁶Ra is produced by decay of ²³⁰Th, the regeneration time for ²²⁶Ra in the sediments is very long, on the order of hundreds to thousands of years. The sediment diffusion source is therefore a small term that is approximated based on sediment ²³⁰Th data from similar estuarine environment (Veeh et al., 1995).

Knowledge of water residence time (T_w) is required for quantifying SGD using the approach described above. The large-scale input of radium isotopes along the coastline and the boundaries of estuaries is akin to a purposeful tracer release, with the short-lived radium isotopes providing

the rate of dispersion based on their decay as they mix away from the source. Both residence time and age are used interchangeably to describe how long water remains in an estuary. One definition of residence time is “the time it takes for any water parcel to leave a given water body through its outlet to the sea”, usually relative to an arbitrary reference point within the system (Monsen et al., 2002). On the other hand, age is defined as the time a water parcel has spent since entering the estuary through one of its boundaries, defined for Ra isotopes with the following equation (Moore et al., 2006):

$$T_w = \frac{[F(^{224}\text{Ra}/^{228}\text{Ra}) - I(^{224}\text{Ra}/^{228}\text{Ra})]}{I(^{224}\text{Ra}/^{228}\text{Ra})\lambda_{224}}$$

In this case $F(^{224}\text{Ra}/^{228}\text{Ra})$ is the $^{224}\text{Ra}/^{228}\text{Ra}$ activity ratio (AR) of the input into the system and $I(^{224}\text{Ra}/^{228}\text{Ra})$ is the $^{224}\text{Ra}/^{228}\text{Ra}$ AR of the estuarine sample. The decay constant for ^{224}Ra is represented as λ_{224} . The application of this model requires knowledge of the $^{224}\text{Ra}/^{228}\text{Ra}$ AR of input, which in this case was derived from the average ratio measured in groundwater along the estuary boundary. The estuarine residence time averaged 18 and 16 days for April and October 2009, respectively.

Objective 4: To determine the distribution of macroalgal biomass using underwater video and hydroacoustic survey. (Grizzle, Foster, Riegl and Coen)

Areas within San Carlos Bay and offshore of Sanibel Island were surveyed using towed-video and hydroacoustics methodologies by the University of New Hampshire, Nova Southeastern University and SCCF. The overall objective was addressed in three phases from 2008 through 2010. These included an initial mapping and methods development effort in October 2008 (Phase I). With the approval of the funding agencies and co-PIs a second mapping effort was initiated in April-May, 2009 (Phase II). Finally, an additional towed-video survey (Phase III) was conducted in May 2010 to assess predictions of macroalgae absence/presence and to add an additional biogenic layer (e.g., worm tubes, pen shells, etc.) that was relevant to potential macroalgal attachment and growth.

Towed-video and hydroacoustic methods were used in complementary fashion to provide as much spatial coverage as practical. Supervised classification of hydroacoustic data provides a high density of along-track seabed classifications (approximately 1 record every 2 meters) at a greater spatial coverage than towed-video. Expert classification of video imagery, however, is far more straightforward and unambiguous than acoustics when water clarity is sufficient, and allows for a more detailed characterization of the benthos. For example, hydroacoustics is not well-suited for detecting low densities of infaunal and epifaunal biota (e.g., pen shells and worm tubes), but quantification by towed-video is easily accomplished. Towed-video also can be used in sub-meter depths, whereas hydroacoustics are limited to 1-2 m water depth.

The hydroacoustic data were acquired with a BioSonics DT-X echosounder and a multiplexed single-beam digital transducers operating at 38 and 418 kHz. The hydroacoustic surveys by NOVA ranged from nearshore depths of 2 m to as far as 11 km offshore. The towed-video surveys by UNH included sub-meter depths within San Carlos Bay and extended as far as 24 km offshore (approximately 15 m depth). The hydroacoustic and towed-video data were classified into one of five (total of 5) visually-apparent categories of seabed roughness, reflecting the variable potential of the seabed to act as a macroalgae attachment site. Classes 1 and 2 consisted primarily of unconsolidated mud and sand sediments, and are least suitable for macroalgal attachment and growth. Class 3 is a marginal substrate for a macroalgal “bloom”, consisting of packed sand and large, intact shell debris. Classes 4 and 5 offered the best conditions for macroalgal attachment and growth. Class 4 consisted of either unconsolidated shell hash or exposed rocky bottoms. Class 5 consisted primarily of submerged aquatic vegetation (SAV), mainly seagrasses.

Eleven acoustic parameters derived from the 38 and 418 kHz signals were submitted to a novel multi-pass Discriminant Function classification scheme to refine the training dataset into end-member structural and biological elements. The majority (approximately 80%) of acoustic classifications were of soft bottom sediments (Classes 1-2), but there were two significant expanses of rough seabed thought to be suitable for macroalgae attachment. These two areas covered a total of 19 km², within which approximately 56% of the hydroacoustic ‘records’ were classified as “rough” (Classes 3, 4 and 5). The first was a large area of seagrass beds and “live hard-bottom” in the mouth of San Carlos Bay, where large amounts of macroalgae were variably present during the April-May 2009 surveys. The second was offshore of Lighthouse Point, near the mouth of San Carlos Bay. This area is located near a large sand bar that extended from the beach to approximately 6 km offshore. Along the west side of this sandy area was substantial acreage of moderate to high bottom “roughness”, mostly in the form of unconsolidated, shelly hash. The average depths of these two acoustically-rough areas were only 5.0 and 4.0 m, so sufficient irradiance to initiate a macroalgal ‘bloom’ would be likely much of the year. These textured and shallow areas on or near the mouth of San Carlos Bay are presumably potential source areas for the initiation of macroalgal biomass (attachment and growth). Under the appropriate conditions algae could be readily transported onto the areas’ beaches, especially given the close proximity to the islands beaches.

In contrast, the areas further offshore in the Gulf of Mexico were classified predominantly as soft sediments with low bottom “roughness” based on both the hydroacoustic and video surveys. The area offshore of Redfish Pass had a moderate (approximately 22%) proportion of “rough” acoustic classifications out to 5 km offshore, but from 5-10 km offshore the bottom was classified as >95% soft sediments. The other two Gulf of Mexico areas of focus were acoustically classified as >95% soft sediments from nearshore to 11 km offshore. The towed-video transects over a larger area of focus indicated there were relatively small areas that harbored large concentrations of shelly and/or “live hard-bottom” occurring sporadically at

distances greater than 10 km offshore. Further assessments of these survey data in the context of available nutrients and ambient light levels will be needed to fully assess the bloom potential for these offshore sites, but it would appear that the open Gulf of Mexico waters around Sanibel-Captiva are probably not a major source of drift macroalgae.

The following tasks were addressed chronologically to meet the overall goal for this objective:

Phase I (2008): Preliminary Studies On-site: Plan Development (Initial site visit)

Task 1: Work with contractors during their assessment using hydroacoustic and video to meet the overall project objective.

Task 2: Work with contractors to provide the best approach(es) for concurrent hydroacoustic and underwater towed- video mapping.

Phase II (2008-09): Seafloor Mapping Around Sanibel-Captiva Islands

Task 3: Seafloor mapping along multiple transects in the vicinity of Sanibel-Captiva Islands and off Fort Myers Beach (i.e. portions of Lee County).

Task 4: Collaborative assessment of video imagery, hydroacoustic data with ground-truthing.

Phase III (2009-2010): intensive mapping of additional areas

Task 5: Seafloor mapping at additional sites using an adaptive approach.

Task 6: Collaborative assessment of video imagery, hydroacoustic data with ground-truthing by video and diving.

The hydroacoustic data collected during the October 2008 (Phase I) and April-May 2009 (Phase II) surveys were post-processed using the same methodology. Therefore, the hydroacoustic methods for Phase I and II are presented together below as a single study. The towed video methods for Tasks 1–4 are presented following the hydroacoustic information.

Hydroacoustic Study Area (Phases I and II)

Hydroacoustic surveys were conducted in the nearshore waters of Sanibel Island, FL. Six areas encompassing the local diversity of benthic habitats were surveyed, including sites offshore Sanibel Island and within San Carlos Bay. The surveys were conducted in two segments; a methods-development exercise conducted from October 6 to 8, 2008 (Phase I, Tasks 1-2) and a larger-scale survey on May 10-22, 2009 (Phase II, Tasks 3-4). Three areas were surveyed in 2008; a 7 x 2 km plot alongshore Lighthouse Point (400 m spacing), a 6 x 1 km plot offshore Redfish Pass (200 m spacing), and a 1,500 m meander through seagrass in Pine Island Sound (Fig. 4.2). Five areas were surveyed in 2009; an additional 7 x 2 km plot adjacent to the 2008 Lighthouse Point plot, a 10 x 5 km plot offshore Fort Myers Beach (1600 m spacing), a 10 km transect offshore Tarpon Bay Road, a 9 x 1.6 km plot offshore Dinkins Bayou (800 m spacing), and a 9 x 3.2 km plot within San Carlos Bay (800 m spacing).

Hydroacoustic Survey (Phases I and II)

The survey was conducted from a 7.5 m v-hull boat with a 0.5 m draft, an average net speed of 4.5 knots (vessel plus drift). Hydroacoustic data was acquired with a BioSonics DT-X

echosounder and two multiplexed, single-beam digital transducers with full beam widths of 10° (38 kHz) and 6.4° (418 kHz), operated at 5 Hz sampling frequency and 0.4 ms pulse duration (see Figs. 4.1 and 4.3). The 38 and 418 kHz transducers ‘ensonified’ a roughly-circular area of seabed with diameters equal to approximately 17% and 11% of water depth, respectively. Global positioning data were collected with a Trimble Ag132 dGPS, differentially corrected against a WAAS signal to achieve positioning accuracies less than 0.9 m horizontal dilution of precision. The dGPS signal was interfaced with navigational software to provide real-time monitoring of vessel position with respect to geo-referenced imagery and pre-planned survey lines.

Hydroacoustic Processing (Phases I and II)

Hydroacoustic data were processed with BioSonics Visual Bottom Typer (VBT) seabed classification software (Version 1.0) to obtain values of E1' (time integral of the squared amplitude of the leading edge of the 1st echo waveform), E1 (2nd part of 1st echo), E2 (complete 2nd echo), E0 (pre-bottom backscatter of 1st echo), and FD (or “fractal dimension”, a measure of shape irregularities of 1st echo waveform) (see Fig. 4.1), as per Burczynski (1999). The E1' setting was adjusted so that E1 would capture only the trailing edge of the first echo, maximizing its sensitivity to scattering components of the seabed. Other user-defined settings include; time-varied gain = $20\log R$, minimum data processing threshold = -80dB, 5 pings per report, and energy filter = 50%. VBT computes FD as the Hausdorff dimension of the first echo (e.g., Mandelbrot, 1982), simplified by gridding the waveform into ‘box’ dimensions (e.g., Lubniewski and Stepnowski, 1997). The acoustic energy parameters E0, E1', E1, and E2 were log-transformed to improve normality (e.g., Sokal and Rohlf, 1981).

Normalizing to Reference Depth. The current version of VBT does not normalize echo length to a reference depth, i.e. adjust the sampling rate to effectively adjust the width of E1' and E1 bottom sampling windows (in units of samples) to maintain a consistent first echo division as the echo stretches and flattens with increasing depth (e.g., Dommissie et al., 2005). Purely from the standpoint of echo length, E2 should not require normalization to a reference depth provided the bottom sampling gate is adequately wide to capture the entire second echo across the range of depths. However, all acoustic parameters except E0 were significantly correlated with depth. To ameliorate the effect of depth contamination, the log-transformed acoustic energy parameters and FD were empirically normalized to median survey depth. The raw VBT output of all survey sites dominated by unconsolidated sediment (e.g., Redfish Pass) were combined and sorted by depth. Median values of acoustic parameters were then computed for each block of 1,000 records. These sites were characterized by relatively flat, sedimentary, uncolonized seabeds, which made it reasonable to assume that depth was the main factor affecting echo shape. This data was then binned into 18 ranges of depth and logarithmic polynomials were fit to each of the acoustic parameters (Fig. 4.4). Depth-normalization factors were applied to each hydroacoustic record, calculated as the ratio of model-predicted acoustic energy at actual depth divided by the model-predicted acoustic energy at median survey depth. The E1 of both frequencies had

inflection points around 2 m, with very steep slopes on the shallow side of the curve (see Fig. 4.4). To avoid the high degree of uncertainty associated with the inflection point and steep slope, the depth-normalization models were constrained to depths greater than 1.75 m, and survey data shallower than 1.75 m were rejected during quality analysis.

Quality Analysis. The log-transformed and depth-normalized hydroacoustic survey records were subjected to a series of QA filters to identify and remove “irregular” hydroacoustic returns. The following QA process was conducted individually for each survey site so as to emphasize removal of anomalous within-site records, so as not to key on genuine between-site variation. The first filter checked the differential depth between successive pings, removing waveforms that contacted the seabed at shallow angles, typically caused by excessive vessel roll. The next filter removed records with depths less than 1.75 m or greater than the 99.5 percentile. The 99.5 percentile setting rejected the anomalously deep records within a survey site, which are frequently the result of misshapen waveforms. The final filter addressed outliers by removing records for which any of the ten acoustic parameters fell beyond either the 1 or 99 percentile.

Hydroacoustic Training Dataset. 62 ground-validated hydroacoustic samples were collected within the study area for the training dataset, collected with the vessel drifting in idle (Fig. 4.5a). Each sample consisted of 30 seconds of concurrent hydroacoustic and video files, acquired with a drop camera trailing just behind the ‘ensonified’ area. Videos were reviewed post-survey and assigned visually-apparent areal cover of structural (e.g., mud, sand, shell, hard bottoms) and biological elements (e.g., turfy algae, upright macroalgae, seagrass, corals (e.g., scleractinians, alcyonaceans), bivalves (e.g., pen shells), and polychaete worms. Areal cover was used to assign training samples into one of five categories of visually-apparent seabed roughness, ranging from low to high and intended to represent the potential for macroalgae attachment (Table 4.1, Fig. 4.6). For example, the “low” roughness areas (e.g., Classes 1 and 2), mainly represented by smooth soft sediment (mud/sand mixtures) with little or no shell or small rocks, had little hard substrate suitable for attachment and growth of macroalgae. In contrast, the “high” roughness areas (e.g., Classes 4 and 5), represented by hard, rocky bottoms or seagrasses, had substantial amounts of substrate suitable for macroalgae.

Multivariate Classification. Discriminant analysis (DA) is an eigenanalysis technique (i.e. matrix-based) that determines the linear combination of independent variables that maximizes discrimination between predefined groups. The supervised classification workflow was divided into four major segments: (1) a series of multivariate analyses [Principle Components Analysis (PCA) → K-means clustering → multidimensional scaling (MDS)] to refine the assignment of each training sample to one of the five classes, and to remove outlying hydroacoustic records; (2) an exploratory DA to arrive at the final class assignment of training samples, and to reject training samples that did not conform to their assigned class; and (3) a series of three descriptive DA’s to refine the training dataset into end-member records and produce a set of classification functions, and (4) a predictive DA to classify survey records using the classification functions of

the 3rd-Pass descriptive DA (Fig. 4.7). The eleven independent variables (38 and 418 kHz log E0, logE1', logE1, logE2, FD, and 418 kHz depth) were entered stepwise into the DA with prior probabilities of group membership computed from group size.

PCA + K-means + MDS of Training Dataset. The overall 62 hydroacoustic samples comprising the “training dataset” were submitted to the same series of QA filters as the survey data. Next, the individual 38 and 418 kHz datasets were merged into a single dataset (57 of the original 62 samples remained after QA/Merge). The final form of the training dataset was arrived at using a series of multivariate techniques, in which some samples were rejected outright and others were re-assigned to another acoustic bottom class (Fig. 4.7). First, the eleven independent variables were standardized by $(x-\mu)/\sigma$ for equal weighting and submitted to a PCA. The first 10 Principle Components (PC), accounting for 98.3% of variance, were submitted to a K-means clustering algorithm to separate the training data into 16 clusters (a number arrived at by trial and error). Records belonging to disproportionately small clusters were regarded as outliers and removed from the training dataset. The remaining records were then submitted to another PCA+K-means analysis (again, $k = 16$). For each of the 57 training samples the proportion of records falling into each of the 16 clusters was computed (hypothetically, a training sample might have 25% of its records fall into cluster 4, 25% into cluster 12, and 50% into cluster 16). This matrix (columns = K-means cluster membership, rows = training samples) was submitted to an MDS analysis (obtained from a Bray-Curtis similarity matrix). Training samples judged to be outliers in the 2D MDS plot were rejected outright (i.e. samples that not only grouped apart from other samples of the same bottom class, but also from the samples as a whole). Training samples were re-assigned if (1) they were located among another bottom class in the 2D MDS plot, and (2) the initial visually-apparent class assignment could reasonably be overturned to the class indicated by the MDS plot.

Exploratory DA. The final arrangement of the training dataset was achieved by an exploratory DA, using the post-MDS training dataset. As a check against the number of subjectively chosen *a priori* groups, an approximation of the optimum number of groups was obtained using one of the many stopping rules developed for clustering algorithms. Milligan and Cooper (1985) reported the variance ratio criterion (VRC) of Calinski and Harabasz (1974) to be amongst the best performers in a simulation study of 30 stopping rules. To compute the VRC, the first 5 PC of the last PCA analysis were submitted to K-means cluster analysis. For each value of k , the VRC was computed as the maximum between-cluster variance divided by the minimum within-cluster variance. Calinski and Harabasz (1974) suggest that the first local maximum of VRC is an informal indicator of the optimal value of k .

Descriptive DA. The post-exploratory-DA training dataset was submitted to a series of three descriptive DAs to: (i) refine the heterogeneous training samples into “pure” structural and biological elements; (ii) examine how the independent variables contribute to discrimination between groups; and (iii) generate a set of Fisher’s linear discriminant functions (FLDF) for

classification of survey records into one of the five pre-defined bottom classes (Fig. 4.7). Training records submitted to the 3rd-Pass descriptive DA were tested for critical DA assumptions because: (a) ecological data frequently violate DA assumptions (e.g., Williams, 1983); (b) it is useful to assess the discriminatory power of individual hydroacoustic variables, judging by the canonical functions obtained from the descriptive DA; and (c) unequal variance-covariance matrices distort plots of canonical functions (e.g., Lachenbruch et al., 1973; Krzanowski, 1977; Wahl and Kronmal, 1977; Williams, 1982).

The assumption of normal multivariate distributions was assessed by ratios of ‘skewness’ and ‘kurtosis’ to their respective standard errors. Homogeneity of variance and covariance was assessed by comparison of between-group variances and similarity of log determinants, respectively. Significance of the Discriminant Function Analysis (DA) was tested by a Chi-Square transformation of the Wilks’ Lambda score. The critical DA assumption of mutual exclusivity of groups was impinged upon by “mixed” training samples acquired over heterogeneous benthos. This violation was addressed by extracting end-member records from the mixed training samples in a series of three descriptive DAs. Only records that: (1) correctly classed by the Discriminant Analysis; and (2) exceeded a minimum probability of group membership were passed onto the next DA. This process also removed any remaining outliers, to which DA is particularly sensitive. Outliers frequently result from intrusion of environmental and hardware factors, e.g., ship wakes, excessive pitch and roll, co-mingling of echoes, and can be separated from the main data cloud by orders of magnitude.

Predictive DA. Discriminant analysis generates a set of Fisher’s linear discriminant functions (FLDF) for each group, based on the linear combination of independent variables providing the best discrimination between groups. The FLDF from the 3rd-Pass descriptive DA of the training dataset were used to classify survey records (Fig. 4.7). For each record, group scores were computed as the sum of the product of FLDF coefficients and independent variables plus a constant. Records were classified as the group with the largest score.

Accuracy Assessment. An external accuracy assessment was conducted using only samples that were not included in the training dataset, since predictive accuracy will always be greater using the training dataset than for a new dataset (e.g., Kachigan, 1986; Huberty, 1994). A total of 117 ground-validation samples were collected inline with the survey by intermittently slowing to idle speed, deploying a weighted video camera overboard, and simultaneously recording sonar and video for a period of 30 to 60 seconds. The Trimble dGPS latitude and longitude and UTC time were burned onto the recorded video for post-survey synchronization with hydroacoustic data. As with the training dataset samples, videos were reviewed post-survey and assigned visually-apparent areal cover of structural (mud, sand, shell, hard bottom) and biological elements (e.g., turf, macroalgae, seagrass, scleractinians, alcyonaceans, pen shells, and polychaete worm tubes). Areal cover was used to assign training samples into one of five categories of visually-apparent seabed roughness (Table 4.1).

The ground-validation data was subjected to the same VBT post-processing, depth-normalization, and quality assurance as described previously for the survey data. Of the 117 in-line ground-validation samples collected, 89 remained for accuracy assessment. These 89 samples were constituted of a total of 3,398 individual hydroacoustic records (approximately 45 records per sample). Each of the 3,398 records was classified into one of the five bottom classes of Table 4.1 using the same Fisher's linear discriminant functions that used to classify the survey data. The ground-validated class of each of the 89 samples was then computed as the mode of the DA-predicted class.

The overall accuracy, producer's accuracies, and user's accuracies were computed directly from a confusion matrix of ground-validated (columns) versus DA-predicted (rows) classifications (Story and Congalton, 1986). The overall accuracy (P_o) was calculated as the sum of the major diagonal (i.e. correct classifications, divided by the total number of ground-validation samples). Overall accuracy was adjusted to the number of groups using the Tau coefficient for equal probability of group membership, T_e (e.g., Ma and Redmond, 1995). Tau is a measure of the improvement of the classification scheme over a random assignment of samples to categories, bounded between -1 (0% overall accuracy for 2 map categories) and 1 (100% accuracy for any number of categories). Each diagonal element was divided by the column total to yield a producer's accuracy and by the row total to yield a user's accuracy. The producer's and user's accuracies provide different perspectives on classification accuracy. The producer's accuracy (errors of omission/exclusion) indicates how well the map classified a particular category (i.e. the percentage of times that substrate known to be Class 1 was correctly classified as "Class 1"). The user's accuracy (commission/inclusion error) indicates how often map categories were classified correctly (i.e. the percentage of times that a sample classified as Class 1 was actually Class 1 and not one of the other four classes).

Underwater Towed-Video (Phase I)

In addition to the video imagery obtained in conjunction with sonar data and used for ground-truthing and seabed classification (as described above), video imagery was obtained independently in two areas following the general methods of Grizzle et al. (2005, 2008 and Fig. 4.2). This was done for two major reasons. First, to compare seabed classification schemes resulting from expert classification of video stills along continuous transects with the along-track seabed classifications derived from hydroacoustics. Secondly, to determine what role independently collected video might play in conducting Phase II studies; more specifically, the aim here was to determine if video could be used independently to expand the scope of the project by surveying areas further offshore than the hydroacoustic vessel could safely navigate. This would allow greater spatial coverage of the survey while staying within the original budget. Field surveys were conducted on October 2, 3, and 6, 2008 using a SeaViewer Model 550 color video camera deployed in towed mode on a custom-designed stainless steel sled, with GPS data and video imagery continuously recorded. A Garmin (Model 76CSx) GPS unit was used with WAAS mode activated, which typically yields positional accuracy of 3-5 m. A diver was also

deployed in several areas to obtain samples of benthic organisms and information on seabed characteristics not directly discernable in the imagery.

Underwater Towed-Video (Phase II)

Independent towed video mapping was conducted over the period April 26 – May 8, 2009. The video surveys included San Carlos Bay but focused on areas greater than 10 km offshore. A total of sixty-three transects ranging from about 200 m to greater than 1 km in length were recorded in 2009. The imagery was processed and classified to provide as much detail as possible, focusing on information relevant to assessing the potential for macroalgal growth and production.

Collaborative assessment of video imagery, hydroacoustic data with ground-truthing by video and diving. (Phase III)

The overall aim of the two final objectives was to test the accuracy of previous mapping products (Tasks 3 and 4) by sampling for the presence/absence of seasonal drift macroalgae at a time when the probability of a bloom was high. Underwater video imagery (see Task 1 for methods) was obtained May 25-27, 2010 along a total of ninety-two (92) short (typically <100 m in length) transects in three general areas that had been previously mapped: offshore of Sanibel Island from Redfish Pass to near Lighthouse Point; offshore of Estero Island; and in San Carlos Bay. The bottom was also inspected by snorkeling at sites where additional information was needed on bottom type or composition. Sample points were assigned by random stratification to three levels of irradiance-modified acoustic bottom classes. Phase I and II hydroacoustic records were adjusted to bottom depth by application of an irradiance modifier to the acoustic bottom roughness (Classes 1-5, Table 4.1) classification. Three levels irradiance modifiers, based on the quantity of subaerial irradiance at depth, were selected. In ascending order of available irradiance at depth, Level 1 = 0-33%, Level 2 = 33-66%, and Level 3 = 66-100%. For each hydroacoustic record, irradiance at depth was computed from the measured acoustic depth, assuming a vertical attenuation coefficient of 0.12 m^{-1} ; on this basis, level 1 equated to bottom depths greater than 9m, level 2 to 3.5 - 9m, and level 3 to 0 - 3.5m. The numerical value of acoustic class (1-5 in ascending order of bottom roughness) was multiplied by the irradiance modifier (1-3 in ascending order of available irradiance) and binned into three levels (bin 1 corresponds to the lowest combination of roughness and irradiance, bin three the highest).

Objective 5: To determine the distribution and productivity of attached macroalgal biomass and the conditions that are favorable for uncontrolled growth, including the examination of substrate attachment. (Milbrandt, Parsons, Ceilley and Coen)

Distribution and abundance of macroalgae

Thirteen stations were established in June 2008 to conduct quantitative sampling of macroalgal communities, to collect sediment cores (Objectives 1, 2), and to collect water samples for nutrient analyses (Objective 1). Stations were visited bimonthly for a total of 12 sampling events

concluding in June 2010. The study area included the area North from Captiva Pass to the southern boundary of Wiggins Pass (Fig. 5.1). Stations were established inshore and offshore, from Sanibel down to Fort Myers Beach in an effort to address gradients related to nutrients and freshwater (decreasing away from the Caloosahatchee River) and to ensure locations near Sanibel and Fort Myers Beach were included. Given the lack of information about macroalgae, a diversity of habitats was sampled to ensure a broad, yet thorough examination of the benthic habitats. Several adjustments were made to increase the diversity of the habitats encountered and to improve the overall characterization of the region. Information surfaced to indicate that areas with seagrass trapped macroalgae (Riegl et al., 2005b) in the Indian River Lagoon. Station GOM16 (Fig. 5.1) was established in January 2009 to sample a patchy seagrass habitat, while several stations near the Sanibel lighthouse were discontinued because of overlap in habitat types with existing stations. Generally, the northward and southward transects were paired; there were sampling stations nearshore (n = 6) and offshore (n = 5). A station in the lower estuary was used previously by several of the other investigators (CES11, Site 4 in Objective 1 and 2). Nutrient and sediment samples were also collected upstream in the Caloosahatchee (Objectives 1 & 2), however, the focus of Objective 5 was the lower estuary and offshore regions.

Stations were visited bimonthly to conduct quantitative sampling of the macroalgal communities and to characterize the seafloor habitats (Figs. 5.2, 5.3). At each station, a 100-m lead-core, transect line was deployed parallel to shore. Samples were collected from 20 random quadrats placed along a 100-m transect line. Sediment samples were collected at the end of each dive. A non-rigid one m² quadrat was used to visually estimate percent cover within the quadrat and then all attached macroalgae was collected and placed into a mesh bag (part # 2534725, West Marine). Once all of the attached macroalgae was collected within the quadrat (Lobban and Wynne, 1981), the densities of macroinvertebrates were determined. Whenever possible, a voucher organism was brought to the surface and identified. Macroalgae was transferred to Ziploc™ bags (for identification and tissue nutrient analysis) or to 50 ml centrifuge tubes containing ambient seawater (for PAM fluorometry analysis). The Ziploc™ bags were stored on ice and the centrifuge tubes were stored at ambient temperatures in the shade for transport back to the laboratory. Upon returning to the laboratory, Ziploc™ samples were stored at 4°C and sorted and identified within 2 weeks of collection. The centrifuge tube samples were examined within four hours of delivery to FGCU (see PAM fluorometry below).

Macroalgal samples were first sorted by morphotype, and then identified to the lowest taxonomic level using Dawes and Mathieson (2008) primarily, but also Littler and Littler (2000). Voucher specimens were preserved in 5% formalin in seawater and sent to Dr. Clinton Dawes (University of South Florida), as needed. Dr. Dawes produced three reports (see Appendix 5.2). Additional specimens were catalogued at the laboratory by pressing and drying on herbarium paper (part # 663211, Carolina Biological Supply).

Standing macroalgal biomass (Kain, 1975; Holm, 1978; Zeiman et al., 1989) was used to quantify relative macroalgal species abundances. After sorting and identification to the lowest taxonomic level, fresh weights were determined with a Mettler-Toledo Analytical balance (model # AG204). Samples were dried to constant weight at 60°C in a Fisher Isotemp 500 Series drying oven (model # 516G, Fisher Scientific Inc.) and reported in g DW m⁻². All macroalgal data, including the sample ID number and the morphological characteristics used to identify a given specimen (e.g. internal cell structure, X-section morphology, branching pattern, and morphology) were entered into a dedicated Microsoft Access 2003 database. Once dried, a sub-sample of each species was reserved for stable (C, N) isotope analyses (see below).

For each station, individual graphs of monthly mean total biomass (g DW m⁻²) over time for all species (cumulative sum in a given quadrat) were plotted. Fresh weight (g FW m⁻²) and dry weight biomass (g DW m⁻²) for each species were summed for each quadrat along a given transect. Mean percent cover per quadrat was also plotted for each station (n = 20), as recorded by divers swimming along each transect. Individual species plots were generated with SigmaPlot (ver. 10.0) for those species that were found on area beaches by SCCF or previous investigators.

In order to compare species composition by station, the relative percent contribution of each species to the total transect biomass by station and date is displayed as a series of pie charts (SigmaPlot, Ver. 10). Biomass of individual species collected from a given transect were used to conduct a multivariate analysis using PRIMER (Ver. 5). Unidentified samples and other algae collected non-quantitatively were excluded. A Bray-Curtis similarity analysis on square-root transformed biomass was projected into two-dimensional space using multi-dimensional scaling (Clarke and Warwick, 2001). Each sample containing algae was assigned to a group in PRIMER and was a designation based on the location and whether or not there was abundant algae typically collected at that location (e.g. abundant, offshore; abundant, inshore; rare/absent, offshore; rare/absent inshore; rare/absent nearshore). Diversity measures were calculated (PRIMER, Ver. 5) including Shannon-Wiener diversity index (H') and the total number of species for each station was tallied. Additionally, the physical data were related to the species composition data using the BIOENV function in PRIMER (ver. 5). This procedure uses a Spearman rank coefficient to compare the physical data to a similarity matrix generated from the species and biomass as explained above by the Bray-Curtis analysis.

Underwater Video Analysis

Analysis of underwater video for the determination of seafloor cover has been used as a low cost option for large scale sampling (Leonard and Clark, 1993). The advantage of utilizing underwater video is that the entire transect can be analyzed frame-by-frame, where divers are limited to sub-sampling. This analysis increases the area sampled significantly, thus allowing for characterization at higher spatial resolution. Video of the entire length of the 100 m transect was recorded using a camcorder system (model # DCR-TRV 950 Sony Corp.) equipped with an

Amphibico Dive Buddy Plus 950 underwater housing, which included a laser scale (15 cm spread, waterproof underwater dive laser, Beam of Light Technologies, Inc., Clackamas, Oregon) mounted to the underwater lights (Oceanoptics Inc., Cape Coral). A fixed-length aluminum rod was attached to the camera housing for a 53 cm distance from the lens to the seafloor. The videographer swam at approximately 0.16 m per second, or 10 m per minute. Upon returning to the lab, the videos were transferred from the MiniDV tape to a memory drive via Pinnacle Studio software (ver. 12). Exported files then had date and time stamps manually added using the program vDTS. Videos were then transferred back to Pinnacle, where title frames and notes were added to each video file corresponding to date and tape number. Videos were saved as MPEG-1 files. Individual videos were stored, and backed-up to a 1 TB hard drive, titled by date of trip. Backup DVDs were also created to ensure data would not be lost. Using Corel™ software, fisheye lens distortion was examined, but because of minimal distortion, frames were not converted prior to analysis.

While identification of individual macroalgal species was generally not possible from the underwater video, generalized morphological types or functional groups were distinguishable, as were the invertebrate densities (eg. urchins, penshells, corals). A functional group analysis was performed using a modified approach from Steneck and Watling (1982). This type of aggregation is often used when simplifying ecological studies that utilize a large number of individual species. However, instead of characterizing according to herbivore preference and characteristics of mouth parts, macroalgae biomass samples were characterized using groups separated by observable morphological characteristics. Species were grouped according to the following specific morphologies: (1) filamentous alga species included those species that were deemed morphologically “filamentous”, generally not having much vertical height, and were finely branched including algae like *Polysiphonia* spp. and *Hincksia* spp.; (2) “corticated” algae species had a more highly branched morphology, with greater vertical height, and contained algae like most of the *Gracilaria* sp. and *Hypnea* sp.; (3) “foliose” algae species were generally firmer and flattened, and included the *Caulerpa* sp.; (4) “flattened” algae species that were more finely branched and flattened, and included primarily *Dictyota* in this analysis; and (5) “leathery” algae were grouped according to height and thickness of the thallus and included primarily the *Sargassum* species found. Functional analysis was also used to characterize collected specimens for a cross method comparison. While individual species could not be identified, general morphological characteristics were determined using the functional groups outlined above as a general guide.

Area analysis with video is an accurate, alternative option for analysis of percent cover of benthic characteristics (Tkachenko, 2005). Preliminary analysis included evaluation by three methods, specifically by two programs, Vidana[©] and Coral Point Count with Microsoft Excel Extensions (CPCE) from National Coral Reef Institute (NCRI), as well as a grid analysis technique using Corel™ options to overlay the grid (Fig 5.5). The Vidana[©] program utilized a point count technique, after overlay of colored polygons. After outlining the species of interest,

one hundred points were over-laid and presence/absence of the original colored polygons are tallied by the program, and presented as percent cover. CPCE included the option of using a calibration to a known distance to calculate actual areas. Calibration by using the lasers to find pixels per cm was performed (15 cm between the lasers, an average of 10 pixels per cm) and species of interest were outlined. CPCE then calculated the area based on the previous calibration. The grid option available using the Corel™ PaintShop software overlaid a grid on the JPEG file that effectively split the area into a 108 square grid (12 x 9 squares). The grid was then used in a similar manner as the diver quadrats for analyzing percent cover. If there was macroalgae within one square, it was tallied as present, if no algae were seen inside the box, their absence was noted. Each program required that the video file be broken into individual frames as JPEGs before analysis. Using Pinnacle (Ver. 12) software, each video was broken down into JPEG files. A total of 21,600 frames were analyzed, with an average of 150 frames per transect. The video frames had a viewing area of 0.7 m² (average 72 X 68 cm rectangle).

All three programs were used to analyze 300 identical video frames. A One-Way ANOVA was performed with total percent cover as the dependent variable and software type as the independent variable. The COREL program had significantly higher values for percentage cover ($p < 0.001$). Point count techniques have been shown previously to overestimate percent cover, as well as presence/absence determination (Leonard and Clark, 1993). CPCE and Vidana were not statistically different, however CPCE was the most conservative for mean percent cover (median of 40.5% CPCE; vs. 44.0% Vidana). Therefore, CPCE was picked as the final means of analyzing data because of the more conservative cover estimates.

Growth Rates

Growth rates were estimated from the rate of increase in total biomass. The following formula was used to determine daily growth rates (DGR) following Dawes (1998):

$$\text{DGR} = \frac{(w_f - w_i)}{\text{day}} \times 100 \quad (\text{Eq 5.1})$$

where w_i is the initial weight; w_f is the final weight.

Opportunistic Collection of Beach-stranded Macroalgae

While collecting stranded algae on the beach was not an explicit component of this objective, there were several opportunistic beach collection events. Beginning in January 2008 (before the contract was signed), several trips to Sanibel beaches were made to collect and subsequently identify macroalgal species. A timeline explaining the opportunistic beach sampling can be found in the Appendices. Samples were collected non-quantitatively from Fort Myers Beach and elsewhere in May 2010 during a beach stranding event.

Measured Detachment Force

Given that attached macroalgae are likely the major source of the stranded algae on the local beaches, a determination of the force needed to detach an algal thallus from the predominant

substrates was conducted. Several worm tubes (*Diopatra cuprea*) were collected in July 2009 from CES11 where there was abundant macroalgae attached to the worm tubes. A hammer and chisel were used to collect limestone with attached algae from GOM12. A set of 3 Pesola spring scales (No. 93009, Forestry Suppliers, MS.), of varying spring tension were used in combination with a modified clip. The clip was modified with rubber to prevent physical damage to the thallus from the clip. The clip was attached to the base of the thallus on the primary branch of the alga. The spring was pulled vertically until the alga detached from the substrate. The detachment force in g was converted to Newtons (N) with the assumption that the rate of acceleration of gravity was 9.8 ms^{-2} . The detachment force along with the identity of species and other notes were recorded. All species were pooled for the analysis. A one-way ANOVA was used to test whether the differences in mean detachment force were significant. For all statistical tests, significance was accepted at $p < 0.05$.

Physical parameters

Surface and bottom water quality data were collected at each station, including DO (mg/l and %), temperature, specific conductance, pH, depth, salinity and turbidity with a Hydrolab Quanta with turbidity (Model # QD00202, Hydrolab Quanta, Austin, TX). Hydrolab sensors were calibrated using Hach calibration standards (Hach Company, Loveland, CO) at the beginning of each sampling event. Filtered water (0.2 μm) and 40 NTU Formazin Turbidity Standard (Hach, Model # 2461-49) were used for a two point calibration of turbidity. A two point calibration was also used for pH with 7 and 10 buffer solutions (Hach Model # 2283656 and 2283556). Distilled water and 47.6 mS cm^{-1} conductivity standard (Hach Model # 013650HY) was used to calibrate conductivity. Dissolved oxygen was calibrated in water saturated air on the bench.

Downwelling scalar irradiance (I_z) was measured at two depths using two, 2π irradiance sensors (Biospherical Instruments Inc., San Diego, CA) mounted at 0.64 m depth interval (Fig. 5.4). The sensors were submerged for at least 30 s at each station, recording at 1 Hz. Whenever possible, light was collected between 1000 and 1400 when the sun angle was at a maximum following recommendations by Steward et al. (2005). Data was downloaded into a Microsoft Excel spreadsheet via DeLogger Lite software (Biospherical Ver. 2.2.0.0), upon returning to the lab. Downwelling scalar irradiance (I_z) for each sensor was averaged for 20 s, where the first and last 5 s are trimmed to avoid error associated with instrument deployment and retrieval. Light attenuation coefficients were calculated using Beer's Law (Kirk, 1994). Using Beer's Law (Eq. 5.2), the unitless $K_{d\text{PAR}}$ values are reported. Higher values indicate greater attenuation (scattering/ absorption).

$$\ln(I_z(\text{deep})/I_z(\text{shallow})) * (z_2 - z_1) = K_{d\text{PAR}} \quad (\text{Beer's Law}) \quad (\text{Eq 5.2})$$

The irradiances (I_0) from the rooftop sensor, the $K_{d\text{PAR}}$ measured at each station, and depth (z) were used in Beer's Law to solve for I_z at the seafloor. Rooftop irradiances were preferred because they represented the light field for that time of year and were not influenced by the particular time of day or sea state. The average irradiance ($n = 5$) between 1000 and 1400 for

the sampling date were used to calculate the irradiance at depth. Percent light at depth was calculated as the product of the incident solar radiation (I_0) recorded from 1000 to 1400 on the sampling date, K_{dPAR} , and the depth recorded at each station using Beer's Law (Eq 5.2).

All physical parameters were correlated to macrophyte biomass and percent cover using a Pearson correlation analysis. The coefficients along and significance are reported.

Biological Parameters

Observations of the epifaunal benthic community were recorded within each quadrat. Invertebrate macrophytes were enumerated into broad categories, such as pen shell, short spined urchin, etc. Additional notes were recorded describing the seafloor sediments and other features. Voucher specimens were collected as needed and confirmed identity back at the lab or on the surface. Pearson correlations were used to relate macroalgal biomass and percent cover to the densities of the macrophytes (SPSS Ver. 13).

PAM Fluorometry Analysis

The Phyto-PAM measurements were using the following procedures. All macroalgae collected in centrifuge tubes were kept in the dark in a cooler for at least 4 hours after collection (including on the boat) to dark-acclimate the algae prior to PAM fluorometric analysis. Dark acclimation is necessary to obtain accurate estimates of F_m (Maxwell and Johnson, 2000). Triplicate readings were made on each algal sample analyzed. Three separate fragments (approximately 2 g wet wt) were removed and individually inserted into quartz cuvettes (46 mm H x 15 mm OD) containing approximately 3 ml of instant ocean seawater of comparable salinity. The cuvette containing each fragment was placed in a Phyto-ED Emitter-Detector unit attached to a Walz Phyto-PAM Power Control Unit. Once background fluorescence (F_0) readings stabilized (after approximately 10 seconds), the saturating pulse of light was applied to measure F_m . The Phyto-PAM software then calculated QY. F_0 , F_m , and QY values were recorded for each sample in an MS Access 2007 database, along with pertinent station, sample collection date, and species identification information. Algal samples were identified after PAM fluorometric analysis, typically within two weeks of collection. Identification was based primarily on Dawes and Mathieson (2008) with supplemental reference to Littler and Littler (2000) as needed.

Stable Isotope and Tissue Nutrient Analysis

A subset of algal samples was selected for stable isotope and tissue nutrient analysis. Sample selection was based on those species having the best spatial coverage for any given sampling event or with the best temporal coverage at any given location. Efforts were made to select three replicate algal samples whenever possible (e.g., three *Gracilaria mammillaris* samples from GOM12 in March 2010). Additional samples were chosen to study stranding events and to monitor algal responses to water releases through Franklin Lock.

Once samples were selected, they were acidified and ground following procedures outlined in Parsons et al. (2008). Three to 10 mg of dried, ground algal sample was weighed out into tared

Costech tin capsules (Part # 041061) on a Mettler microbalance. Replicate subsamples were also weighed out in the same manner. Sample identification information and weights were recorded on an Excel spreadsheet, which was then sent to the Stable Isotope Facility at the University of California at Davis for analysis along with the samples.

At the Stable Isotope Facility, samples were analyzed utilizing their established protocols outlined below. Solid materials were analyzed for ^{13}C and ^{15}N isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (irMS; Sercon Ltd., Cheshire, UK). Samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered cobaltous/cobaltic oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flowed through a water trap (magnesium perchlorate) and an optional CO_2 trap (for N-only analyses). N_2 and CO_2 were separated on a Carbosieve GC column (65°C , 65 mL/min) before entering the irMS.

During analysis, samples were interspersed with several replicates of at least two different laboratory standards. These laboratory standards, which were selected to be compositionally similar to the samples being analyzed, have been previously calibrated against NIST Standard Reference Materials (IAEA-N1, IAEA-N2, IAEA-N3, USGS-40, and USGS-41). A sample's preliminary isotope ratio was measured relative to reference gases analyzed with each sample. These preliminary values were finalized by correcting the values for the entire batch based on the known values of the included laboratory standards. The data generated from these analyses included $\delta^{13}\text{C}$, %C (% of dry wt), C content (mg dry wt), $\delta^{15}\text{N}$, %N (% of dry wt), and N content (mg dry wt). Atomic C to N ratios were calculated by dividing the C content by 12 amu and N content by 14 amu. Stable isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and C:N were used in subsequent analyses.

Any material left over from the above analysis was subjected to tissue phosphate analysis to provide additional data on the nutrient content of the algae. Samples were extracted in 1 M HCl and then analyzed spectrophotometrically for orthophosphate using the phosphomolybdate blue method (see Objective 1 above). The total particulate phosphate (TPP) data were used in subsequent analyses.

Statistical Analyses of Algal Physiologic Data

The triplicate QY data were averaged for each species from each sample collection. The QY data were correlated against the tissue nutrient data ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, C:N, TPP) for those samples where QY and tissue nutrient data were collected. The QY data were also averaged for each station from each sampling event to compare the average station QY against environmental parameters including benthic values for salinity, temperature, and light (I_z) using regression analysis. Lastly, the cyclical nature of some of the parameters (i.e., temperature, light, QY) were examined using trigonometric equation analysis to study the lags that are inherent to many cause-effect relationships using procedures outlined in Parsons et al. (2010).

Artificial Reef Surveys

The distribution and biomass of attached macroalgae was determined from representative artificial reefs and natural hard-bottom reefs near Sanibel Island and Fort Myers Beach. A seasonal, as well as a spatial pattern, was hypothesized, therefore sampling events were conducted during the peak of the typical dry season and wet season over the two-year study period. FGCU researchers conducted surveys with SCUBA support and assistance from the Volunteer Scientific Research Team (VSRT), a 501(c) not-for-profit dive club who have been assisting Lee County biologists for nearly 20 years with fish surveys and reef mapping. During late summer and fall of 2008, we located three suitable natural limestone ledges and three appropriate artificial reefs to monitor for the duration of the study. The final artificial reef sites that were selected represent popular recreational fishing and diving locations and include the G-H reef complex (sunken barge), the Edison Reef (bridge debris), and Sherman's reef (sunken barge). Natural reefs were selected based on size, accessibility, and proximity to artificial reefs and include limestone ledges that protrude 1-2 meters off the benthos and located in 19 meters, 17 meters and 11 meters water depth. The depths of artificial reefs and natural ledges placed limitations on the length of time for standard SCUBA diving with regular compressed air and for covering all sites in a reasonable time frame. GPS coordinates were recorded for each site and are presented in Table 5.1.

Species presence/absence and community composition of macro-algae was determined from samples collected by divers along transects at each of six locations. At each station, a 30m to 50m transect was stretched across the reef. Reef samples always consisted of 10 quadrats along each transect but transects varied in length based on the amount of artificial reef available for sampling at a given location. Three divers swam along the transect line and at stratified points along each transect (n=10), a 1 m² quadrat was placed on the bottom. All attached macroscopic algae falling within the quadrat were collected by divers and placed into numbered mesh bags. Samples were stored in sea water at 4°C to minimize decomposition and returned to the FGCU laboratory for identification and enumeration. Upon returning to the laboratory, samples were identified to the lowest taxonomic level possible using Littler and Littler (2000) and Dawes and Mathieson (2008). Typically this was to genus or species level with voucher specimens retained for confirmation by Dr. Dawes.

Data Analysis

Overall macroalgae abundance for each sampling event was determined by measuring total wet weight biomass for each transect sample. Individual species abundance on the reefs was estimated by overall coverage using frequency of occurrence in quadrats along each transect where the minimum recorded abundance is one (1/10) and maximum abundance is ten (10/10). Statistical analysis was conducted using univariate and multivariate techniques to assess diversity using several metrics and community structure respectively. Bray-Curtis similarity matrices were used to construct hierarchical cluster dendrograms with SIMPROF random permutation test for significance (Clarke and Gorley, 2006). We also used non-metric

multidimensional scaling (MDS) ordination plots to visualize relative similarity/dissimilarity of all samples, by season, by reef type, and by site. Similarity percentage tests (SIMPER) were used to identify the taxa or species that were the most important contributors to the similarity within a group of samples and the dissimilarity between groups of samples.

Objective 6: To determine the ecological consequences of the accumulation of nuisance algal biomass by measuring the decomposition rates and nutrient recycling on area beaches. (Everham and Ceilley)

To address this objective, three field and one laboratory studies were established: 1) bimonthly beach monitoring to determine background levels of algae deposition; 2) response to deposition events to characterize biomass accumulation; 3) decomposition experiments on the beach to determine rate of biomass loss; and 4) laboratory decomposition experiments to quantify nutrient release from beach deposition. We established 16 beach monitoring sites throughout Lee County, sampling from July 2008 to July 2010. We responded to five deposition events. We ran eighteen decomposition experiments on area beaches. We ran four sets of laboratory decomposition experiments.

Bimonthly Sampling

We conducted bimonthly sampling starting in July 2008 and continuing through July 2010. We established 16 sites (Table 6.1) throughout Lee County. Starting in the Spring of 2009 the timing of the beach sampling has been coordinated with the off-shore monitoring of transects to capitalize on the possible connections between offshore and beach deposition observations. For each monitoring sample, the site was visited, the deposition was characterized using deposition intensity scale of 0 (no algae on the beach) to 5 (deposition event). If algae were present, a random sample of biomass was collected for identification.

Event Response

A toll-free number was established at FGCU for the general public to report any beach deposition of macroalgae (866-724-3428). Prompted by calls to the hotline, or by contact from City or County staff, we responded to deposition events. For each event, a measurement was taken of the linear extent of the deposition. A sample of the freshest available algae was selected at random along the deposition to allow species identification. At random points along the deposition, the width and depth of the deposited algae was measured. As each event typically involved multiple depositions over a period of days, zones of deposition, based on the degree of drying of previous depositions, were identified and included in the data collection. Biomass samples were collected from random locations within each deposition zone using a 0.25 m² quadrat. All algae within the quadrat were collected, down to the sand (Fig. 6.1). The width of the algae mat offshore was estimated at each sampling point, and a biomass sample in the surf zone was collected using one or more passes of a net (area 0.1125 m²) to collect floating algae.

The biomass samples were dried and weighed to allow estimates of biomass per unit area. The dimensions of the entire deposition were then used to estimate total biomass of algae deposited.

Decomposition - Beach

The original protocol required establishing beach decomposition experiments only after a large deposition event. We modified this protocol to collect masses of algae for lower intensity events, or to utilize algae collected offshore to allow these experiments to be conducted. For each deposition experiment, a 1 m² section of the beach was staked out, similar to the turtle nest markings. Algae, either from collection along the shore, or from off-shore sampling, were placed in four quarters of the plot, in roughly equal masses (Fig. 6.2). Wet mass of the samples was recorded. A subset of the algae was taken, to determine the relationship between wet and dry mass. The four samples were collected after one week, two weeks, four weeks, and eight weeks. Samples were returned to the laboratory, dried and weighed to determine rate of decomposition.

We ran eighteen experiments over the study area (Table 6.2), six during the wet season in 2009, six during the 2009-2010 dry season, and the last six during the dry season to wet season transition in 2010.

Decomposition – Laboratory

We used aquaria as microcosms to quantify the release of nutrients during algae decomposition (Fig. 6.3). Each aquaria was partly filled with 10 liters of beach sand. Measured masses of algae were placed in three replicate aquaria, with fourth as a control, with sand but no algae. A sample of the algae used was weighed and dried to determine the percent water content, to allow conversion to dry biomass. A measured quantity of seawater was flushed through the system and both the ingoing water and the effluent was collected for analysis of nutrient content. For the pilot study, seawater was flushed through once per week for eight weeks. The preliminary results indicated that the bulk of the nutrient release occurred during the first two weeks, so subsequent trials used a higher frequency of sampling (once per three days) for a shorter time period (three weeks). Concentrations of nitrate/nitrite, ammonia, ortho-phosphorus, total phosphorus and total nitrogen were determined using an autoanalyzer. Following the pilot experiment we ran three replicate trials. The final trial was run with sterilized sand, to attempt to quantify the role of microbial communities in sequestering nutrients during decomposition.

Objective 7: To verify and calibrate a hydrodynamic model for the system to address the potential fate of nutrients that are generated and exported from the Caloosahatchee River and the fate of detached macroalgae as they enter the near shore waters around Sanibel Island and to develop a sediment transport model and implement the coupled sediment and hydrodynamic model for the same time periods as the hydrodynamic model. (Fugate)

The Regional Ocean Modeling System (ROMS) was chosen to simulate and predict the hydrodynamics and sediment transport in the region of interest. ROMS is widely used and respected by the scientific community, is open code, and has a robust support group. Unlike other proprietary models, the ROMS model that has been developed for this project will be available for use and modification by the funding agencies and supported by the ROMS users group long after the completion of this specific project.

The model

The Regional Ocean Modeling System (ROMS) was used to model the hydrodynamics and sediment transport for the Caloosahatchee Estuary and the adjacent continental shelf. ROMS is a free-surface, hydrostatic, fully three-dimensional numerical model that uses a curvilinear horizontal grid and a terrain following vertical grid. Details of the model governing equations and solution algorithms are widely published (e.g. Warner et al., 2005 and www.myroms.org) and are not included in this report. However, some of the details of the model are included below so that future investigators can replicate the results from this report.

The ROMS allows for the use of many different turbulence closure schemes, such as the k-epsilon, k-omega, Mellor and Yamada 2.5, and the generic length scale (GLS) methods. The initial formulation was set up using the GLS method with the Kantha and Clayson stability function and horizontal smoothing of buoyancy/shear. After comparing initial results with observed, it was found that the traditional Mellor and Yamada 2.5 method simulated the observed periodic stratification better than the GLS method, and so this method was used in model simulations reported here.

Multiple horizontal and vertical advection schemes are coded into ROMS. These formulations use the MPDATA advection schemes for the horizontal advection of sediment and for the advection of other tracers (temperature/salinity). The MPDATA advection scheme is a non-oscillating always positive advection scheme. The vertical advection of sediment (sediment settling) is calculated using the piece-wise parabolic method (PPM). This method allows calculation of settling over several grid cells and so lessens the Courant-Friedrichs-Lewy constraint. Essentially, this ensures that the size of individual grids is appropriate for accurate calculations over the chosen time step of the model.

The open boundaries use a gradient boundary condition for 3D momentum and tracers. Flather and Chapman boundary conditions are set for the 2D momentum and the free surface,

respectively. Gradient boundary conditions for the other open boundary specifications do not give spurious current flow near the boundaries, so are found to be appropriate. Any landward sides to the grid are set to closed boundaries.

Seabed shear stress calculations are made using the Sherwood, Signell, and Warner bottom boundary layer formulation within the ROMS. The formulation of Madsen (1994) is used to calculate the wave current interaction and their influences on the shear stress. Roughness is based on the grain roughness (Nikaradse), the sediment transport roughness (such as from saltating grains), and the bedform roughness. Bedforms characteristics (ripple height/length) are calculated based on Wiberg and Harris (1994). These shear stress calculations are used to suspend sediment and are fed into the momentum equations to influence water flow.

Particle tracking is coupled to the ROMS hydrodynamic modules and is computed using a fourth order Runge-Kutta integration method. This replicates passive neutrally buoyant LaGrangian drifters and is numerically much more efficient than using Eulerian methods such as tracer concentrations (Dias et al., 2001). While the currents are computed only discretely at the faces of the grid, the particles are allowed to move continuously throughout the grid space.

Grid

The model grid is curvilinear and uses 200 X 250 horizontal grid and 20 vertical layers creating a grid space of 1,000,000 points. Vertical layers are spaced based on V-stretching and V-transform parameters of 3 and 1, respectively, which are recommended (per ROMS forum/wiki) for shallow water applications. This configuration produces higher resolution at the surface and bottom boundaries, where horizontal currents are expected to change most in the vertical direction. The grid extends out onto the continental shelf and to the north and south of the Caloosahatchee Estuary (Fig. 7.1).

Bathymetry data was provided by the SFWMD. In cooperation with the South Florida Water Management District, in 2002 the U.S. Geological Survey conducted a bathymetric survey of the Caloosahatchee Estuary. Using a boat equipped with GPS and depth-sounding equipment (SANDS), the USGS measured the bottom elevation at more than 140,000 locations within the estuary. In 2003 the USGS measured bottom elevations at some 480,000 locations in the Lower Charlotte Harbor area (Matlacha Pass and San Carlos Bay) and Estero Bay. As part of the same project, NASA also collected bathymetric elevations using an experimental Lidar technique (EAARL). All bottom elevations are relative to the North American Vertical Datum of 1988 (NAVD 1988). Unlike the older datum, in which "sea level" was arbitrarily defined as zero elevation, the NAVD values are geocentric and thus are not dependent on sea level. For this part of Florida, the Gulf shoreline corresponds to an NAVD elevation of approximately 1.15 feet. For offshore areas that were not part of the recent survey, bathymetric elevations were filled in with data from the NOAA Coastal Relief Model (CRM), which is based on historical bathymetric surveys. Coastline data were obtained from the NOAA National Geophysical Data Center Coastline Extractor (<http://rimmer.ngdc.noaa.gov>).

The bathymetry was interpolated onto the curvilinear grid and then underwent some 2nd order Shapiro filters to smooth out grid cell irregularities while keeping the same total basin volume. After each filter, the bathymetry of the inlets was replaced by the measured bathymetry in order to keep from artificially shallowing the inlets significantly. This model grid contains greater than 600 m resolution in the Gulf of Mexico, and ~300 m resolution within the Caloosahatchee Estuary. The entire grid does not plot well on standard paper size, but the region around San Carlos Bay is plotted in Fig. 7.2 to show some representative grid cells. Because the deep upstream section of the river extends significantly eastwards from the rest of the grid, it would contribute an enormous amount of extra calculation and CPU time to execute the model with the natural orientation. In order to reduce the time required for the model, the upstream section was aligned at an angle directed northwestward within the grid. This modification does not affect the hydrodynamics but does produce contour maps with a coastline different from what a typical nautical chart would look like.

The model is run at 10 second time steps for a total of 388800 iterations (approximately 45 days), grid cells are allowed to alternate between wet and dry using a critical depth of 0.1 m during the wet season. Dry season runs have the critical depth set to 0.3 m. The parameters of interest are written after every hour's worth of time steps (360) to output files for analyses.

Forcing

The major forcings of the model are wind, tide, and freshwater discharge. Winds are obtained from the Page Field General Aviation Airport located close to the Caloosahatchee Estuary. Wind speed and direction are hourly mean values and are applied uniformly over the grid. Missing wind speed and directions are interpolated linearly from the nearest available good data points. Wind speed and direction distributions are shown in Figs. 7.3 a and b for the wet season and dry season runs. During the wet season, the strongest winds came episodically from the west, but the most common winds were generally weak and from the east (25% of the time). Dry season winds were more varied in their direction and generally were more intense. Tides are forced with the Oregon State University OTPS V7.2 tidal prediction software. The M2, S2, N2, K2, K1, O1, P1, Q1, and M4 constituents are used. Data for freshwater inflow from the Franklin Locks and Dam (S-79) in the Caloosahatchee River and for the Peace River in Charlotte harbor are obtained from the USACE through the DBHYDRO database maintained by the SFWMD. The amount of freshwater discharge at S-79 is augmented by 20% to account for freshwater discharge from sheet flow and other sources within the tidal extent of the river (Peter Doering, personal communication). Time series of the river discharge levels during the primary simulations are shown in Fig. 7.4. Other forcings of secondary importance include air pressure, relative humidity, and temperature, all also obtained from Page Field.

All currents are initialized to 0 m/s. Temperature is initialized to 18.5 °C and ocean salinity is initialized to 35 psu. Salinities within the Caloosahatchee are set close to those that were observed at surface and bottom from SFWMD water sensors. The river was divided into 3

sections, and the salinity in each section was set so that the water column approached that of the ocean in a stepwise fashion. This initialization to near observed values greatly reduces the time that the model needs to “spin up” to realistic values. The sediment bed is initialized using 3 grain sizes uniformly distributed on the seabed. Each sediment class represents ~33% of the sediment at each location in the model grid.

Model Simulations

The model simulations covered a range of conditions. All of the simulations were done during the dry season or the wet season of 2008. This year and the time interval of the wet season run were chosen because it encompasses one of the more significant macroalgal stranding events which occurred in July 2008. Each simulation was run for 45 days, time to allow the model to spin up and simulate both neap and spring tides within each run. The dry season runs were from February 1 to March 16, and the wet season runs were from June 22 to August 5. The first 100 hours when the model is coming to equilibrium, or “spinning up” were omitted from the animations that are included in these results. Because the macroalgal stranding event did not occur during the maximum discharges, a further simulation was performed to estimate residence times during these high discharge events. This simulation ran from July 22 to September 7.

The sediment transport model, as the hydrodynamic model with which it is coupled, is from an Eulerian viewpoint, i.e., it predicts accretion, erosion and other processes within each cell and does not “know” where sediment that enters the cell originally came from, nor where sediment that leaves the cell will ultimately end up. In order to investigate where water that originates in one place, e.g. the Caloosahatchee River is transported, it is necessary to use a particle tracking model, which is from a Lagrangian viewpoint. Essentially, neutrally buoyant particles, or “drifters” are released at specified locations and followed through the simulation. Rather than being associated with specific grid cells, the drifters may move continuously within and across grid cells. Within the Caloosahatchee Estuary, 657 drifters were released at 219 locations, with a drifter released at surface, bottom, and mid level for each of the locations (Fig. 7.5). The drifters provide an estimation of where neutrally buoyant material such as dissolved organics, or near-neutrally buoyant particles might be transported. It is difficult to predict the size and density of estuarine aggregates, however, very small aggregates composed of mostly organic material may behave as if they were neutrally buoyant and drifter paths give some insight into the potential transport of these very small porous organic particles as well as the transport of dissolved material. After consultation with the project scientists, locations outside of the river where macroalgal stands were discovered became of interest. Eight locations were provided, and at each location, five drifters were released near the bottom (Fig. 7.6). The five drifters were separated vertically by a very short distance (0.01 m in shallow locations and 0.1 m in deeper locations). The release of 5 drifters very near each other gave an idea of the dispersion that might be expected from many clumps of algae torn from close but not identical locations. The cluster of drifters at the artificial reef sites were released every 7 days and followed throughout

the simulation. This spatial and temporal distribution resulted in a total of 240 additional drifters released from the eight artificial reef sites.

Additional simulations that fix the wind direction were performed at the request of project scientists. In one simulation, the wind direction was consistently southwesterly, and in the other the wind was consistently northwesterly. In order to simulate the natural oscillations of the wind speed, especially diurnal variation, the observed wind magnitudes were preserved for the forcing, and only the wind direction was held constant.

Objective 8: To evaluate existing water quality models, watershed plans and/or assessments for the Caloosahatchee and Estero Bay watersheds to help guide nutrient reduction strategies for managing drift algal blooms in Lee County waters. (Parsons, Loh and Everham)

The original plan for this objective was “To develop a water quality computer model of the West Florida Shelf (WFS) in the Fort Myers region...” Due to the existence of a comprehensive nutrient model recently developed by DEP, the need for another nutrient model for this area is currently not essential. Therefore, to allow us to draw conclusions relative to the original intent of the modeling efforts of this objective, “... predictive studies on the effects of high flow river run-off on the development of red tides and macroalgal growth,” we evaluated the simulation results from existing hydrodynamic and water quality models such as the HSPF & EFDC as well as all available watershed assessments including: the Southwest Florida Feasibility Study; the Caloosahatchee River Watershed Protection Plan; the Tidal Caloosahatchee Draft TMDL; and the Estero Bay Watershed Assessment. In addition, nutrient data collected from the synoptic surveys in Objectives 1, 2 and 3 were used to calculate loadings and residence times of nutrients in the Caloosahatchee Estuary in order to identify potential nutrient loading “hotspots”. A comparison of the hydrologic conditions between 2003 – 2007 and 2008 – 2010 was done to help elucidate the differences between these two periods. Average monthly freshwater discharges and rainfall at S-79 for 2003 – 2005, 2005 – 2007 and 2008 – 2010 were calculated and the percent difference calculated. A list of specific resource management recommendations geared towards nutrient reduction strategies for managing drift algal blooms in the waters of Lee County was also developed.

Objective 9: To identify the role of macroalgal grazers in determining macroalgal distribution patterns. (Coen and Provost)

Summary of Events 2003-2010

We compiled data from relevant reports here in southwest Florida and generated an overview of all available historical stranding information available for our area from 2003-2010 (Appendix

9.1). This included: (1) actual releases from upstream at the two locks at S-77 and S-79; (2) significant harmful algal blooms (HABs) that might impact grazers and other organisms; (3) *Enterococcus*-associated beach closures from the FL Board of Health website for local beaches; (4) named hurricanes or tropical storms; and (5) other anecdotal or relevant information. We used all available sources including area reports, City of Sanibel logs, FWRI and other searchable websites and other grey literature such as newspaper articles. Surprisingly little site- or species- specific information was available for this critical task.

Field Sampling to Assess Potential Grazers

Trawls. In October 2009 and April 2010, we used a 16' otter trawl sampling to assess macroalgal, invertebrate and finfish populations and to more extensively sample for potential grazer species for further study. Trawls samples were taken in Tarpon Bay, Pine Island Sound, San Carlos Bay and the Gulf of Mexico noting any sea urchins, and other potential known finfish and invertebrate grazers over several days. In April 2010, sites were resampled by trawl, including the addition of new sites mostly at inshore areas based on concurrent algal observations. Algal densities and community composition were recorded, as well as any seasonal changes in grazer composition and occurrence. Material at that time was also being sampled by FGCU (for isotope analyses before and after, Dr. M. Parsons) given the soon to occur freshwater releases upstream of the sampling area.

Another survey was made on September 10, 2010, and included sites east and west of the Sanibel Causeway (Fig. 9.1). Urchins were seen in 1-2 individual per m² densities, along with pen shells, and gravel type bottom. Drift algae was not seen at these sites, and urchins were past the deep edge of the seagrass.

In-Water Assessments. Staff members were also towed behind the boat for a rapid survey of areas near the Sanibel Causeway, in the seagrass near the Sanibel toll booth. Snorkeling also took place at several sites around the island, including Captiva Pass, Redfish Pass, Bunch Beach, Tarpon Bay and Blind Pass. Observations made include density and presence of herbivores, including urchins, and bottom type (Fig. 9.1).

Lab Feeding Trials

Two methods were used to examine feeding habits of the sea urchin, *Lytechinus variegatus*. One was offering a single species per trial, and the other was using a multiple choice experiment with three species of algae per urchin. Details follow here by experiment type.

Single Species. Several trials using algae common to inshore and offshore/nearshore stations were completed. Single species trials include 10 replicated single urchins being fed 1g for each algal species. Six species have been used including several common offshore/nearshore (*Caulerpa racemosa*, *Gracilaria blodgettii*, *Agardhiella subulata*, *Botryocladia occidentalis*) and inshore (*Acanthophora spicifera*, *Spyridia filamentosa*, *Hypnea musciformis*) species. Single urchins (n = 10 replicates per species) were placed in 1L containers and offered 1g (± 0.3 g) wet

weight of algae (Fig. 9.2). A control of 1g of algae with no urchin in 1L container was also run. Urchins were allowed to feed for 4 hours, and wet weight of the algae left was taken (after patting dry). Urchins were starved for 24 hours prior to experiment, and housed in one of two 300 gallon flow-thru tanks. Sea urchins were cycled through each experiment, so that no urchin was used twice for any experiment. One large tank (Fig. 9.3) was used to house all urchins, another to house the urchins being starved prior to any experiment. Urchins were collected from around Sanibel for the single species trial. Other areas used for collections included the Gulf of Mexico RECON site for prior multiple species trials. Urchins were fed a variety of algae collected from Bunch and Dixie beaches, as well as seagrass from wrack lines when algae was scarce on beaches. Salinities were kept at around 30 psu for the duration of the experiments, and temperatures ranged from 22-26°C depending on season. Water changes of tanks occurred every month, with a 50% water change. Wet weight of each urchin is measured prior to feeding, and feeding amount is then standardized to overall average weight. Urchin feeding was calculated using standardized urchin wet weight (n = 80 urchins, mean wet weight 88.82 g). Final values were tabulated as algae eaten in four hours by average-weight individual sea urchin.

Multiple Species. Trials using several species simultaneously, or multiple choice experiments, were also run. Algal species utilized were chosen based on their abundance in sampling offshore and inshore stations, and spanning a range of structural differences. The species included *Gracilaria tikvahiae* (a corticated, common inshore red species), *Gracilaria mammillaris* (a flattened, common inshore and offshore red species), and *Dictyota cervicornis* (a flattened, common offshore brown species). Urchins (total of n = 18 trials) were placed in containers holding approximately 1g (± 0.3 g) wet weight of each of the three algal species, which were placed in random order, with equal probability of a sea urchin's arrival (Fig. 9.4). Individual urchins were allowed to feed for no more than 4 hours, and wet weight of each alga per species was quantified. Observations were made every 15 minutes during the first 2 hours of a 4 hour trial to assess urchin behavior and selection order.

A third experiment for a longer time period was also run. This experiment was run in 38 liter (10 gallon) tanks with air stones, where urchins were allowed to feed for a total of 16 hours. Approximately 8g (± 0.3 g) of four other macroalgal species (three red algae, *Botryocladia occidentalis*, *Agardhiella subulata*, *Solieria filiformis* and a green *Caulerpa racemosa*) were placed in the four corners of an aquarium, with an urchin placed in the middle to start the trial (see Fig. 9.5). Observations were made every 15 minutes or until the urchin arrived at one algae. Wet weight of each algal species left (patted dry) after 16 hours was quantified as above.

Grazer Enclosure and Enclosure Experiments

Urchin-Enclosures Experiments. Two simple experiments were run to assess urchin survival and grazing pressure. Survival experiments were run with 1 cage at several sites (n = 4, RECON sites) with 3 urchins per inclusion cage. Cages were constructed using 1¼ inch mesh (Industrial Netting Inc., XB113-48X50) and ¾ inch PVC with side-outlet 90s (see Fig. 9.6). Cages were 48

cm by 48 cm, and were 35 cm tall. Cages were affixed to the bottom using 3/8" x 24" rebar within the PVC legs, reinforcing the legs and allowing the cages to be installed more easily. The legs were then attached by ladder cable ties to the rebar. Cages were pounded into the sediment using a sledge, with approximately 35 cm hammered into the sea floor.

The Foundation's River Estuary Coastal Observing Network (or RECON) sites were selected because of real-time salinity and other water quality data available (see <http://recon.sccf.org/>). Cages were left for 1 month, and checked every other week for urchin survival/escape.

Grazer-Exclosure Experiments. The second set of caging experiments was aimed at determining potential algal growth in macrograzer-excluded footprints vs. adjacent areas with macrograzers present (e.g., sea urchins and other larger herbivores such as parrotfish). Exclusion cages were deployed at several sites (Fig. 9.7, total of 8 sites) where urchins were typically seen, but had little or no algae ever observed. Several sites from Objectives 1 and 5 were utilized, including four at GOM10, GOM01, GOM03 and GOM04. One additional and new site (see Fig. 9.7) was selected from information derived from the hydroacoustics video mapping work (Fig. 9.7) as another potential good algal recruitment habitat, designated Light 1 (see Appendix 5.2 for additional station descriptions). Additional sites included one at Redfish RECON, another at the Blind Pass RECON, and the final one at the Gulf of Mexico RECON (see Figs. 4.2, 9.7 and 9.8). Three cages were deployed at each site, for a total of 24 exclusion cages. Replicate exclusion cages were checked approximately every three weeks for algae growth inside and outside of cage, cage condition, fouling, and the presence of any urchins in three, 1 m² (overall area) quadrats adjacent to and outside of the caged areas. Data on the presence of invertebrates (urchins, pen shells, worm tube densities) and algal percent cover were also collected when possible (e.g., visibility, weather, currents). If algae were present, subsamples were taken for enumeration and identification. We used ranges following Fig. 9.9 to assess percent cover.

Study Site Descriptions for Urchin Cage Enclosures and Exclosures

Light 1. This station was utilized for the exclusion cages (Fig. 9.7). Cages were deployed on July 7, 2010, and visited on July 28, August 19, September 17, and October 13, 2010.

Redfish RECON. Exclusion cages were deployed June 9, 2010, and visited July 13, August 9, August 17, and September 14, 2010. Cages were located at the base of several RECON stations (Fig. 9.7). Three cages per site were visited every month.

Shell Point RECON. This site was chosen just for the inclusion survival sites, as real-time salinity data is available for all RECON stations. This site is located at the estuarine end of the Caloosahatchee, and it was expected that the urchins would have come into contact with greatly reduced salinity levels (Figs. 9.7, 9.10, 9.11).

Blind Pass RECON. This site was utilized for both the inclusion survival sites, as well as the exclusion sites. Exclusion cages were deployed June 9, 2010, and revisited on July 8th, August

17th, September 14th, and finally October 20, 2010. Grazer exclusion cages were located at the base of the piling where the RECON station was attached.

Gulf of Mexico RECON. This site (Fig. 9.7) was also utilized for both the inclusion and exclusion sites. Exclusion cages were deployed May 27, 2010, and revisited July 14th, August 14th, September 17th and finally retrieved on October 20, 2010. This site had numerous urchins and pen shells found sporadically, along with sandy bottoms. Four cages were used for the exclusion site, as the high currents were sufficient to unearth the mesh. The fourth cage was the survivability cage that was cleared of urchins and left in case.

GOM10. See Appendix 5.2; station descriptions, for bottom cover information. Exclusion cages were deployed June 7, 2010, and revisited July 28th, August 19th, and finally September 17, 2010.

GOM04. See Appendix 5.2; station descriptions, for bottom cover information. This site was located off of Sanibel, right outside of Blind Pass (Fig. 9.7). This site was deployed on July 7, 2010, and revisited July 28th, August 19th, September 17, 2010 and retrieved on October 13, 2010.

GOM03. See Appendix 5.2; station descriptions, for bottom cover information. This site was deployed on July 7, 2010, and visited July 28, August 19, Sept 17 and October 13, 2010. This site (Fig. 9.7) was re-named GOM03A in Objective 5 and was located closer to the beach than GOM03.

GOM01. See Appendix 5.2; station descriptions, for bottom cover information. This site was deployed on July 7, 2010, and visited July 28, August 19, Sept 17 and October 13, 2010.

Results and Discussion

Objective 1: To determine whether Lake Okeechobee or basin sources are more important to macroalgal biomass and productivity with an emphasis on those associated with organic and benthic nutrient sources. (Loh and Brand)

Fig. 1.3 shows water flow from Lake Okeechobee along the Caloosahatchee River through control structures S-77, S-78 and S-79 from May 2008 - June 2010 into the Caloosahatchee Estuary. Water discharges into the estuary showed typical seasonal flows of high flows during the wet season (August – October, 2008, June – October, 2009 and April – June 2010) and low to no flow during the dry season (May – July, 2008, November 2008 – May 2009 and November 2009 – March 2010). Arrows indicate actual Gulf of Mexico bi-monthly synoptic surveys for Objectives 1, 2 and 5. Monthly surface and bottom salinity along the Caloosahatchee Estuary from May 2008 – June 2010 is shown in Fig. 1.4. Salinity was higher during S-79 low flow periods and was lower during S-79 high-flow periods. All hydrographic data collected during the synoptic surveys for the estuary and GOM are given in Table 1.3.

Dissolved nutrient and organic carbon concentrations were similar in magnitude as those measured from previous studies (ERD, 2003; Boyer, 2005; Loh, 2008). Dissolved inorganic nitrogen and phosphorus (DIN and DIP, respectively) concentrations along the Caloosahatchee River increased during the wet season and decreased during the dry season (Fig. 1.5), corresponding with water flows, although variability was high. This decrease in inorganic nutrient concentrations were more marked during the November 2008 – May 2009 season compared with the November 2009 – March 2010 season (Figs. 1.3 and 1.5). Dissolved organic carbon (DOC) concentrations also showed similar seasonality (Fig. 1.6a) although the November 2008 – May 2009 dry season saw higher DOC concentrations than the wet season preceding it. Dissolved organic nitrogen (DON) concentrations were fairly constant during the study period (Fig. 1.6b) and made up of ~80% of the total dissolved nitrogen pool for the Caloosahatchee River. This was also similar to findings from previous studies (ERD, 2003; Boyer, 2005; Loh, 2008). Dissolved organic phosphorus (DOP) concentrations showed similar trends as DIP concentrations (Fig. 1.6c) and is comprised of ~30% of the total dissolved phosphorus pool.

Downstream of the Caloosahatchee River, within the Caloosahatchee Estuary, dissolved organic matter (as DOC, DON and DOP) and dissolved inorganic nutrients (as DIN and DIP) concentrations were generally higher at the upstream site (Site 1) compared to the San Carlos Bay site (Site 4) (see Figs. 1.7 and 1.8). Dissolved inorganic N concentrations in the Caloosahatchee Estuary did not differ between surface and bottom water samples (Figs. 1.7a and b) and showed similar seasonal trends as for salinity at these sites (higher concentrations during wet season, lower concentrations during dry season). Dissolved organic N again made up the majority of the total dissolved nitrogen pool (Fig. 1.7) and showed similar seasonal trends as for DIN. There were also no differences in DON concentrations between surface and bottom waters

(Fig. 1.7c and d). There were also no differences between surface and bottom DOC and DIP concentrations (Fig. 1.8). Dissolved organic C and DIP concentrations also showed the same seasonal trends as DIN and DON concentrations (Fig. 1.8).

Out in the coastal Gulf of Mexico, dissolved organic matter and dissolved inorganic nutrient concentrations were lower than those found within the Caloosahatchee Estuary. Dissolved inorganic N concentrations were fairly similar between sites and seasons (Fig. 1.9a), albeit showing a slightly wider spread during the dry season. Dissolved inorganic P concentrations showed similar site-to-site and seasonal patterns as for DIN (Fig. 1.9b). Dissolved organic C, DON and DOP concentrations also showed no differences between sites and seasons (Fig. 1.10). Dissolved organic N again made up the majority of the total dissolved nitrogen pool while DOP and DIP comprised similar fractions of the total dissolved phosphorus pool (Figs. 1.9 and 1.10).

The highest abundance of microalgae was upstream near Lake Okeechobee (Fig. 1.11), suggesting that this is a major source of nutrients for algal production in the system. The station numbers on the x-axis are given in Fig. 1.1b. There was an increase in chromophyte algae (probably diatoms) as river water flowed into the estuary (Site 1 in Fig. 1.1a) indicating a shift in the species composition of the algal community (Fig. 1.12) from freshwater to brackish to saline waters (Tolley et al., 2010). Cyanobacteria were most abundant upstream near Lake Okeechobee (Fig. 1.13), again suggesting that this is a major source of nutrients for algal production. With the exception of surface waters at Site 2 (from Fig. 1.1a), microalgal abundance shows dilution with increasing salinity in the estuary (Figs. 1.14 and 1.15), indicating that freshwater is a greater source of nutrients than seawater in this area. There is an increase in microalgal abundance at Site 2 (from Fig. 1.1a) that may suggest a local input of nutrients here.

Nutrient-addition bioassay experiments indicate that nitrogen is the limiting nutrient throughout the system (Fig. 1.16). Nitrogen bioassays show the highest amount of nitrogen that is available for algal growth in the freshwater reaches of the Caloosahatchee River (Fig. 1.17), suggesting that there is a greater source of nutrients in the river compared to the estuarine reaches of the CRE. When comparing these bioassay microalgal abundances with the available nutrient data, the inorganic nutrients present in the system is not enough to account for those microalgal growth (Fig. 1.18). However, when taking into account the entire total dissolved nitrogen pool, bioassay microalgal abundances approaches the amounts of theoretical microalgal abundance (Fig. 1.18). This indicates that there is a potential for DON to be available for microalgal growth. As plants and algae typically utilize inorganic forms of nitrogen, these organic nitrogen compounds must be broken down into utilizable inorganic compounds by microbial (or photolytic) processes before it can be made available for algal growth. Bioavailable nitrogen shows dilution with increasing salinity in the estuary (Figs. 1.19 and 1.20), indicating that freshwater is a greater source of nitrogen than seawater in this area.

Microalgae are generally more abundant nearshore than offshore in the Gulf of Mexico, particularly near the mouth of the Caloosahatchee Estuary in both surface and bottom waters

(Figs. 1.21 and 1.22). These data indicate that the C-43 canal rather than offshore sources is the major source of nutrients generating algal blooms. Cyanobacteria are also more abundant near the mouth of the Caloosahatchee Estuary (Figs. 1.23 and 1.24).

Bioassays indicate that nitrogen is the limiting nutrient throughout these waters (Figs. 1.25 and 1.26). Nitrogen bioassays show the highest amount of bioavailable nitrogen near the mouth of the Caloosahatchee Estuary (Figs. 1.27 and 1.28), suggesting that the Caloosahatchee River is the major source of nitrogen generating algal blooms in the system.

Abundance of benthic microalgae on the sediment surface is highest in the Caloosahatchee Estuary and declines going offshore (Fig. 1.29), indicating the major source of nutrients to benthic microalgae is derived upstream from the Caloosahatchee River.

Objective 2: To determine if benthic nutrient flux derived from degradation of organic matter is a significant contributor to drift algal blooms. (Loh and Brand)

Daily dissolved inorganic (as NO_2^- , NO_3^- and NH_4^+ and DIP) and organic (DON and DOP) nutrient and dissolved organic carbon (DOC) fluxes from sediment microcosm experiments were calculated for June 2008 – 2010 for three long-term stations; GOM04, GOM06 and GOM16. In general, GOM04 sediment comprised mainly of very poorly-sorted fine sands (Fig. 2.1) with 45% of the sediment comprising of shell hash greater than 1 mm (Table 2.1). GOM06 sediment comprised mainly of very poorly-sorted very fine sands (Fig. 2.2) with 34% of the sediment comprising of shell hash greater than 1 mm (Table 2.1). GOM16 sediment comprised of moderately well-sorted fine sands (Fig. 2.3) with 94% of the sediment comprising of sediment smaller than 1 mm (Table 2.1). Sediment samples for GOM04 contained high amounts of carbonates that interfered with the organic matter content analyses at this site. However, organic matter content for the other two sites was available. GOM06 sediment had organic matter content (average = $8.59 \text{ mgC gdw}^{-1}$ and $1.09 \text{ mgN gdw}^{-1}$, Table 2.1) that was higher than the sediment at GOM16 ($2.33 \text{ mgC gdw}^{-1}$ and $0.309 \text{ mgN gdw}^{-1}$, Table 2.1) indicating that there is a possible accumulation of organic matter at GOM06. Ratios of C:N of the sedimentary organic matter were similar between the two sites (~9, Table 2.1), suggesting a predominant marine source of organic matter. Stable carbon and nitrogen isotope signatures for sedimentary organic matter at both sites were also similar (average $\delta^{13}\text{C} \approx -19\%$; $\delta^{15}\text{N} \approx 2\%$) and is also indicative of a marine source (Table 2.1).

Sediment fluxes presented have been subtracted from water column controls. Positive fluxes indicate flux out of the sediment while negative fluxes indicate flux into the sediment. For DIP, positive fluxes from the sediment were generally balanced by negative fluxes into the sediments during the course of the year (Fig. 2.4a). This is true regardless site. However, the magnitude of the exchange of fluxes measured were greater at GOM06 and GOM16 compared with GOM04

(Fig. 2.4a). Ammonium (NH_4^+) fluxes however, were mainly positive (Fig. 2.4b) and were highest at GOM06. However, there were two significant negative flux events at GOM04, which occurred during the two driest sampling period of the study (May 2009 and May 2010; Fig. 2.4b). Ammonium fluxes were also the highest of the four nutrient measured, and the predominant nitrogen species. These fluxes of NH_4^+ from the sediment is probably due to the degradation of both dissolved and particulate organic matter in the sediment (see DOC fluxes below).

Nitrite (NO_2^-) fluxes were very small and exhibited the same pattern in terms of positive versus negative fluxes annually (Fig. 2.5a). There were also no differences in the magnitude of NO_2^- fluxes between sites. Similarly, NO_3^- fluxes also showed the same pattern but with a higher magnitude compared with NO_2^- fluxes (Fig. 2.5b). Nitrate (NO_3^-) fluxes at GOM06 were slightly lower than those in GOM04 or GOM16. The magnitude of NO_3^- fluxes was comparable with DIP fluxes.

The magnitude of DON fluxes were the highest of all the N fluxes (Fig. 2.6a) and were highly variable between seasons and sites. However, as with DIP, NO_2^- and NO_3^- fluxes, positive fluxes from the sediment were generally balanced by negative fluxes into the sediments during the course of the year. With the exception of the June 2008 experiment at GOM04, DOP fluxes also showed a similar pattern of positive fluxes being balanced by negative fluxes over the course of year (Fig. 2.6b). In June 2008, there was a large sink of DOP into the sediment at GOM04, although there was no concomitant release of DIP into the water column (compare Figs. 2.6b and 2.4a). This suggests that the DIP released was quickly taken up, presumably by benthic microalgae at this site. For DOC, these experiments indicate that the sediment is a sink of this dissolved component (Fig. 2.7). As the degradation of DOC in the sediments usually releases inorganic nutrients, these results are in agreement with the inorganic nutrient fluxes that we see in this study.

*Objective 3: To determine the submarine groundwater flux of nutrients to coastal waters.
(Charette)*

Groundwater Radium

Groundwater radium concentrations are among the highest we have observed in over 10 years studying submarine groundwater discharge (Tables 3.1 and 3.2). As there are no known anthropogenic sources of radium in this region, the key influence appears to be deposits of phosphorite, a naturally occurring phosphate-bearing mineral that also contains appreciable quantities of uranium and its decay products. It is also notable that radium was enriched in groundwater irrespective of salinity and wet vs. dry season: the average ^{226}Ra for April and October 2009 was 780 and 580 dpm 100 L^{-1} , respectively. As such, our box model results will likely reflect the low salinity component of submarine groundwater discharge.

Surface Water Radium

Samples for four isotopes of radium (223, 224, 226, and 228) were collected from the Gulf of Mexico, through the estuary salinity gradient, and along the river to its origin at Lake Okeechobee. Concentrations were only a factor of 2-5 times lower than groundwater, which suggests significant groundwater-surface water exchange. Spring values were 50-100% higher than fall values, indicative of seasonal changes in the groundwater flux to the estuary. The average ^{226}Ra for the estuary was 340 and 160 dpm 100 L^{-1} , respectively.

Box Model for Radium-derived Submarine Groundwater Discharge

Box model results using the parameters outlined above yielded radium-derived SGD rates of $3.3 \times 10^6\text{ m}^3/\text{d}$ (April) and $1.3 \times 10^6\text{ m}^3/\text{d}$ (October) for the Caloosahatchee Estuary (Table 3.3). As an independent check on these values, we conducted a water balance for the watershed. The water balance was based on the product of average daily rainfall (m/d) for the year preceding the study (e.g. for April 2009, we averaged rainfall from April 2008-April 2009), the watershed area (m^2), and evapotranspiration (% of rainfall lost to evaporation and uptake by plants = 75%). We then assumed that this net recharge value must be balanced by loss to the estuary. This calculation returned values of $4.3 \times 10^6\text{ m}^3/\text{d}$ (April) and $2.8 \times 10^6\text{ m}^3/\text{d}$ (October), however, they are considered an upper limit as we did not correct for aquifer withdrawals and input landward of the Franklin Lock. An estuary salt balance, a function of the estuarine freshwater inventory and residence time, produced total freshwater fluxes of $3.9 \times 10^6\text{ m}^3/\text{d}$ and $4.4 \times 10^6\text{ m}^3/\text{d}$ for April and October, respectively. Strictly speaking, the salt balance value should equal the Franklin Lock flux plus the freshwater SGD flux. For April, the sum of these inputs are 30% higher than the salt balance while for October they are 30% lower. Such a discrepancy is not unreasonable given the uncertainty and assumptions associated with the various methods.

Groundwater nutrient fluxes to the Caloosahatchee Estuary

The central goal of this objective is the application the radium approach to determine groundwater nutrient fluxes. The importance of coastal groundwater discharge in delivering dissolved nutrients, such as nitrate and phosphate, to coastal waters has often been overlooked, primarily because it is difficult to estimate. The problem lies in the fact SGD had been difficult to quantify using traditional methods such as seepage meters since the discharge is often patchy and may vary with time. The approach via the radium tracer is relatively simple: the nutrient flux is the product of the Ra-derived SGD flux and the average nutrient concentration in the groundwater endmember:

$$F_N = F_{SGD} * N_{gw}$$

where N is the nutrient of interest and N_{gw} is the mean concentration of the nutrient in groundwater. However, many of these calculations do not take into account the potential nutrient transformations that may occur in the subterranean estuary (Moore, 1999). These include such processes as denitrification, sorption of phosphorous to Fe (hydr)oxides, and

desorption of ammonium during seawater intrusion. Our study therefore has focused on sampling wells located as close to the location of discharge as possible, i.e. at the estuarine land-water interface and not at inland wells.

We focused on three classes of macronutrients: total dissolved nitrogen (TDN, includes both organic and inorganic forms that pass a 0.45 μm filter), dissolved inorganic nitrogen (DIN, includes nitrate, nitrite and ammonium), and phosphate (PO_4 , includes inorganic dissolved phosphorous only). Except for phosphate, the average groundwater nutrient concentrations were similar for April and October (TDN = 73 and 79 μM ; DIN = 42 and 33 μM ; PO_4 = 2.2 and 5.3 μM). In this sense, the groundwater-derived nutrient fluxes generally scaled with SGD (Table 3.4). For TDN, the groundwater flux to the estuary in April (3410 kg/d) and October (1400 kg/d) were of the same order of magnitude as the flux through the Franklin Lock. However, the flux of DIN, a significantly more bioavailable form of nitrogen, was almost 7-times higher for groundwater than from the river. Like TDN, groundwater phosphate fluxes were of the same order of magnitude as the river.

Objective 4: To determine the distribution of macroalgal biomass using underwater video and hydroacoustic survey. (Grizzle, Foster, Riegl and Coen)

Hydroacoustic Tasks (Phases I and II)

QA of Training Dataset

Of the 62 (total $n = 6,951$) ground-validated hydroacoustic training samples collected within the study area, 57 ($n = 5,296$) remained after QA and merging of the 38 and 418 kHz hydroacoustic datasets (Fig. 4.7). Most were rejected because they did not exceed the minimum depth filter of 1.75 m. Remaining outliers were removed by performing a PCA+K-means clustering analysis ($k = 16$) and rejecting records belonging to four disproportionately small clusters, which accounted for only 0.26-0.62% of the total records. The membership of the remaining fourteen clusters ranged from 1.3 to 22.9% of the total records. Following PCA+K-means outlier removal, 5,209 records belonging to 57 hydroacoustic samples remained in the training dataset.

Final Arrangement of the Training Dataset

The initial assignment of the 62 training samples was done on the basis of visually-apparent seabed characteristics, after reviewing the accompanying video files. Prior to submitting the training dataset to the multi-pass descriptive DA, these bottom type assignments were re-evaluated in two steps. The 11 independent variables of the 5,209 records passing the PCA+K-means outlier removal step were again submitted to PCA, and the first ten PC were clustered into homogeneous groups ($k = 16$) using a K-means algorithm. The percent membership amongst the 16 K-means clusters was computed for each of the 57 samples. This matrix (columns = K-means cluster membership, rows = training samples) was submitted to an MDS analysis, obtained from a Bray-Curtis similarity matrix (see Fig. 4.8a). Three training samples (#s 25, 59, and 62) were

found to: (a) lie far outside their respective class groupings, and (b) bear no resemblance to the bottom class with which they comingled in 2D MDS space. Since their location within the MDS plot could not be reconciled with their visually-apparent characteristics, these samples were rejected outright from the training dataset. Eight of the remaining 54 training samples were found to comingle among other classes (#s 7, 8, 23, 24, 35, 38, 39, and 47). Because the initial visually-assigned class and the class with which they comingled differed by only one unit, and the definition of these classes was subjective and somewhat arbitrary, these eight samples were re-assigned. The newly arranged training dataset (54 samples, $n = 4,901$, Fig. 4.8b) was submitted to an exploratory DA as a final check prior to multi-pass descriptive DA. Four samples (#s 11, 32, 50, and 53) were rejected from the training dataset due to gross misclassification. The remaining 50 training samples (total $n = 4,634$) were submitted to the multipass Descriptive DA.

Optimum Number of Classes

As a check against the number of subjectively chosen *a priori* groups, an approximation of the optimum number of groups was obtained using the variance ratio criterion (VRC), one of the many stopping rules developed for clustering algorithms. For each value of k , ranging from 3-20, the VRC was computed as the maximum between-cluster variance divided by the minimum within-cluster variance. Calinski and Harabasz (1974) suggest that the first local maximum of VRC is an informal indicator of the optimal value of k . The first local maximum, and hence the suggested optimum number of groups, was observed at $k = 4$ (see Fig. 4.9). However, the plot of VRC also suggests that $k = 5$ is a reasonable number of groups, as this was the last value of k prior to the beginning of a monotonic decline beyond $k = 6$.

Exploratory DA versus K-means Clustering

The first local maxima of the VRC criterion suggested the optimum number of clusters was 4, or less favorably, 5. Table 4.2 is a comparison matrix of the exploratory DA classifications ($k = 5$) versus the PCA+K-means clusters ($k = 5$). In other words, the exploratory DA classification (Bottom Classes 1-5) of each training record is compared to the PCA+K-means cluster, obtained from the VRC optimum clusters analysis. For ease of interpretation, the comparison matrix was standardized to a constant number of 100 exploratory DA cases (Table 4.2b). Going across rows, it can be seen that 4 of the 5 K-means clusters were dominated by a single DA bottom class. The standardized comparison matrix suggests that Bottom Classes 1, 2, and 5 are all unique classes, and that Bottom Classes 3 and 4 form a 4th class. This reinforces the suggestion of the VRC criterion of an optimum number of 4 groups. However, given the diametrically-opposed design of the two algorithms (i.e. maximizing between-group variation of predefined groups (DA) versus minimizing within-cluster variation of undefined groups, or K-means), this level of agreement is strong evidence that the five pre-defined bottom types shown in Table 4.1 approaches an optimal balance between the number of bottom types and the capability of the hydroacoustics to differentiate between them.

Multi-Pass Descriptive DA (Supervised Training Catalog)

The five-group training dataset was submitted to a series of three descriptive DAs to: (i) refine the heterogeneous training dataset in “pure” end-member structural and biological elements; (ii) examine how the independent variables contribute to discrimination between groups, and (iii) generate a set of Fisher’s Linear Discriminant Functions (FLDF) for classification of survey records into these “pure” classes. The overall (internal) classification accuracy was 82.5% for the 1st Pass DA (n = 4,634), 98.2% for the 2nd Pass DA (n = 3,358), and 99.9% for the 3rd Pass DA (n = 3057) (Fig. 4.7). The effect of refining the training dataset by multiple passes through discriminant analysis is evident in the plots of the Discriminant Functions of the unrefined versus 3rd pass training data (see Fig. 4.10). It can also be seen how the higher-order discriminant functions allowed for differentiation of the most similar habitats (Classes 3, 4 and 5). The use of multiple frequencies improved the discriminatory power, although it was clear that the 418 kHz signal provided the most information. The main contribution of the 38 kHz signal was discriminating Class 4 from the other four bottom classes, in which the 38 kHz FD played a major role in the fourth discriminant function. The power of dual-frequency multivariate classification can also be seen in the GPS trackplots of individual 38 and 418 kHz acoustic parameters offshore Lighthouse Point (Figs. 4.11 and 4.12). The consistent Class 4 (unconsolidated “shell hash”) acoustic classifications within the delineated boundary west of the sand spit are achieved by the joint contributions of several acoustic parameters.

Robustness of the Training Dataset

The proportion of rejected records was equitably distributed amongst the individual training samples comprising the five classes, suggesting the independent variables represented spatially and temporally consistent seabed characteristics of the predefined groups (Fig. 4.13). If the proportion of rejected records had not been equitably distributed amongst training samples, i.e. if a few samples within a class passed through the three DAs unscathed while most others were heavily filtered, it would follow that the acoustics were not keying in on the diagnostic structural and biological elements but rather some superfluous and unrecognized variable. The proportion of rejected records was not equitably distributed between bottom classes, as evidenced by the range of 1st Pass DA producer’s accuracies. The high predictive accuracies of Classes 1 and 2 was presumably due to the relatively homogeneous nature of these habitats compared to the “rougher” classes (i.e. large expanses of uncolonized mud and sand were common), whereas hard bottom or SAV tended to be quite patchy. This necessitated the use of variable thresholds of probabilities of group membership (PG,i) in the multi-pass DA workflow (Fig. 4.7), tightening the constraints on Classes 1 and 2 and relaxing those of Classes 3, 4 and 5, to prevent over-prediction of the “purer” classes.

Testing for Significance

All 11 independent variables (38 and 418 kHz logE0, logE1’, logE1, logE2, and FD, 418 kHz depth) were found to be significant by forward stepwise DA, using Mahalanobis distance (MD) as the criteria and the probability of F for entry and removal (P = 0.05 and 0.10, respectively). The

MD is the distance of a case from the centroid of a group, in units of standard deviations, measured in n-dimensional attribute space (n = 11 here). The 3rd Pass descriptive DA model was found to be statistically significant ($P < 0.001$) based on the Chi-Square transformation Wilks' Lambda. The magnitude of Wilks' Lambda for the four discriminant functions (DF) utilized in the overall model was 0.016 (i.e. only 1.6% of the total variance in DF scores was not explained by differences among the groups).

Predictive DA (Classified Survey Trackplots)

The 103,544 survey records that passed QA were classified into 1 of 5 Acoustic Bottom Classes using the Fisher's linear discriminant functions obtained from the 3rd Pass descriptive DA. The classified acoustic trackplots are shown overlying bathymetry obtained by the South Florida Water Management District (SFWMD, see Fig. 4.14). The five classes are arranged in ascending order of roughness, and hence greater potential for acting as a drift macroalgae attachment site. Fig. 4.15 displays the supervised classification of towed-video transects collected independently in May 2009, alongside the acoustic classifications. The towed-video and acoustic classifications are in general agreement, although there appears to be a calibration bias regarding Classes 1 and 2. In the offshore Gulf of Mexico sites, what was acoustically-classified as Class 2 was judged to be Class 1 in the towed-video transects, but this is a minor difference, as both classes are soft bottom sediments unsuitable for macroalgae settlement. There were two areas within San Carlos Bay where the acoustic and video trackplots crossed (Fig. 4.15, Insets A and B). The two methodologies can be seen to generally agree on the transitions from smooth to rough bottom classifications.

Between-Site Comparisons (Acoustic Bottom Class and Depth)

Factors influencing an areas potential to produce a large-scale drift macroalgae event include seabed texture (availability of attachment sites for drifting macroalgae), spatial expanse (adequate surface area to generate required biomass), irradiance at depth, and nutrient availability. The hydroacoustic survey directly addressed the first two factors: (1) the supervised classification scheme utilized a training dataset categorized by visually-apparent "roughness"; and (2) the classified GPS trackplots allowed for demarcation of acoustically "roughness" areas. The hydroacoustic survey indirectly addressed irradiance by providing bathymetry along with estimations of seabed "roughness" (the attenuation coefficient of photosynthetically-active radiation being the missing part of the equation) with distance from the mouth of the Caloosahatchee also potentially useable as a rough indication of nutrient availability, with the exception of submarine groundwater discharge. General trends of acoustic "roughness" are evident in the classified trackplots (see Fig. 4.14). These visual trends were quantified by computing the proportions of acoustically-derived "roughness" and bathymetry for each of the six hydroacoustic survey sites (Fig. 4.16). The sites within San Carlos Bay and offshore of Lighthouse Point have the largest proportions of "rough" acoustic classifications (Classes 3, 4 and 5). Inside of San Carlos Bay, the rough areas consisted mainly of seagrass beds and areas of "live hard-bottom".

Large amounts of macroalgae were variably present during the April-May 2009 surveys. Offshore Lighthouse Point, there were substantial areas with moderate to high bottom roughness, mostly in the form of bivalve shell debris, on both sides of the large sand spit extending from the beach to approximately 6 km offshore. The requirements for a macroalgae bloom appear to be met in both of these areas. The San Carlos Bay and Lighthouse Point sites are both characterized by relatively shallow depths; the average depth of records classified as “rough” (Classes 3, 4 and 5) was only 4.0 m for San Carlos Bay and 5.0 m for the Lighthouse Point site. Both sites are situated along the outflow of the Caloosahatchee River and Estuary, which would presumably satisfy the nutrient requirements for a macroalgal bloom event. For example, if a macroalgal bloom were to occur in these areas, the bulk of the macroalgae could easily be transported onto the islands’ Gulf beaches given their close proximity to these areas.

In contrast, the sites in the open Gulf of Mexico waters (e.g., off Redfish Pass, Tarpon Bay Beach and Dinkins Bayou) were characterized as consisting of “uncolonized” or sparsely-vegetated mud and sand sediments in relatively deeper waters out to a distance of 11 km (acoustic) and 24 km (video) offshore. The site offshore Redfish Pass had a moderate (approximately 22%) proportion of “rough” classifications out to 5 km offshore, but from 5-10 km offshore the bottom was classified as greater than 95% soft sediments. The other two Gulf of Mexico sites were classified as having greater than 95% soft sediments from nearshore to 11 km offshore. These relatively homogenous habitats consist of unconsolidated sediments suggesting that the areas offshore from Sanibel and Captiva islands may not be a major source of drift macroalgae.

Spatial Extents of Acoustically Rough Areas

To be the source of a drift macroalgae event, a site must provide an area of rough seabed large enough to produce adequate biomass, in addition to adequate attachment sites, irradiance, and nutrients. For this example, two acoustically-rough areas (Classes 3, 4 and 5) were demarcated and their areas were computed within a GIS environment (see Fig. 4.17). These areas were the backside of the sand spit offshore Lighthouse Point (7.1 km²) and an area within the mouth of San Carlos Bay (12 km²). The hydroacoustic records within these two areas classified as consisting of 62 and 52% “rough”, respectively, compared to just 19% for the other records lying outside these two areas (see Fig. 4.17). For the example of the Lighthouse Point site, if drift macroalgae covered 33% of the delineated area (2.3 km²), and 10% of that algae washed ashore, the total coverage would be 0.23 km² of drift macroalgae (at the in situ density). This is approximately equal to the area of exposed beach between Lighthouse Point and Tarpon Bay Road (7 km x 35 m = 0.24 km²).

Accuracy Assessment

Accuracy assessment was conducted using samples withheld from the training dataset, since predictive accuracy will always be greater using the training dataset than for a new dataset (e.g., Kachigan, 1986; Huberty, 1994). A total of 117 ground-validation samples were collected inline

with the survey by intermittently slowing to idle speed, deploying a weighted video camera overboard, and simultaneously recording sonar and video for a period of 30-60 seconds. Of the 117 inline ground-validation samples collected, 89 (or 76%) remained for accuracy assessment. Samples were rejected from the accuracy assessment for a number of reasons, including: (a) depths less than the 1.75 m minimum; (b) those not passing the quality analysis filters; or (c) the seabed did not fit neatly into one of the five predefined bottom types. It was for the first reason that Class 5 (abundant SAV) was not included in the accuracy assessment, as all but one sample was too shallow. The overall predictive accuracy (Po) for the 89 ground-truthing samples was 80.0% for the four Seabed Classes (Table 4.3). The Tau coefficient for equal probability of group membership (Te) was 0.73 (i.e. the rate of misclassifications was 73% less than would be expected from random assignment of hydroacoustic records to one of the four classes).

Hydroacoustic and Underwater Towed-Video Tasks (Phase I)

Classification Scheme

The video imagery from all areas was assessed to determine the major bottom types identifiable, and to estimate the level of spatial heterogeneity of bottom types in each area. A video-based habitat classification matrix (see Table 4.4 and Fig. 4.18) was developed for comparison to the original seven acoustic seabed classes obtained from unsupervised classification of the Phase I hydroacoustic data (Fig. 4.19). The two new schemes were quite similar, the major difference being that more subclasses could be detected with video. After a February 2009 presentation of the initial hydroacoustic classification for Phase I only data, the group decided to reduce the number of classes in favor of greater predictive accuracy. Ultimately, a five class scheme was produced for Phase I and II hydroacoustic and towed-video classification (Table 4.1, Fig. 4.6).

A general conclusion of the Phase I studies was to use hydroacoustic methods within as much of the survey area as possible to about 10 km offshore, and to use towed video mapping at greater distances, to a maximum of approximately 20 km offshore. This allowed maximum use of survey time for both methods, and expansion of the overall survey area.

Hydroacoustic and Underwater Towed-Video Tasks (Phase II)

Hydroacoustic Surveys

Hydroacoustic surveys were conducted in the nearshore waters of Sanibel and Captiva Islands, Florida. Six areas encompassing the local diversity of benthic habitats were surveyed, including sites offshore Sanibel Island and within San Carlos Bay. The surveys were conducted in two segments: (1) a methods-development exercise conducted October 6-8, 2008 (Phase I); and (2) a larger-scale survey on May 10-22, 2009 (Phase II, Fig. 4.2). Three areas were surveyed in 2008: (1) a 7 x 2 km plot alongshore Lighthouse Point (400 m spacing); (2) a 6 x 1 km plot offshore Redfish Pass (200 m spacing); and (3) a 1,500 m meander through seagrass in Pine Island Sound. Five areas were surveyed in 2009: (1) an additional 7 x 2 km plot adjacent to the 2008 Lighthouse Point plot; (2) a 10 x 5 km plot offshore Fort Meyers Beach (1600 m spacing); (3) a 10 km transect offshore Tarpon Bay Road; (4) a 9 x 1.6 km plot offshore Dinkins Bayou (800 m

spacing); and (5) a 9 x 3.2 km plot within San Carlos Bay (800 m spacing). The majority (80%) of acoustic classifications were of soft bottom sediments (Classes 1 and 2), but there were two significant expanses of “rough” seabed thought to be suitable for macroalgae attachment (see Fig. 4.16). These two areas covered a total of 19 km², within which around 56% of the hydroacoustic records classified as “rough” (Classes 3, 4 and 5, Fig. 4.17). The first was a large area of seagrass beds and “live hard-bottom” in the mouth of San Carlos Bay, where large amounts of macroalgae were variably present during the April-May 2009 surveys. The second was offshore of Lighthouse Point, near the mouth of San Carlos Bay, situated near a large sand spit that extended from the beach to approximately 6 km offshore. Along the west side of the sand groin there were substantial areas of moderate to high “bottom roughness”, mostly in the form of unconsolidated shelly hash (e.g., see ASMFC, 2007).

The average depths of these two acoustically-rough areas were only 5.0 and 4.0 m, respectively, so sufficient irradiance to initiate a macroalgal bloom would be likely much of the time. These textured and shallow areas on or near the mouth of San Carlos Bay are presumably potential sources for macroalgae attachment and growth, and presumably could be readily transported onto the beaches under the right conditions given the close proximity to the shoreline. In contrast, the areas in open Gulf of Mexico waters were classified predominantly as soft sediments with low bottom roughness based on both the hydroacoustic and video surveys.

The site offshore of Redfish Pass had a moderate (about 22%) proportion of “rough” acoustic classifications out to 5 km offshore, but from 5-10 km offshore the bottom classified as >95% soft sediments. The other two Gulf of Mexico sites were acoustically classified as >95% soft sediments from nearshore to 11 km offshore. The towed-video transects indicated there were small areas with large amounts of shell and “live hard-bottom” that occurred sporadically greater than 10 km offshore (Figs. 4.14 and 4.15). Further assessments of these survey data in the context of available nutrients and ambient light levels will be needed to fully assess the bloom potential for these offshore sites, but it would appear that the open Gulf of Mexico waters around Sanibel-Captiva are probably not be a major source of drift macroalgae.

Underwater towed-video

Independent towed video mapping was conducted over the period 26 April – 8 May 2009, a total of 63 transects ranging from about 200 m to >1 km were recorded. The video surveys included San Carlos Bay, but focused on areas greater than 10 km offshore (see Fig. 4.15). The resulting classified imagery indicated several areas with high macroalgal biomass, in general corroborating the findings of the hydroacoustic surveys in the San Carlos Bay area (see above). Another major finding was the dominance of soft bottom areas in offshore areas (Classes 1 and 2).

Collaborative assessment of video imagery, hydroacoustic data with ground-truthing.

The hydroacoustic and video data yielded similar distributions of visually-apparent seabed roughness. At this time, three major trends with respect to macroalgae patterns are evident.

First, both methodologies revealed large areas within San Carlos Bay with high bottom roughness, and thus high potential for attachment of macroalgae (Fig. 4.15). Inside the Bay, these areas consisted mainly of seagrass beds and “live hard-bottom”, and during the April to May 2009 there were large amounts of attached and drift macroalgae. Second, the general area at the mouth of San Carlos Bay was particularly heterogeneous with respect to bottom roughness (yellow polygon in Fig. 4.17). The SFWMD bathymetric base map used in Figs. 4.14 and 4.15 shows what appears to be a large sand spit extending about 6 km offshore from Lighthouse Point (see white polygon in Fig. 4.17). The classified hydroacoustic data for this bathymetric feature confirmed that it is mainly soft sediments with low bottom roughness. However, substantial bottom areas covered by Classes 3 and 4 occurred on both sides of the spit, and nearly 0.8 km² of bottom area of Class 5 occurred just off the Point. The overall area covered by Classes 3, 4 and 5 probably represents a major potential source for macroalgae that could easily be washed up on the beaches under some storm conditions. Finally, most areas in open Gulf of Mexico waters from nearshore to approximately 20 km off the island appear to consist mainly of unconsolidated soft sediments with low “bottom roughness”, with most areas surveyed assigned to Classes 1 or 2 using the hydroacoustic or towed video data (Fig. 4.15). However, there were areas with large amounts of shell (Class 3), and “live hard-bottoms” (Class 4) that occurred sporadically in several areas greater than 10 or so km offshore. This trend suggests that the open Gulf of Mexico waters around Sanibel-Captiva may not be a major source of drift macroalgae.

Hydroacoustic and Underwater Towed-Video (Phase III)

Because no major macroalgal events were observed during the Phase I and II surveys, the overall aim of Phase III activities was to test the accuracy of previous mapping products from Phase II by sampling for the presence/absence of seasonal drift macroalgae at a time when the probability of a bloom was high. Unfortunately, macroalgal cover at the time of the Phase III towed-video survey was moderate, precluding a definitive assessment of macroalgae site predictions. However, the visually-classified towed-video stills were useful as additional biogenic layers (e.g., worm tubes, pen shells, etc.) relevant to the potential for macroalgal attachment and growth (Fig. 4.20). A common pattern emerged for the spatial distribution of pen shells, urchins, and macroalgae. The greatest abundances were found in the live hardbottom within San Carlos Bay (irradiance-modified Class 3) and adjacent to Lighthouse Point (irradiance-modified Class 3), and bivalve shell debris on either side of the sand spit offshore Lighthouse Point and offshore Fort Meyers beach (irradiance-modified Class 2).

Objective 5: To determine the distribution and productivity of attached macroalgal biomass and the conditions that are favorable for uncontrolled growth, including the examination of substrate attachment. (Milbrandt, Parsons and Ceilley)

During the two-year study, there were no large-scale beach stranding events equal in magnitude to the events that were photographed and described from 2003-2007. Despite the lack of

catastrophic macroalgal strandings, we found several new lines of information about the spatial and temporal patterns of macroalgal populations and characterized the types of habitats where they commonly occur. When the detailed information from the 13 stations is combined with large-scale habitat mapping efforts (Objective 4), the area's most likely to be sources of macroalgae and times of year when it is expected to wash up on area beaches are better understood.

Study Design and Station Selection

The stations varied widely in depth, sediment character, biology, distance from the Caloosahatchee, and macroalgal community composition (Table 5.2). While only a small percentage of the total seafloor was sampled, the diversity and descriptions of habitats encountered were integral to the seafloor mapping efforts undertaken in Objective 4. At each station, the prominent feature were recorded in field notes was determined, as well as the amount of potential attachment substrate. Detailed descriptions, including video screen captures, of each station are given in Appendix 5.2.

At the beginning of the study in 2007, little was known about types of algae growing offshore of Sanibel, the locations they are found, or what times of year were supportive of luxuriant growth. A list of all of the species collected can be found in Table 5.3. During the large-scale beach strandings of the early 2000s or shortly thereafter, several species lists were compiled by Dawes (2004), Bartleson et al. (2006) and Lapointe and Bedford (2007). These species commonly found on the beaches (Table 5.4) were likely to be the most abundant species growing in benthic habitats at the time of collection. Because there were 96 total species of macroalgae collected during this study (2008-2010), only those species found previously on beaches were highlighted for the analysis. These species were the most abundant species collected. For comparison, 221 taxa were identified for the Tampa Bay estuarine system, an area considered to have a luxuriant and diverse algal flora compared to other estuaries (Dawes 1982). However, many of the species collected in this study were only collected one time and are therefore not indicative of overall population patterns and trends. The trends and patterns from the most abundant species were highlighted.

There were a total of 96 macroalgal species collected and identified during the two-year study (see Table 5.4). Most of these species are branching red algae (Division Rhodophyta), with 12 species of brown algae (Division Phaeophyta), 14 total species of green algae (Division Chlorophyta) and 1 common cyanobacteria (Division Cyanophyta). A total of 20 macroalgal species were collected and identified on area beaches since 2003. These were collected either during this study or in previous studies (Dawes 2004, Bartleson et al. 2006, Lapointe and Bedford 2007). Most of the common species are branching red algae (Rhodophyta), but there are also brown algae (Phaeophyta) and green algae (Chlorophyta). Many of these same species were also commonly collected at the fixed stations (Fig. 5.1). Color plates of these common species are provided in Appendix 5.3.

The sampling stations were chosen at the beginning of the study based partly on previously collected underwater video in 2006 (J. Evans, City of Sanibel) with an emphasis on three transects with increasing distance from the presumed major nutrient source in the region, the Caloosahatchee. The character of the seafloor and other unique features are described. The diversity of habitats was largely unknown at the beginning of the study and the enormous potential area of the continental shelf made choosing sampling stations difficult. In the end, general locations were chosen based on depth and distance from the Caloosahatchee and were subsequently re-visited throughout the two year study. Stations were added to incorporate additional potential macroalgal habitat after discussions and related data collection by the hydroacoustic and underwater video analysis (see Objective 4). A station was discontinued (GOM09) after it was determined that the expense and difficulty of sampling were not adding value to the study as it was largely featureless with no macroalgae. Safety of the samplers and equipment was also a concern.

Much of the effort at stations where algae were rarely collected or never collected during the 12 sampling events was directed toward characterizing the seafloor and collecting samples for the other investigators. It was critical to re-visit stations with little to no macroalgae because of the possibility that macroalgae would occur at these stations if there were large amounts of algae on the beaches. Given that there were no large-scale bloom events during the study period, it is suspected that the stations routinely sampled with little to no macroalgae (9 of 13) could support macroalgal growth. Extensive descriptions and characterizations of the epibiota at these stations were used in Objectives 4 and 9 and should be helpful if/when the next large-scale event occurs. There are stations, for example that were far from shore and were largely featureless, with low potential for macroalgal attachment and growth. Other stations, however, had abundant large shell hash and living habitat (e.g. pen shells) which could provide abundant attachment substrates and should be considered as higher potential for the growth of macroalgae. More extensive investigations of these stations are not necessary unless there is another large-scale macroalgal event.

The stations where underwater video showed evidence of abundant macroalgae prior to the start of this study (provided by J. Evans, City of Sanibel) yielded little to no macroalgae during this study. These stations also had abundant potential grazers (e.g. *Lytechinus variagatus*). There were also abundant potential attachment sites (e.g. large shell, pen shells) and many of these stations, while not producing abundant macroalgae during the study period, were located in areas where longshore and tidal currents are conducive to macroalgal transport to the beaches when tested in the hydrodynamic model (see Objective 7).

There were four of 13 stations where macroalgae were routinely collected in moderate abundances. Additional investigations of these locations and identification of other potential source areas based on their location and biological characteristics may be needed. Histograms of these stations (Figs. 5.6 – 5.7) indicate that during the study period, macroalgae were

infrequently collected and observed in the large target area of the nearshore continental shelf. For inshore stations with macroalgae, there were 218 quadrats ($n = 250$) with less than 25 g FW m^{-2} macroalgae collected at CES11 and at GOM16, there were 168 quadrats ($n = 250$) with less than 25 g FW m^{-2} collected. For the offshore, live bottom stations, there were 176 quadrats ($n = 250$) with less than 25 g FW m^{-2} at GOM12 and at GOM11, there were 230 quadrats ($n = 250$) with less than 25 g FW m^{-2} . Macroalgae were routinely rare or absent throughout the study period at the remaining stations including; GOM01, GOM02, GOM03, GOM04, GOM05, GOM08, and GOM10 (See Appendix 5.4 for plots of biomass, percent cover by individual station).

The greatest amount of algae collected from a quadrat at CES11 was 371 g FW m^{-2} (27 g DW m^{-2}) collected in January 2010 where most of the biomass in the quadrat was *Spyridia filamentosa* (263 g FW m^{-2}). The greatest biomass at GOM16 was 409 g FW m^{-2} (37 g DW m^{-2}), collected in May 2010 and was also mostly *Spyridia filamentosa* (393 g FW m^{-2} , 31 g DW m^{-2}). For the offshore/live bottom stations, the greatest biomass at GOM11 was 504 g FW m^{-2} , collected in July 2009 and was mostly the red alga, *Botryocladia occidentalis* (454 g FW m^{-2} , 40.86 g DW m^{-2}). At GOM12, the greatest biomass was 704 g FW m^{-2} (55 g DW m^{-2}), also collected in July 2009 with a majority comprised of both *Caulerpa racemosa* (194 g FW m^{-2} , 22 g DW m^{-2}) and the red alga, *Halymenia pseudofloresia* (258 g FW m^{-2} , 10 g DW m^{-2}). As mentioned previously, little to no algae was collected at the other sentinel stations. However, there were notable amounts of unattached, drifting algae at stations GOM06 and GOM07, as quantitated by the underwater video analysis (see below), but was not collected in quadrats because of the difficulty in sampling a known area of the seafloor while the macroalgae drifted past. Samples were collected and brought back to the lab for identification. The total biomass from the individual stations (all species combined) is presented as dry weight biomass per quadrat (Appendix 5.4), wet weight biomass per quadrat (Appendix 5.4) and by percent cover (Appendix 5.4).

The number of species was consistently higher at stations where algae was frequently collected (CES11, GOM16, GOM11, GOM12). Diversity indices were summarized for each sampling event and station in Tables 5.5 and 5.6. The greatest number of species in a transect occurred at GOM12 in July 2009 and June 2010, when a total of 38 species were collected and identified. The greatest number at GOM11 was 15 in July 2009. The greatest number of species collected at CES11 was 19 total in March 2010 while the greatest number at GOM16 was 16 in May 2009. Diversity (H') was highest at the offshore stations GOM11 and GOM12 and lower at the other stations. The seasonal differences between inshore and offshore/live bottom were reflected in diversity (H'). CES11 had highest diversity in January and March in both years during the study, while GOM11 and GOM12 had higher diversity in July and in both years.

There were two distinct community types as identified by a statistical analysis of macroalgal species composition and abundances (MDS, PRIMER, ver. 5). One community type associated

with inshore stations near the Sanibel causeway and a second associated with offshore live bottom and patch reef habitats. Large amounts of macroalgae were comparatively rare, but did occur seasonally at these four stations. All stations and all macroalgal biomass data were analyzed together using PRIMER. The details of the analysis are described in the methods. The results of the MDS (see Fig 5.8) indicate that there are two distinct assemblages, as alluded to previously. A One-Way ANOSIM was used to test the significance of these assemblage differences. The results indicate significant differences (Global $R = 0.241$) between groups; inshore, offshore ($R = 0.357$, $p = 0.01$); inshore, other ($R = 0.259$, $p = 0.01$); offshore, other ($R = 0.116$, $p = 0.02$). The differences are greatest between inshore and offshore live bottom, while there are smaller differences between offshore live bottom and the other stations. The species responsible for these differences among groups were determined using PRIMER (SIMPER, Ver. 5). Inshore species responsible for the community differences were *Acanthophora spicifera*, *Champia parvula*, *Dasya* spp., *Gracilaria tikvahiae*, *Hypnea spinella*, *Lomentaria baileyana*, and *Spyridia filamentosa* (Table 5.7). The most species defining the live bottom, offshore macroalgal communities included; *Agardhiella subulata*, *Botryocladia occidentalis*, *Euचेuma isiforme*, *Gracilaria* spp., *Hypnea spinella* and *Solieria filiformis*.

Inshore Macroalgal Communities

Samples collected at inshore stations GOM16 and CES11 were similar in composition, as expected. These two stations were located near the Sanibel causeway and shared similar sand to silt sediments and had similar irradiances and salinities. These inshore habitats were often characterized by other biogenic features; including patchy seagrasses or tube-building polychaetes (e.g., *Diopatra cuprea*, Chaetopteridae). GOM16 had patchy seagrasses, which has been demonstrated elsewhere to trap drift macroalgae (Riegl et al. 2007) and encourage growth of psammophytic algae (Brown 2001, Dawes 2004). CES11 had abundant tube-building polychaetes (*Diopatra cuprea*) capable of providing settlement substrates for macroalgae (Thomsen and McGlathery 2005).

At station CES11, there were peaks in July 2009 and in February 2010 (see Appendix 5.4). At GOM16, there were peaks in June 2009 and July 2010 (Appendix 5.4); the peak in July 2010 was caused by a single species, the red alga, *Spyridia filamentosa*. At CES11, the peak biomass occurred early in the spring (January to March) in *Champia parvula*, *Lomentaria baileyana*, and *Sargassum* spp. (Figs. 5.9-5.11). The other species encountered were rare, typically with one or two months of one species having a large proportion of the total biomass but that species was not collected in any other months. The daily growth rate (DGR) for all species at CES11 from January to March 2009 was 2% and from November 2009 to March 2010 DGR was 5% (DGR calculation is described in the methods).

At GOM16, there was an early spring peak in *Lomentaria baileyana*, *G. tikvahiae*, and *Spyridia filamentosa* (Figs. 5.12-5.14). It is not surprising that the patterns would reflect those found at nearby CES11. The mapping group demonstrated significant macroalgae associated with

seagrasses around the Sanibel causeway and GOM16 was made a permanent station in January 2009. Given that this station was not established until after the first hydroacoustic mapping (see Objective 4) in January 2009, therefore the fall of 2008 was not captured. Daily growth rates between March and May 2010 was high (5%), an indication that macroalgae can grow quickly at these inshore stations.

Upon further review of individual species plots from CES11 and GOM16, it became apparent that the peak biomass at inshore stations occurred earlier, between January and May during both years sampled. *Champia parvula*, *G. tikvahiae*, *L. baileyana*, and *Sargassum* spp. had peak biomasses between Jan. and May at CES11 in both years of the two year study. While *Dasya* spp., *H. spinella*, and *L. baileyana* had peaks between Jan. and May at GOM16. In addition, extra trawl sampling for the grazing experiments (see Objective 9) yielded unusually large amounts of macroalgae from around the causeway further supporting the occurrence of an early spring to early summer peak in biomass. Other similar studies in the Indian River Lagoon, FL, also documented peak biomass in early spring (Virnstein and Carbonara 1985, Benz et al. 1979). It is thought that shading by seagrass blades, low salinities, and growth inhibition in summer caused by excessive temperatures (greater than 31 °C) are reasons for why macroalgae are not abundant inshore in late summer and fall. The lack of macroalgal abundance in the late summer and fall are not consistent with higher nutrient loads and the hypothesis that land derived nutrients are causing macroalgal blooms (Lapointe and Bedford 2007).

Salinities recorded in the early spring were typically above 32 and the K_d PAR was typically lower in the early spring and summer than during the late summer and early fall. While global correlations between macroalgal biomass and either salinities or temperatures were not significant, there does appear to be a consistent pattern in the amount and timing of macroalgal biomass at inshore stations. Flow from S-77 and S-79 and potential nutrient loading indicated that the early spring runoff in 2009 was from the Caloosahatchee drainage (as opposed to Lake Okeechobee) while the majority in 2010 was from Lake Okeechobee. However, this did not seem to translate into differences in macroalgal abundances, and there was less biomass during the early spring to early summer in 2010 than in 2009 at CES11.

Seasonal variability at all stations was also evident in temperature collected over the study period (Fig. 5.15). Temperature maxima (31°C) occurred in July 2009 and the lowest temperature was recorded in March 2010. Given that the sampling events were 2 months apart, these summary plots show a snapshot of the actual water conditions for one day out of two months. Greater resolution temperature data, as provided by SCCF's River, Estuary, and Coastal Observing Network (RECON), indicated one day to week-long periods where the temperatures at Shell Point (inshore) were a few °C higher than in the nearshore Gulf of Mexico (Figs. 5.16, 5.17). These occurred from January 1 through March 25, 2009 and again from January 1 through March 25, 2010 and correspond with the maximum biomass for several inshore macroalgal species. While there were no global correlations between macroalgal biomass and temperature

(see Table 5.8), there were long periods of consistently warmer temperatures for days to weeks as recorded at inshore RECON stations. The temperatures inshore were up to 2-3 °C warmer than the Gulf of Mexico during the spring after cold fronts. It is possible that macroalgal growth on the seafloor was inhibited at stations (e.g. off of Sanibel beaches) in the Gulf of Mexico while growth at inshore stations was more vigorous in the shallow and warmer water.

Inshore macroalgal communities have been well-documented in west Florida, including their contribution as habitat for juvenile fishes and invertebrates (Hooks et al. 1976). However, the transport mechanisms and processes responsible for drift algae movement and connectivity within the inshore bays and to the beaches and Gulf remain poorly understood. Decomposition rates are relatively rapid with up to 65% of the original algal mass degrading after 25 days due to the lack of structural polysaccharide complexity (Sassi et al. 1988). These high rates can provide an important supply of organic and inorganic nutrients that can be rapidly assimilated by other macroalgae, phytoplankton, phytobenthos and zooxanthellae in reefs. Additional investigations of the inshore stations, including nutrient addition experiments and determining the enabling conditions for growth are needed.

Not all effects of the inshore macroalgal community are beneficial as large accumulations can lead to oxygen deficiency and decreased diversity in the benthic community (Norkko et al. 2000). Excessive abundances of macroalgae in the inshore can also decrease the amount of available light in seagrass beds (Williams Cowper 1978). Additional investigations of inshore areas during a large scale bloom are possible to determine the adverse effects of large ‘tumbleweeds’ of drift algae on seagrass growth and anoxia. This information requires a manipulative sampling approach, not used in this study whose primary aims were to investigate macroalgal patterns and trends over a large area.

Offshore, Live Bottom Macroalgal Community

The second macroalgal community type, as distinguished in the MDS analysis (PRIMER, ver. 5), was found at stations with live bottom and limestone patch reef. This habitat ‘type’ is likely to be quite variable within the study area and was found at GOM11 and GOM12. There are large platform reefs with low relief, higher relief shelves and features, and hard substrates with a thin veneer of carbonate and siliceous sediments. The offshore, live bottom and patch reef habitats were characterized by an abundance of hard and soft corals. While the stations chosen to sample were representative, all types of live bottom habitat were not likely characterized. However, the bathymetry and towed underwater video recorded correlations between shallower sand covered limestone versus the deeper exposed limestone which may be useful for identifying additional locations not visited during this study (see Objective 4).

Peak biomass offshore at stations GOM11 and GOM12 occurred mid to late summer (Figs. 5.18-5.20). The species with peak summer biomass included: *Agardhiella subulata*, *Botryocladia occidentalis*, *Hypnea spinella*, *Gracilaria tikvahiae*, *Sargassum* spp., and *Solieria filiformis*. The daily growth rate (DGR) for all species at GOM11 from May 2009 to July 2009 was 60% with

total average biomass of 74 g FW m⁻² m⁻² (n = 20; SE = 20). Biomass in June 2008 was 34 g FW m⁻² d⁻¹ (n = 30; SE = 10) but growth rates could not be determined because the station was not sampled in previous months. Peak biomass at GOM12 also occurred in July through September, later than the peak inshore periods. *Agardhiella subulata*, *Botryocladia occidentalis*, *Champia parvula*, *Gracilaria* spp., *Hypnea spinella*, *Sargassum* spp., and *Solieria filiformis* had peak biomass in mid to late summer, suggesting a broad scale temporal pattern separating the offshore/live bottom habitats from the productive inshore habitats (Figs. 5.21-5.24). Daily growth rate (DGR) between May 2009 and July 2009 GOM12 was also high at 79% increasing from 5 g FW m⁻² d⁻¹ (n = 20; SE = 4) to 227 g FW m⁻² d⁻¹ (n = 20; SE = 33) in the two month period. Another estimate of DGR from March to June 2010 at GOM12 was 18. Overall, the offset in peak biomass between inshore and offshore habitats has implications for the timing of strandings on area beaches and may help explain the seemingly stochastic occurrence of beach strandings historically (see timeline, Objective 9).

This study is the first comprehensive examination of the macroalgal community and the associated live bottom habitats since the historic Hourglass expeditionary study (Joyce and Williams 1969). The live bottom on the shelf typically had abundant macroalgae and a diverse and abundant epibiota. The epibiota included hard and soft corals, large barrel sponges, tunicates, and a variety of other invertebrates. The macroalgal community was also more speciose with a greater number of species and greater Shannon-Wiener diversity (H') at GOM11 and GOM12 than at CES11 or GOM16. The algal community at live bottom stations also had a greater variety of morphologies, including branched, sheet-like, and flattened types with a greater number of green and brown algae. The assemblage collected in this study is somewhat similar to that described in the 1960s (Dawes 1969) but the results of this study have much greater spatial and temporal resolution regarding the habitats offshore.

Many of the macroalgae collected from the limestone patch reefs had well developed discoid and rhizoid holdfasts. Those species, such as *A. subulata*, *B. occidentalis*, *Caulerpa* spp., *E. isiforme*, and *G. mammillaris*, were rarely collected inshore and accounted for the differences in species composition between the two community types determined in the MDS. The well-developed holdfasts translated into greater forces needed to dislodge algae from the limestone. As stated above, the flow velocities from the predictive hydrodynamic model could be used to determine the instances where macroalgae would become dislodged if the drag coefficient for a given species were known. Empirically, the determination of drag coefficients for complex morphologies (i.e. highly branched red) requires a flume, however, there are empirical and estimated drag coefficients available in the literature (e.g. Carrington 1990, Dudgeon and Johnson 1992).

Macroalgal Communities from Two Minor Beach Strandings

The timing of peak biomass has important implications for when and where macroalgae washes up on Sanibel's beaches. The timing of the peak biomass at offshore, live bottom stations

(GOM11, GOM12) occurred from July to November 2009. As mentioned above, the peak biomass at inshore stations (CES11, GOM16) near the causeway was from January to May. A beach stranding event (during March 2010) was sampled during the second year of the study. The events were limited to a small area of the beach and were sampled opportunistically, not as part of the concerted effort in Objective 6. We used PRIMER to determine whether the macroalgal community on the beach was similar to nearby sampling stations. The samples were analyzed using the presence/absence function and MDS in PRIMER. The results indicated that the species composition on the beach was most similar to GOM16 and CES11 (Fig. 5.25). From this single event in March 2010, it appeared that the macroalgal community washed up on the beach in March 2010 appeared to match more closely with the composition at the inshore stations, near the causeway.

Other more recent beach strandings (November 2010; Evans, pers. comm.) were composed of primarily live bottom associated species, and it is possible strong winds and lower temperatures caused offshore macroalgal communities to be dislodged and transported to the beaches. In requested simulations for Objective 7, neutral density ‘floats’ released at GOM11 and GOM12 were eventually transported to Sanibel’s beaches through longshore currents in the simulations. The mapping efforts in Objective 4 identified the spatial extent of several live bottom areas with potential to support macroalgal growth. If beach stranding events on the southern and west-end beaches of Sanibel are reoccurring, there may be the need for further investigation. Future sampling efforts should focus on more frequent sampling intervals and/or manipulative elements (e.g. nutrient addition; Smith et al. 2001).

Drifting Macroalgae

Fragmentation or loss of large portions of an attached alga is common. Macroalgae growing in calm water often become brittle and have a propensity to fragment (Norton and Mathiesson 1983). These unattached fragments are difficult to quantify when surveying a quadrat of known area and using the surveying and collection techniques described above.

The primary purpose of analyzing the underwater video, as stated previously, was to sample the entire length of the transect rather than at the 20 sub-sampled quadrats where attached macroalgae was collected. There were several dates and sampling events, as mentioned in the methods section, in which the quality of the video was not sufficient for recognizing the outline of the algae or enumerating invertebrates (see Table 5.1). This unavoidable consequence of using underwater video certainly has limitations in the overall comparisons made, but actual diver observations were made at the same stations and dates despite the poor visibility. The inshore stations (GOM16, CES11) were particularly problematic due to the large amount of colored dissolved organic matter (CDOM) and suspended material in the water.

The general trends described for the diver collected percent cover and underwater video percent cover were consistent between the methods at most locations and times with the exception of GOM06 and GOM07 in March 2009 (Appendix 5.4). These stations are located nearshore along

Estero Island and Bonita Beach (see Fig 5.1). There was very little attached macroalgae collected and reported at these locations and the results are reflected in the biomass plots (Appendix 5.4). A Kruskal-Wallis one-way ANOVA indicated significant differences in cover between the underwater video analysis and underwater observations of cover at these two stations, but not at other locations ($p < 0.001$). The underwater video analysis indicated that in March 2010, there was a maximum mean macroalgal cover of 36% ($n = 159$) at GOM06 and 8% ($n = 155$) at GOM07. Average percent cover by underwater observers was 1% ($n = 20$) at GOM06 and 0% ($n = 20$) at GOM07. In reviewing the video, large pieces of drift macroalgae were apparent in the video that were not collected in quadrats. An alternative approach, such as the analysis of underwater video was necessary. When there is a large amount of unattached, algae drifting in the water column, the quadrat collection method underestimated the drift biomass. Underwater video appropriately quantified the large drifting fragments. The total macroalgae percent cover for each year were reported along with the occurrence of other prominent benthic biological features, including sponges, hard corals, and soft corals (Appendix 5.4).

Underwater video analysis on the same transects sampled by divers did also detect differences in macroalgal morphology during the study period. Using a modified approach from Steneck and Delthier (1996) originally designed for investigating grazing preference, the functional groups of the macroalgae were determined to enlighten the study design of Objective 9. While valuable for determining discrepancies with other methods, the underwater video and analysis was primarily used to provide a more complete picture of the study site with a greater number of replicates than two divers using quadrats at random points on the transect.

Relationships Between Physical, Biological Parameters and Macroalgal Abundance

The physical data collected bi-monthly at each station were not predictive of the total macroalgal biomass. This was a surprising finding given the seasonal patterns apparent in the biomass plots of individual macroalgal species plots. Of all the physical parameters measured, temperature and light should be strongly correlated with algal biomass based on previous physiological studies (Lobban et al. 1985). Temperature had the greatest correlation coefficient with total biomass and percent cover, but this correlation was not significant (Table 5.8). Pearson correlation between dry weight biomass and temperature was 0.113; between fresh weight biomass and temperature, 0.131; and between percent cover and temperature, 0.143. Another potentially important physical parameter often highly correlated with temperature is salinity (Fig. 5.26). There were large differences in salinity at the inshore stations, as expected given the proximity to freshwater sources in the Caloosahatchee Estuary. In the lower estuary, salinities during the summer months are lower than in the Gulf of Mexico due to rainfall and watershed freshwater input (see RECON website; <http://recon.sccf.org>). The landfall and subsequent release of water associated with TS Fay drove salinities down at the inshore stations from September through November 2009 while salinities in the Gulf of Mexico remained at 37. Unusually high salinities were evident in the Gulf of Mexico at the beginning of the study period (June 2008). During the early

winter of 2009-10, salinities at inshore stations were 32, which is high for an estuary. Salinity, however, explained little or no variation in macroalgal biomass or percent cover (Table 5.8).

Irradiance at depth (I_z) was not correlated to biomass which was somewhat surprising (Table 5.8). However, the measurement of irradiance at one point in time and space does not characterize the light field experienced by an alga. Sun angle, day length, sea state, and shading are all potential causes of variation in light availability not captured by a single estimate of irradiance. Plots of irradiance at depth where macroalgae were routinely collected indicated large variability and indicated that high irradiances were not necessarily required for macroalgal growth. There was greater light attenuation at inshore stations than other stations in the nearshore and offshore (Fig. 5.27-5.29).

However, photosynthetic efficiency and productivity may provide an indication of the physiological effects of irradiance on macroalgae, as opposed to using the total biomass. The sampling design was intended to study a wide range of habitats and conditions over a relatively large potential area (photic zone from Redfish Pass to Wiggins Pass).

The occurrence of macroalgae was strongly related to the type of substrate (see Objective 4) and the biological parameters measured. There was a significant negative correlations between macroalgal biomass (Pearson correlation coefficient; FW, $r = -0.186$; DW, $r = -0.217$) and *Lytechinus variegates* density (Table 5.9). This finding has implications for the role of grazers in areas with little to no macroalgae (see Objective 9) and the lack of grazers where there are abundant macroalgae.

There was also a significant negative correlation between macroalgal percent cover and pen shells ($r = -0.185$; Table 5.9). This suggests that pen shells are not an important settling location for macroalgae as suggested previously (Perry 1936). However, the rough bottom that is created by pen shell populations is likely to trap drifting macroalgae and could be a substrate for macroalgal attachment. There were several occasions where macroalgae was loosely attached to pen shells but was difficult to quantify in a quadrat. The analysis of underwater video revealed abundant drifting and loosely attached macroalgae at GOM06 and GOM07 in March 2009. At both of these locations, strong tidal currents and close proximity to the tidal passes led to the occurrence of drift macroalgae. This phenomenon of large amounts of drifting detached macroalgae was not evident at other nearshore stations with pen shells (e.g. around Sanibel), and may be a function of the tidal current and overall hydrodynamics south of the Sanibel causeway (see Objective 7). For these stations, the analysis of underwater video provided new information not captured using the quadrat and underwater collection techniques. Further examination of GOM06 and GOM07 are needed when Fort Myers Beach macroalgal strandings occur, as these stations are likely to support luxuriant macroalgal growth and large amounts of drifting algae.

Measured Detachment Force

As the role of substrate type in the spatial distributions of macroalgae became increasingly important, the detachment force required to remove attached algae from two predominant attachment substrates were compared (Fig. 5.30). All macroalgal species were combined for this analysis in order to provide a comparison of the substrates. The macroalgal species were either loosely attached or attached by large discoid holdfasts and the differences among species with regard to attachment mechanism was not very different. As the results from this study would suggest, the same species are not found in all locations. Worm tubes (0.40 Newtons, n = 30) had a lower mean detachment force than limestone (0.65 N, n = 30). The results of the t-test indicated that the differences were borderline significant (p = 0.07). Additional replication would likely demonstrate a p-value of less than 0.05.

As demonstrated in Hog Island, VA (Thomsen and McGlathery 2005), *Diopatra cuprea* facilitates algal persistence against hydrodynamic forces such as tidal currents or storm surge by providing a stable attachment substrate. While the strength of macroalgal attachment to *Diopatra cuprea* was less than that measured for limestone, the force required to remove the macroalgae was 0.4 N and depending on the amount of drag and velocity (Denny 1995, Shaughnessy et al. 1996), there would be instances when the hydrodynamic forces would not be sufficient to cause detachment. The exact velocities needed to cause macroalgae to become detached can be determined from the literature or if the drag coefficients of the macroalgal species can be estimated or measured empirically (Vogel 1994). Additional coupling to the hydrodynamic model would require additional experimentation with a flume to measure empirically the drag coefficients of complex morphologies of the locally occurring species. These local species are typically highly branched and less conducive to predicted measurements of drag based on surface area compared to simple morphologies (i.e. sheet, blade).

PAM Fluorometry Results

One hundred and sixty seven algal samples were analyzed by PAM fluorometry, comprising 48 species (and one mixed sample) with the following characteristics (Table 5.10). *Botryocladia occidentalis* was analyzed most often (14 separate occasions), followed by *Solieria filiformis* (12), and *Agardhiella ramosissima* (11). Ten species were analyzed at least five times, and thirty three were sampled at least twice. Sixteen were only analyzed one time.

Rhodophytes comprised the majority of samples analyzed (133), followed by phaeophytes (15), and chlorophytes (13). *Gracilaria* was the most common genera analyzed (39 times), followed by *Agardhiella* (20), *Botryocladia* (14), and *Solieria* (12). All other genera were analyzed fewer than 10 times (Table 5.11). CES11 contributed the most samples to PAM fluorometry (40), followed closely by GOM12 (37), and GOM11 (32). Seven stations had fewer than ten samples analyzed over the course of the project (Table 5.12). At least ten samples were collected and analyzed every month of the study (Table 5.13). July 2009 had the most samples analyzed (25), followed by May 2009 (16). All others had between 10 – 15 samples analyzed.

Statistical analysis of quantum yield (QY) data I: testing for spatial, temporal, and taxonomic differences

Ideally, QY data would have been collected for a multitude of species across all locations and sampling events. Examination of the data collected as summarized in the above tables, however, indicates that QY analysis was dominated by a few species (Table 5.10) from four genera (Table 5.11) collected at five stations (Table 5.12). Temporal coverage, on the other hand, was consistent for the most part (Table 5.13). As the most statistical power is gained when one can account for variability (in our case caused by differences between species, locations, and/or time), we concentrated our statistical efforts on those data with the best temporal and spatial coverage; specifically the top six species shown in Table 5.10 and the top four genera from Table 5.11. We also tested for differences in QY between classes, seasons, and location (inshore versus offshore) to provide a more complete picture of the variability observed in the QY data that could then be interpreted in relation to the environmental conditions and parameters of the estuarine and coastal ecosystems in question.

An analysis of QY differences versus location (inshore versus offshore) for the six most common algal species indicates that *Solieria filiformis* was the only species to show a significant difference ($p = 0.028$), in which QY was higher (on average) at inshore versus offshore locations (Table 5.14). The other five species showed no significant difference in QY relative to location. In a similar fashion, *Solieria filiformis* had significantly higher QY values during the dry season versus the wet season (Table 5.15). The other five species showed no significant difference in QY relative to season.

In order to better examine the interaction between location and season, two-factor ANOVAs were computed using the Generalized Linear Model algorithm in SPSS ver. 17.0. The QY data had to be analyzed by genera rather than species to obtain enough data to meet the necessary criteria (i.e., inshore-wet vs. inshore-dry vs. offshore-wet vs. offshore dry) to run the test. The top four genera were analyzed, and results indicated that *Agardhiella* and *Gracilaria* had significantly higher QY values during the dry season at inshore locations, with no significant seasonal differences at offshore locations. Additionally, there were no significant differences between locations. These results suggest that at inshore locations, algal growth is more favorable during the dry season, whereas seasonality may not play as significant of a role at offshore locations. The other genera did not display significant differences between locations or seasons (Table 5.16). The QY data were also grouped by algal class (rhodophytes, phaeophytes, and chlorophytes) to provide an even more robust dataset to test for location and seasonal differences in QY, and no significant differences were seen (data not shown).

The next set of analyses in this section tested for differences in QY relative to taxonomic differences. Two approaches were used: 1) a paired t-test, where the QY values were compared among species or genera, or classes for those data having matching sample dates & locations

(thereby accounting for spatial and temporal differences); and 2) three-factor ANOVA, where genus is included as a factor in addition to season and location as in previous analyses.

The QY values of the six most common species listed in Table 5.10 were compared using paired t-test analysis (Table 5.17). The results indicate that only three of the 15 comparisons were significant, with *Agardhiella ramosissima* having lower QY values than *A. subulata*, and *Botryocladia occidentalis* having higher QY values than both *Gracilaria mammillaris* and *Solieria filiformis*. Paired t-test results among the four most common genera listed in Table 5.11 indicated that there was no significant difference in QY values among the genera (data not shown). There were significant differences in QY values among the three classes of algae, however. Rhodophytes consistently had lower QY values than both phaeophytes and chlorophytes, suggesting physiologic differences among the three algal groups (Table 5.18).

Overall, the genus of the algae does not appear to be a factor in determining QY (Table 5.19), although location (inshore vs. offshore) does play a significant role (inshore QY values are higher than offshore). A paired t-test comparing inshore vs. offshore QY values across all sampling dates, however, demonstrates that there is no significant difference in QY values ($p = 0.824$), but that the inshore values are highly correlated to the offshore values ($r = 0.808$; $p = 0.005$), indicating that offshore and inshore algal populations may be responding to larger, regional drivers, rather than those restricted to each location. This premise will be explored in more detail below.

Statistical analysis of quantum yield (QY) data II: testing for the role of water parameters

The QY data were examined in relation to the water quality data (specifically bottom temperature, bottom salinity, and bottom light intensity (I_z)) to determine if and how these variables affected the QY of the algal population as a whole. The QY values from all specimens analyzed from a particular station and sampling event were averaged, and these values were then compared to the water quality data collected at the same location and time. Prior to analysis, however, the QY data and water quality data were three-point averaged (i.e., each data point was averaged with the value immediate below and above it) to smooth trends (reduce noise) in the data. Results indicate that QY was negatively correlated with temperature ($r^2 = 0.42$; $p < 0.0001$; Fig. 5.31), suggesting that higher temperatures may be a stressor for the algae (Eggert et al. 2006). Quantum yield was positively correlated with salinity ($r^2 = 0.20$; $p = 0.03$; Fig. 5.32), although the relationship is not as clear, likely due to differences in inshore and offshore environments (data not shown). Other researchers have also found that QY responds to salinity (Theil et al. 2007), indicating that low salinities can be a potential stressor to marine algae. Quantum yield was negatively correlated with bottom light intensity ($r^2 = 0.56$; $p = 0.02$) when examined using monthly-averaged data ($n = 11$; Fig. 5.33). When light levels decrease below $20 \mu\text{E}/\text{m}^2/\text{s}$, however, quantum yield values start to decrease, possibly indicating the onset of light limitation. Quantum yields are stable between 15 and $35 \mu\text{E}/\text{m}^2/\text{s}$, suggesting that optimal light intensities for the algae examined in this study lie in that range. Light intensities over 40

$\mu\text{E}/\text{m}^2/\text{s}$ are related to decreasing quantum yield values, possibly reflecting photoinhibition. Haeder et al. (1998) found similar relationships between low QY and high light intensities indicative of photoinhibition.

The above results (QY being negatively correlated with temperature and light, and positively correlated with salinity) can be interpreted as follows. Inshore locations typically have lower salinities, and will often have higher light intensities than (deeper) offshore sites as mentioned above, and their shallow waters will warm faster than offshore waters in the summer. These apparent contradictions indicate that 1) conditions must be suitable for algal growth; i.e., not too much light, not too hot, and not too fresh; and 2) inshore locations must have other beneficial characteristics for algal growth (e.g., nutrient availability).

Although the above regression results indicate significant relationships exist between the water quality variables and QY, the relatively low r^2 values indicate that the goodness of fit could be better. Trigonometric equations were developed to simulate several of the variables (QY, I_z , and temperature) to explore how each variable changes over the course of one year (365 Julian days). To develop the trigonometric equations, the data were averaged by month, and equations were developed and regressed against the averaged data until the one with the best fit was determined. The equations and the associated regression results are shown in Table 5.20. The three simulations (for QY, I_z , and temperature) are displayed in Fig. 5.34. Salinity did not show a seasonal cycle (data not shown), possibly due to the fact that freshwater inflow is regulated and therefore does not adhere to seasonal patterns.

QY peaks between day 65 and 70 (mid-February) and reaches its low between days 252 and 258 (mid-August). Light (I_z) has a similar (but more pronounced profile) that occurs just over a month later, with a peak on day 112 (late April) and the low on day 294 (late October). Temperature variation is more subdued, with a peak on day 188 (early July) and a low on day 4 (early January). Temperature is nearly out of phase with QY, where increasing temperatures correlate with decreasing QY, and vice versa (as shown in Fig. 5.34). A 180 day difference in peaks or lows would be perfectly out of phase; temperature and QY are approximately 120 days out of phase. The lag between QY and I_z suggests that increasing light intensity stimulates algal growth to a point (in mid-February), after which light levels are too high, when coupled with increasing temperatures, may be leading to photoinhibition (and lower QY values). It is not until the fall when temperatures and bottom light intensity decrease enough to allow recovery and subsequent increases in QY (and likely algal growth). Therefore, the conditions most suitable for algal growth would be moderate temperatures ($<25\text{C}$), high salinities (>35), and low/moderate light intensities ($15 - 35 \mu\text{E}/\text{m}^2/\text{s}$). If the peaks in algal biomass in the spring (inshore) and late summer (offshore) reflect higher growth, then it suggests that these conditions may be more common in inshore waters during the spring and offshore in the late summer. While the above growth conditions can be conducive for growth, the other important factor is nutrients, as discussed below.

Statistical analysis of quantum yield (QY) data III: stable isotope analysis and tissue nutrient data

Two hundred and sixty eight samples were processed and analyzed at the Stable Isotope Facility at the University of California Davis. Table 5.21 provides an overview of samples processed. *Solieria filiformis* was analyzed the most of the algal species, although as a genus, *Gracilaria* was subjected to the most analyses. Station selection was dominated by GOM12, but inshore and offshore locations were well sampled. Samples from a variety of dates were also sampled, although 2010 had three of the top five sampling events.

C:N was higher at offshore versus inshore locations when the data from all algae were statistically analyzed (Table 5.22), but more specific tests (*Gracilaria mammillaris*, all rhodophytes, and *Solieria filiformis*) did not exhibit this same significant difference. Rhodophytes and *Solieria filiformis* exhibited higher C:N ratios during the dry season. These results suggest that nitrogen may be more abundant (and therefore sequestered in algal tissue) inshore and during the wet season. Rhodophytes and *Solieria filiformis* also had higher $\delta^{15}\text{N}$ ratios inshore, but significantly higher $\delta^{15}\text{N}$ ratios during the dry season. The $\delta^{15}\text{N}$ ratios were significantly higher during the dry season when the isotope data from all algae were analyzed as well. These results suggest that inshore (and/or during the dry season), algae rely more heavily on regenerated nitrogen. Interestingly, *Gracilaria mammillaris* had no difference in $\delta^{15}\text{N}$ inshore versus offshore, or wet versus dry season. Total tissue phosphate was also significantly higher in the analysis of *Gracilaria*-only data and for all algae, suggesting that phosphate may be more available (or more sequestered) during the dry season.

Regression analysis results indicate that salinity does not appear to be a driver for algal tissue nutrient content, except in the case of $\delta^{15}\text{N}$, which had a negative relationship with salinity (Table 5.23; Fig. 5.35). These results suggest that freshwater inputs are not the source of the phosphate taken up by the algae (Table 5.22), but could be responsible for (some) nitrogen enrichment (although this possibility somewhat contradicts the results presented in Table 5.22 above).

Regenerated nitrogen is likely coming from the sediments, otherwise the $\delta^{15}\text{N}$ values would be significantly higher during the wet season (which they are not). The fact that $\delta^{15}\text{N}$ is negatively correlated with salinity, however, suggests that fresh (or estuarine) waters are a source of ^{15}N -enriched (regenerated N) water. This apparent contradiction can be resolved if the estuarine sediments or submarine ground water discharges are the source of the ^{15}N -enriched nitrogen, or if regenerated nitrogen is coming from a local surface source, such as watershed sub-basins in the Tidal Caloosahatchee (see Objective 8). Total tissue phosphate was significantly higher in algal tissues during the dry season (Table 5.24), suggesting that phosphate may be more available (or more sequestered) during this time, again with the sediment being a likely source. Based on the data gathered for this study, the results suggest that algae will grow best during the dry season (when light is not too intense), and algal biomass will build up when the sediment regeneration pool is large enough to support the biomass.

An additional 29 algal samples were collected, processed, and analyzed for stable isotopes in May 2010 (prior to scheduled releases through S79) and in July 2010 (after 1+ month of ongoing release of water through S79). The samples were collected at Shell Point, in San Carlos Bay, near the Causeway, and in Tarpon Bay. Only attached algae were collected and processed (to ensure algae were not being transported from one area to another between May and July). Efforts were made to collect the same algae from each location in May and July, but only one species (*Gracilaria bursa-pastoris*) was present at one location (Kitchel Key in San Carlos Bay) in both months. ANOVA results indicated there was no significant difference in $\delta^{15}\text{N}$ between May and July ($p = 0.276$), among locations ($p = 0.140$), nor among species ($p = 0.09$). These results suggest that the releases from S79 were not significant enough to alter (increase) the $\delta^{15}\text{N}$ signal present in the lower, tidal Caloosahatchee, as Lapointe and Bedford (2007) suggest would happen (they demonstrated that $\delta^{15}\text{N}$ values increase as one moves upstream to S79). Alternatively, the lack of algae in July prevented the collection of the same species as were collected in May, which could have prevented the detection of an increase in the $\delta^{15}\text{N}$ signal, as different algae can have different $\delta^{15}\text{N}$ signatures (implied by the relatively low p-value of 0.09 above).

Artificial Reef Survey Results

The VSRT volunteer divers and dive boat were only available on weekends, primarily Saturdays, thereby limiting opportunity to conduct reef surveys. Initial problems with water clarity in 2008 following Tropical Storm Fay and early winter storms postponed several reef surveys following unsuccessful dive attempts. As water clarity improved in 2009, transects were established in April and May 2009 and sampling began in earnest and continued through the summer of 2010. All natural and artificial reef sites were quantitatively surveyed twice during the wet season (July-November) and twice during the dry season (April-May) to document temporal fluctuations in species presence/absence and macroalgae community structure.

A minimum of 45 separate species of attached macro-algae were collected and identified from natural and artificial reefs including at least 28 species of Rhodophyta, six species of Phaeophyta, and 11 species of Chlorophyta (Table 5.24). The lowest level of identification (Genus and/or species) and species codes with overall combined frequency of occurrence is included in the Table 5.24. The most abundant and widespread taxa (% frequency of occurrence) within all the reef quadrats included *Botryocladia occidentalis* (43.8%), *Gracilaria mamillaris* (37.1%), *Udotea* sp. (35.8%), *Gracilaria tikvahiae* (23.8%), *Sargassum* sp. (22.1%), and *Euclima isiforme* var. *denudatum* (19.6%). Also relatively common and often locally abundant were the red algae *Gracilaria blodgettii* (15.8%), *Agardhiella subulata* (14.6%), and *Dasya* sp. (12.9%) and the green algae *Caulerpa racemosa* var. *peltata* (14.9%). The greatest biomass was observed to be attached *Sargassum* spp. (mostly *S. filipendula*) with little differences between the natural and artificial reefs in terms of species composition. Anecdotally, urchins (*Lytechinus variagatus* and *Arbacia punctulata*) tended to occur in groups of many

individuals together and were associated with a lack of macroalgae where they occurred even when the same type of substrate nearby was colonized by numerous algal species.

Univariate diversity metrics were evaluated for wet season samples and are presented in Table 5.25. Diversity was calculated to provide another metric by which the algal community structure could be assessed. For the wet season, the highest species richness (22 taxa), total abundance ($n=71$), Margalef richness ($d=4.96$), Shannon diversity ($H'=2.83$), and Simpson diversity (0.945) was observed at natural reef site 35 Ledge on the 8th of August 2009. The lowest species richness (4 taxa) and all diversity values were observed at Sherman's artificial reef on 10 October 2009. Generally speaking, wet season species richness and diversity was higher on natural rock ledges and lower overall on artificial reefs. There also appears to be a trend of decreasing species richness and diversity at artificial reef sites between August and October sampling events. Sherman's reef species richness declined from 14 taxa in August to four taxa in October while Edison reef decreased from 15 taxa to eight taxa over the same time period. Species richness decreased only slightly at GH reef from nine taxa to eight taxa which may be a reflection of the tolerance of those species to changes in salinity or other water quality parameters.

Univariate diversity metrics were also evaluated for all dry season samples (Table 5.26). The GH reef sample for May 2009 contained no macroalgae because of the apparent grazing activity of abundant urchins observed during that sampling event. This sampling event was excluded from univariate and multivariate statistical analyses. Urchins also were found in abundance at natural 35 ledge during May 2009 and may be related to low species richness there. Overall, we observed higher variability in species richness and diversity in the 2009 samples from all sites while the richness and abundance seem to stabilize by May 2010 with relatively high richness (11-17 taxa) and diversity index values at all locations except for GH reef (6 taxa) (Table 5.26).

Multivariate analysis began with creating a Bray-Curtis similarity matrix for comparing algal community structure between all sites and sampling events. A hierarchical clustering of samples using the similarity profile (SIMPROF) random permutation test identified six significant groupings of sites (Fig. 5.36). These are shown in black with lines connecting sites in red as not separable from each other at the 95% confidence level. To the right of the cluster there is one large group of 14 samples that consists mostly of natural ledge samples but with two samples each from Edison and Sherman's artificial reefs. A MDS ordination of all sites and sampling events illustrates the relative scatter of several artificial reef samples from GH, Sherman's, and Edison reefs (Fig. 5.37). The 2-dimensional (2D) ordination stress level of 0.21 indicates that caution should be taken in interpreting the results and a cross-check with the cluster analysis recommended when interpreting results (Clarke and Warwick 2001). However, the ordination of all transect samples revealed the importance of year and season in forming the macroalgae communities on the reefs and as a result, samples were sorted by wet and dry season and by reef type for additional analyses.

A cluster analysis of dry season algae communities using the SIMPROF test identified five significant groups (Fig. 5.38). Reef communities at each site varied between 2009 and 2010 and were significantly different at all sites except natural reef site 53 Ledge. The MDS ordination illustrates the relative similarity/dissimilarity of all dry season samples simultaneously in 2D space with an overlay of significant groupings from the cluster analysis and sites labeled by year (Fig. 5.39). The 2D stress value of 0.11 is reduced to 0.05 in a 3D ordination but the groupings remain the same and are well represented in 2D especially with significant groupings (identified from the cluster analysis with SIMPROF test) superimposed on the plot. Three species of red algae were the most important contributors to the dry season similarity between sites with *G. mammillaris* contributing 25.1%, followed by *B. occidentalis* (16.3%) and *E. isiforme* (12.2%).

A cluster analysis of wet season samples identified 4 significant groups of sites with all natural reefs not significantly different from each other (Fig. 5.40). As a group, the artificial reefs were dominated by the same three species of red algae found in the dry season. On the natural reefs (as a group) the green algae *Udotea* sp. became the most important taxa contributing to 25.7% of similarity between sites followed by *B. occidentalis* (21.2%), *G. mammillaris* (10.0%) and *Laurencia* sp. (9.1%). The MDS ordination of wet season samples clearly shows a tight grouping of all natural reefs along with both Edison reef wet season samples (August and October 2009) and one sample from Sherman's reef collected in August 2009 (Fig. 5.41). Both of wet season samples collected from GH reef and the late wet season samples from Sherman's reef collected in October 2009 show up as outliers and are significantly different communities from each other and all natural reef communities. The 2D stress value of 0.13 along with the overlay of significant groupings from the cluster analysis indicates a good ordination.

From the similarity percentage tests we found that natural reefs shared 45.5% similarity between all sites in the wet season and 34.7% similarity in the dry season samples, with 40.6% similarity between wet and dry seasons. Greater differences were observed between all of the artificial reef samples and they shared 26.0% similarity in the wet season, 28.3% in the dry season, with only 28.4% similarity between wet and dry season samples overall. This indicates that artificial reefs are more different from each other and also change more from wet to dry season than the more stable communities found on natural reefs. This is likely the result of location rather than substrate type since the clustering and ordinations show higher similarity between reefs that are close in geographic proximity to each other (e.g. Edison and 53 Ledge) or have similar depths (Sherman's and 60 Ledge). GH remains an outlier and is closest to Fort Myers Beach and the mouth of the Caloosahatchee River.

The results of wet season and dry season reef surveys in 2009 and 2010 demonstrate seasonal differences in species assemblages. In the dry season there were three species of red algae; *G. mammillaris*, *B. occidentalis*, and *E. isiforme* as the most important contributors to similarity between all reef sites. In the wet season, the green algae *Udotea* sp. became the most important taxa contributing to the similarity between natural reef sites followed by *B. occidentalis*, *G.*

mammillaris, and *Laurencia sp.* A comparison between years also identified some important differences in macroalgal communities with greater similarity between all sites in 2010 and greater dissimilarity in 2009. Some of these differences in 2009 were attributed to the abundance of grazing urchins at GH, Sherman's, and 60 ledge. From the multivariate analysis it appears that geographic location and depth of reefs (as well as season) are important factors in determining the macroalgal community structure at artificial and natural reefs in Lee County, FL.

Objective 6: To determine the ecological consequences of the accumulation of nuisance algal biomass by measuring the decomposition rates and nutrient recycling on area beaches. (Everham and Ceilley)

Bimonthly Sampling

Generally background levels of algae deposition on the beaches were low (Table 6.3). The most commonly occurring red algae taxa, where mechanical damage and decomposition did not preclude identification, included: *Botryocladia*, *Gracilaria*, *Solieria*, and *Lomentaria*.

Event Response

We responded to five algal deposition events. Two, the event on Fort Myers Beach in June, 2008 and the *Sargassum* event on Sanibel in July 2009, were larger (approximately 1050 and 750 tons of wet algal biomass respectively). The other three events were much smaller in spatial extent and total biomass (Table 6.4). The deposition events during the study period were much less intensive and extensive than the events in 2003-2005. The events examined during this study were also relatively ephemeral, with durations of two weeks or less. This reality must be considered in any management decisions regarding mechanical removal of algae from the beach. An immediate response may turn out to be a waste of resources.

Algal biomass was typically too fragmented from mechanical damage resulting from wave action and was usually significantly decomposed, usually precluding an ability to quantify relative abundance of taxa. The algae that could be identified point to an off-shore hard bottom source for red drift algae. A comparison was conducted between all the reef samples and the beach algae "event" samples collected during the winter of 2009-2010, where algae species were identifiable and recorded. The most common species identified on the beaches was in order of frequency of occurrence were the red algae *Gracilaria blodgettii*, *Wurdemannia miniata*, *Gracilaria sp.* and *Halymenia floresii*, and green algae, *Acetabularia sp.* and *Codium isthmocladum*. Data were first transformed to presence/absence and then standardized to account for differences in sampling methods. A cluster analysis using the SIMPROF test found that the beach algae communities were significantly different than all the reef samples and separated from reef clusters at approximately 7% similarity or were at least 93% dissimilar (Fig. 6.4). An MDS ordination of all the reef samples and beach sampling events clearly illustrated the differences between reef algae communities and beach algae communities that were identifiable

(Fig. 6.5). While all of these species were collected occasionally on the reefs, none of the species identified from the beach collections were found to be the dominant taxa on the artificial or natural reefs during this study period. Only *G. blodgettii* was found to be common in any of the sampling events and it appeared to be ephemerally abundant at the Edison reef in May 2009 and the 53 Ledge in September 2009. The natural and artificial reefs do not appear to be playing any role in deposition events.

Decomposition – Beach

Fig. 6.6 shows the mean percent biomass loss for all experiments. No significant differences existed between sites (where replicates existed) or between seasons but this could be due to the higher variability of the percent biomass lost in the first week. Approximately 30% of the biomass is lost in the first week, and approximately 40% of the biomass persists through two months. Therefore the initial decomposition of algae is relatively rapid, but some algal biomass persists for extended periods if not removed by wind, tide, or mechanical means. The persistent algal biomass dries out and has little odor associated with its longer-term decomposition. Since these algae probably were regularly deposited on area beaches historically, algal biomass and decomposition undoubtedly play a role in maintaining invertebrate and microbial communities and is supporting detrital food chains, including shorebirds.

Decomposition – Laboratory

The results of the microcosm experiments are summarized in Fig. 6.7, showing the change in total nitrogen concentrations of effluent water. In all experiments, the nutrient export approached zero after approximately two weeks. In that time the biomass was reduced on average 12% in the aquaria.

By multiplying concentrations by the quantity of effluent water, a total nutrient release was calculated, and related to per unit dry biomass lost (599 mg N/Kg dry biomass decomposed). This relationship was used to scale up and estimate the total nitrogen that could be released from a deposition event, 125-130 kg for the larger events like the June 2008 deposition on Fort Myers Beach. This estimate may be too low, as the field decomposition experiments indicated approximately three times the biomass loss in the field, relative to biomass loss in the microcosms. The larger volume of beach sand and greater distance to pass through sand, may counter balance the nutrient release along a beach.

Initial biomass loss is rapid, and the microcosm experiments suggest that nutrient release is also rapid. However, nutrient release appears to be buffered by sequestration in microbial communities in the beach sand, and the total releases are small relative to other nitrogen sources. Even the largest events during the study period are significantly smaller in estimated nutrient input into the system, compared to river flow and groundwater discharge. Decomposing algae are unlikely to be significant in stimulating further algal blooms or in maintaining a deposition event. This concern should not be used to justify the expense of mechanical removal of algae from the beach.

Objective 7: To verify and calibrate a hydrodynamic model for the system to address the potential fate of nutrients that are generated and exported from the Caloosahatchee River and the fate of detached macroalgae as they enter the near shore waters around Sanibel Island and to develop a sediment transport model and implement the coupled sediment and hydrodynamic model for the same time periods as the hydrodynamic model. (Fugate)

Validation of the Model

Fig. 7.7 compares water elevations and velocities as predicted by the model with those observed by the USGS sensors at Marker 52 near Fort Myers (26 deg 31 min 26.6 sec, -82 deg 0 min 15.95 sec) during the wet season run. Both water elevations and current velocities are modeled well. There is a slight tendency for the model to predict higher ranges and velocities at peak currents but the median discrepancy between observed and modeled currents is only 2.1 % of the observed current speed values. This tendency to slightly over predict the tidal range was also reproduced in an independent model of the area using the EFDC model (Xia et al., 2010) and may be worthy of future research. In any case, the low discrepancy between observed and modeled currents establishes the usefulness and validity of the model as an investigative tool. Similarly, Fig. 7.8 compares observed water levels and current speeds at SCCF's GOM RECON station (26 deg 26 min 28.94 sec, -81 deg 58 min 19.75 sec). The total current speed is shown in this plot rather than the separate velocity components because of the more elliptical nature of the tidal currents. Here there is also a slight discrepancy in modeled water level and current speed, typically about 4.5 % of the observed current speeds.

Variations in salinity associated with freshwater discharges are also predicted well in the mid extent of the river, near downtown Fort Myers (SFWMD sensors, 26.65201 deg, -81.86925, Fig. 7.9), but the model tends to over predict mean salinity by 2-3 ppt at the beginning and end of the wet season simulation. Mixing and stratification are notoriously difficult to predict in partially mixed estuaries with numerical models, and this slight over prediction is unlikely to significantly affect the velocity estimates as is evidenced by the relatively good comparison between prediction by the model and observation shown in Figs. 7.7 and 7.8. The slight over prediction of salinity may be due to underestimating the freshwater discharge into the river. The freshwater flow in the model is forced by input at the S-79 locks. The amount input into the model at S-79 is increased from what was measured by 20%, as discussed in the methods section, to try to account for additional inflow by sheet flow, groundwater, or other sources in the tidal extent of the river. The actual amount of freshwater discharge from all of these sources that is input into the tidal extent of the Caloosahatchee River is not well known (SFWMD personal communication) and is difficult to measure because of vertical and cross channel variations in salinity, and tidal phase lag between the mouth of the Caloosahatchee and the S-79 locks. Measuring this value in the future, and how it changes seasonally is an effort that could help improve the accuracy of numeric models of the Caloosahatchee River.

Wet Season Simulations

The paths of drifters released in the lower to mid section of the Caloosahatchee Estuary after the start of the simulation are shown in animation “wetlowerCalus.avi”. In the animation, the location of the drifters at each point in time is denoted with a red dot. The locations of the drifters in the previous 4 hours are denoted in blue dots. This enables a better visualization of the speed and direction of each drifter. In addition to the obvious tidal motion of the drifters, there is a general tendency of the particles to move to the North and West, although there are a few drifters that pass to the South around Fort Myers Beach.

Another way to visualize and quantify the paths of the drifters is to plot the total number of hours that drifters released from the Caloosahatchee spend in each of the model grids. This is slightly different from the analysis proposed originally because it gives more information about the potential influence of Caloosahatchee dissolved and particulate matter at each region in the study site. Rather than just presence or absence, the total number of hours gives a better indication of the amount of communication of Caloosahatchee water with locations outside of the river. This method is similar to the "transport quotient" method used by Havens et al. (2010) in studying the Tampa Bay estuary. For any given grid cell, for each time step that any drifter is within its area, the cumulative time exposed for that grid cell is incremented by one time step (10 seconds). For these analyses, only drifters from the lower half of the Caloosahatchee are used because those further upstream rarely escape the river during the model simulation. The total number of hours that any drifters from the lower Caloosahatchee Estuary spent in each of the model grid cells is shown in Fig. 7.10. Those cell with higher numbers of hours have higher potential to be exposed to dissolved materials and neutrally buoyant or near neutrally buoyant organic particles. Most of the drifters moved up Matlacha Pass and into Charlotte Harbor. From there they were discharged into the Gulf across the north of Pine Island. Another group of drifters passed to the south of Pine Island and up into Pine Island Sound. During an inadvertent numerical experiment in which Redfish Pass was closed, a much smaller proportion of drifters moved south below the Sanibel Causeway (Fig. 7.21). Figs. 7.11 - 7.13 show the same information by the water level that the original drifters were released. As expected, the surface drifters moved further than those that were released at lower elevations, but there is little difference in the overall patterns (note difference in scales). The difference in hours between surface and bottom drifters is shown in Fig. 7.14, and the overall pattern of surface drifters traveling farther is more apparent. Note that the general path of drifters from Matlacha is up into Charlotte Harbor then out towards the Gulf, so that the slower bottom drifters were still in Charlotte Harbor while the faster surface drifters had already moved towards the mouth and into Pine Island Sound. The results from the simulations with observed wind speeds but oriented at a constant direction are shown in Figs. 7.15 and 7.16. When the winds blew from the northwest there was a large transport of water and drifters along Estero Island and the southeastern portion of Sanibel Island.

The observed winds that were commonly from the east resulted in most of the Caloosahatchee water moving up through Pine Island Sound, and up through Matlacha pass into Charlotte

Harbor. Both winds from the northwest and southwest moved significantly more water onto Fort Myers beach. The same pattern held also for water that was outside of the river as evidenced by the drifter paths. While Fort Myers beach had significant exposure to both water from in and outside the Caloosahatchee, there was little accumulation of drifters on the southern Sanibel shore.

The paths of the drifters released at the locations outside of the Caloosahatchee are shown in the animation "wet_obsreef.avi". This animation also shows clusters of drifters released every 7 days. There is quite a bit more communication of these drifters with the Sanibel and Fort Myers Beaches than those from the Caloosahatchee shown in the previous animation. Fig. 7.17 shows the number of contact hours of any artificial reef drifters released at the beginning of the simulation with each grid cell. The original locations are marked with a purple circle. The results from the simulations with observed wind speeds but oriented at a constant direction for these locations are shown in Figs. 7.18 and 7.19 and in the animations "wetreefNW.avi" and "wetreefSW.avi". As with the drifters originating in the Caloosahatchee Estuary, changing the direction of the winds to come from a constant northwest direction made a big difference in the path of the drifters located outside of the river.

The residence time of the water is computed by taking the average amount of time that each of the 3 drifters that are released at the beginning of the simulation in each of the grid cells remained in the Caloosahatchee during the simulation (Fig. 7.20). Once they leave the mouth, they no longer accrue time, even if they return on the next flood tide. Drifters near the mouth of the river were flushed out in less than 5 days, while those in the mid extent remained in the river between 10 to 20 days. In the middle extent there is a cross channel variation in residence time, with water in the deeper channel being flushed out more quickly. Some drifters on the south bank apparently got “stuck” on the side and did not leave during the entire simulation. This may well replicate the actual behavior of material in the water or may just be an artifact of the model. When the water gets very shallow (<0.1 m) the model grid cells switch to "dry" mode, and model drifters that are in the cell at the time can become abandoned by the model. The drifters are not reinstated into the model after leaving the domain, whether at the open ocean boundaries or at the intertidal land boundaries. More research and verification should be done on this interesting result before making any strong conclusions about extremely long residence times on the south bank. However, the results from the wider shoals on the north bank are more reliable and likely represent a real cross channel variation in residence time in those regions. Further upriver the residence times increase, most over 22 days and some not making it out of the river during the entire 45 day simulation.

Currents, salinity, sediment transport, sediment deposition and sea surface height for the wet season simulation are shown in the animations “allwet20_35.avi” and “allwet10_35.avi”. The two animations have different scales for salinity; the lower scale shows the salinity variations near the Caloosahatchee better, while the higher scale shows salinity variations through the

barrier island passes better. Field investigations of sediment transport were hampered by: 1) very low concentrations of suspended sediment during the first two deployments near Marker 52 on Oct 9-Oct 15, 2009 and at Shell Pt. on October 15-28 did not produce a sufficient acoustic echo to accurately determine the sediment transport 2) the ADCP and ADV were stolen during the third deployment near SR 30 on Jan 20, 2010. These instruments were not recovered. Nevertheless, the results of the model provide a useful qualitative representation of the directions and magnitude of transport and deposition. The sediment deposition panel shows regular deposition by the ebb dominant currents onto the ebb deltas at the barrier island passes. These likely represent significant reserves of sediment just offshore of the passes which are available for cross shore transport into the passes during storms and are important reservoirs upon which the natural beaches depend to rebuild themselves after storm caused erosion. The model also demonstrates the maintenance of the sand shoal spit at the eastern end of Sanibel Island. Longshore transport of sediment along the southwest coast of Sanibel is greatly enhanced when wind direction is from the northeast and the tide is flooding.

High Discharge Results

The primary analyses during the wet season described above focuses on processes just prior to and through a macroalgal stranding event in July. However this event did not coincide with the maximum discharge period in 2008 which was in the middle of August and ranged up to $600 \text{ m}^3 \text{ s}^{-1}$ in the Caloosahatchee and $100 \text{ m}^3 \text{ s}^{-1}$ in the Peace. A further simulation was run during maximum discharges in August 2008 in order to compare peak wet season water residence times in the Caloosahatchee with those during the dry season (Fig. 7.34). Residence times in the mid extent of the river were between 5 and 15 days, about 5 days shorter than those estimated in the lower flows in the previous month. During the much higher flows observed in 2005-2007, the residence time is likely to be much shorter. Drifters in the upper regions did not behave properly, so this portion of the river is not shown in the figure. Because the river is so narrow in this section, it is only represented by one grid cell width, and so is particularly subject to the issues for drifters discussed above. Fig. 7.32 shows the hours of exposure of each grid cell to drifters released in the lower Caloosahatchee. The high discharge pushed many more drifters south of the Sanibel causeway and onto Fort Myers beach. There was also a significant increase in the exposure time of waters in the lower Pine Island Sound to water that originated in the Caloosahatchee. There was also more exposure of Fort Myers Beach and lower Pine Island to drifters that were released from outside of the mouth of the Caloosahatchee (Fig. 7.33).

Dry Season Results

In general, the results from the dry season reflect the slower movement of water out of the Caloosahatchee and reduced along channel mixing of the water. The regions around Matlacha pass were exposed to Caloosahatchee water much more during the dry season than the wet season (Fig. 7.22 and "drylowerCalus.avi"). During the wet season under the observed winds, water moved further north and much more water went into Charlotte harbor. Only during the simulation with winds from the southwest (Fig. 7.24) was there much movement of water from

the Caloosahatchee into Charlotte Harbor. In the dry season simulation with winds from the northwest, almost no water from the Caloosahatchee went into the northern regions of Matlacha Pass or Pine Island Sound.

Drifters outside of the Caloosahatchee (Fig. 7.25 and "dryobs_reef.avi") were much more dispersed than those during the wet season, reflecting the more variable nature of the wind during the dry season. Simulations of these same drifters with winds coming from a constant northwest direction showed a much more directed path (Fig. 7.26 and "dryreefNW.avi") as did those with winds from the southwest (Fig. 7.27 and "dryreefSW.avi"). In contrast to the wet season results, the southern shore of Sanibel was exposed significantly to water that originated in location outside of the Caloosahatchee with observed winds, but interestingly, not with the southwest winds. This is likely to also be a result of the more variable observed winds creating more dispersal of the water.

Residence times during the dry season (Fig. 7.28) show a more bimodal nature than during the wet season with somewhat longer residence times in the mid to upper extent, mostly 35 or more hours. There is a sharp transition in the mid river section from longer to much shorter residence times of about 15 hours or less.

Currents, salinity, sediment transport, sediment deposition and sea surface height for the dry season simulation are shown in the animations "alldry20_35.avi" and "alldry10_35.avi" with different scales for salinity as before for the wet season animations. The largest sediment transport events are clearly associated with strong wind speeds, especially when the wind direction is parallel with the orientation of the southern coast of Sanibel.

A final simulation was run during the dry season with only tidal and river forcing, with no wind forcing. The results from this simulation show that without winds there is much less dispersion of the drifters from the lower Caloosahatchee (Fig. 7.29 and "drylowerCalusNowind.avi"). The animation also shows that although the wind is ultimately the most important forcing variable to the distribution of the drifters, that spring tidal currents can move the drifters a significant distance. Drifters outside of the Caloosahatchee also did not disperse very much without winds and Fort Myers Beach received a lot of exposure to water from the locations inside San Carlos Bay and just offshore (Fig. 7.30 and "dryNowind_reef.avi"). Interestingly, there is little difference in the dry season pattern of residence time in the Caloosahatchee with observed winds and with no wind forcing (Fig. 7.31). The short fetch from most wind directions probably prevents the wind from having a large effect on residence times, instead riverine flow and tidal dispersion are more important to the residence time within the river.

Summary of Numerical Model Results

In general, the results discussed above suggest that outside of the Caloosahatchee River, wind is the primary forcing factor of the water over a time scale of several days, compared to forcing by tidal action or river discharge which operate on a longer time scale. While the fastest currents

are caused by the tide, the ebb and flood currents tend to cancel out the net water motion. Nevertheless, there is a slow net tidal movement that is generally northward, and this net flow (averaged over many tidal cycles) is on a longer time scale than the wind driven currents. The net tidal flow is apparent by the movement of the drifters and sediment during low wind periods. The comparison of the dry season runs with no wind forcing to those with the observed wind show that occasionally strong spring tidal currents will overpower a moderate wind driven current, but this is not the general case. An inadvertent experiment in which Redfish Pass was closed shows that the pass is important not only for flushing of Pine Island Sound, but without the pass, the retention of drifters in the sound made them available for transport southward under the causeway onto Fort Myers Beach and even into Estero Bay during short term wind events. When Redfish Pass is included in the model, near shore and Caloosahatchee River originated drifters move offshore more rapidly, and are not retained within Pine Island Sound or San Carlos Bay to be transported by subsequent wind events. So if a large strand of detached macroalgae is nearshore or within the Bay or Sound, it is likely to be stranded on the coast during a west wind. However, if it is not transported by wind driven currents soon after detachment, it is likely to be moved offshore and northward by the slower moving net tidal current or prevailing winds from the east.

Residence time in the Caloosahatchee River during the wet season ranges from less than 5 days at the lower extent and 10-20 days in the mid river section, to over 45 days in the upper river between Beautiful Island and S79. Residence times during the dry season are longer, with less along channel mixing. This is evident by the much sharper gradient between the long and shorter residence times in the mid river region. During the high flows of 2005-2007, not only were residence times likely much shorter, there was likely also much more density driven circulation as long as the tide was strong enough to allow salt water to intrude into the estuary. The model results demonstrate one of the complex behaviors of partially mixed estuaries. As the horizontal salinity gradient increases (e.g. during the wet season), the net density driven circulation also increases. This moves fresh water at the surface out of the estuary and salt water near the bottom into the estuary faster during the wet season than during the dry season and results in stronger along channel mixing of the water. However, at some point as river flow gets much stronger and is able to stop the intrusion of salty marine water into the estuary, the density driven circulation shuts down and along channel mixing will then be reduced. The fresh water flows that were modeled during 2008 were not strong enough to shut down density driven circulation. Comparison of the model results from the dry season with no winds and that with observed winds shows that in contrast to the water movement outside of the mouth of the Caloosahatchee River, the water movement and residence times are relatively unaffected by winds inside the river, rather, density driven circulation and mixing and freshwater discharge are the primary determinants.

Objective 8: To evaluate existing water quality models, watershed plans and/or assessments for the Caloosahatchee and Estero Bay watersheds to help guide nutrient reduction strategies for managing drift algal blooms in Lee County waters. (Parsons, Loh and Everham,)

We examined the available reports and publications to find data on nutrient inputs into the Caloosahatchee River from within its watershed and from Lake Okeechobee. In conjunction with the model provided, below is a summary of the nutrient loading sources within the watershed. The high loading sources (hot spots) are identified and land use practices associated with that source are described as data allows. Suggested actions for reducing nutrient loads from these sources are then provided.

While there is little debate that Lake Okeechobee is a major source of nutrients to the tidal Caloosahatchee and adjacent coastal waters, the reported proportions due to Lake Okeechobee vary. The variability is at least partly due to data analysis performed. For example, if loadings are reported as daily averages, the large nutrient inputs associated with high discharge events will be diminished through the averaging procedure (i.e., 360 days of low flow will override 5 days of extremely high flow). Cumulative loading estimates (amount per year) better capture the essence of the high flow events, which can account for significant portions of the nutrient inputs for any given year. That being said, we report the values presented in the Caloosahatchee River Watershed Protection Plan (CRWPP 2009) and SFWMD (2005), which are cumulative on an annual basis, and rely on flow data and nutrient concentrations at regularly monitored stations. The data are presented in Tables 8.1, 8.2, and 8.3, respectively.

While Lake Okeechobee is the source of 40% of the total nitrogen (TN) and 24% of the total phosphorus (TP) entering the Caloosahatchee River and Estuary (Table 8.1), the sub-basin watersheds are a significant source of nutrients as well (Fig. 8.1). TN and TP both increase between S-77 and S-78, and between S-78 and S-79 (Table 8.2), corroborating this observation. The West Caloosahatchee sub-basin and the Tidal Caloosahatchee sub-basin are the next two highest sources of TN and TP loading (approximately 25% each) behind Lake Okeechobee (Table 8.1), likely due to agricultural activities and urban/residential development in the two sub-basins, respectively (Table 8.3). After natural areas (32.8%), the next two highest land uses in the West Caloosahatchee sub-basin are citrus (19.7%) and improved pasture (15.9%) as reported in CRWPP (2009). For the Tidal Caloosahatchee, natural areas are most common (36.8%), followed by residential areas (24.7%). Out of the twenty land-use categories listed in CRWPP (2009), natural areas rank 19th as a TP and TN source. Citrus ranks 13th as a TP source, and 9th as a TN source. Improved pasture ranks 11th as a TP source, and 6th as a TN source. Residential areas vary by density (low to high), but rank 14th (low density) or 11th (medium density) as a TP source, and 16th (low density) or 12th (medium density) as a TN source.

Note that the Lake Okeechobee contributions are lower in Table 8.3 versus Table 8.1, especially in terms of TP loading (48 mt/yr versus 104 mt/yr, respectively). These differences are due to

different time frames analyzed; 1995 to 2005 for Table 8.1 and only 2000 in Table 8.3. This difference is pointed out to demonstrate that the influence of Lake Okeechobee can vary from year to year depending not only on lake releases, but local run-off in the watershed.

The West Caloosahatchee sub-basin can be further subdivided into ten local watersheds comprised of seven creeks (or branches) and five canals (La Rose and McPherson 1983). Nutrient loadings from these localized watersheds have not been thoroughly studied, primarily due to the lack of flow data from each creek and/or canal. La Rose and McPherson (1983) present results for the only gauged channel discharging into the West Caloosahatchee sub-basin, Jack Branch. Annual loadings were estimated to be 39.7 mt/yr (TN) and 0.52 mt/yr (TP). These data, however, are from 1979, and may not be relevant today. No other flow (or nutrient loading) data was found for the tributaries and canals of the West Caloosahatchee.

For the Tidal Caloosahatchee, the Orange River supplied the most TN and TP after flow out of S-79 in both the wet and dry seasons (Table 8.4). The Fort Myers South and Central STP were second and third during dry season, respectively, but Telegraph Creek jumps up to second place during the wet season. Nutrient loads are dramatically higher during the wet season, ranging from a 47% to 12,000% increase in loading, with an average increase of 2,113% in TN and 2,708% in TP during the wet season.

Other nutrient loading data for the Tidal Caloosahatchee sub-basin were reported by CHNEP (1999) and are presented in Table 8.5. Again the importance of Orange Creek and Telegraph Swamp (Creek) are indicated, although the numbers vary from those given by ERD (2003). The sub-watershed surrounding Pine Island Sound/Matlacha Pass produce a substantial amount of TN and TP as well, constituting 26% of the TN inputs and 32% of the TP inputs to the Tidal Caloosahatchee, respectively.

In terms of the land use activities contributing to the nutrient loading, range lands are the primary anthropogenic source in the Pine Island Sound/Matlacha Pass sub-watershed (Table 8.6), whereas pastures and range lands are both significant in the three other sub-watersheds (Table 8.7), although residential inputs are also significant in the Lower Caloosahatchee and Orange River sub-watersheds.

Total nitrogen (TN) and phosphorus (TP) loadings from known sources into the Tidal Caloosahatchee are given in Table 8.8. Loadings were from the CRWPP (2009, Table 8.1), the report by ERD (2003, Table 8.4) and those quantified in this study (Objectives 2 and 3). The total nutrient loading from non-point sources was calculated by subtracting the total STP loads from the total Tidal Caloosahatchee loads. Seasonal loadings (for wet and dry seasons) for Lake Okeechobee, the S-4 canal, the East and West Caloosahatchee sub-basins, non-point sources and coastal sources were estimated from total annual loads. The average percentage of total discharges for each season (for the wet season, this is 85% and 83% for TN and TP, respectively,

Table 8.4) was used to calculate the seasonal loadings for each possible source when such seasonal data was not available.

Since this study is one of the first to provide seasonal data for sediment fluxes of nutrients for the CRE, wet season data was not available for sediment nutrient fluxes for tidal portion of the CRE (from S-79 to Shell Point). For Table 8.8, available dry season data was used as an estimate of wet season sediment nutrient fluxes. This estimation is probably an over-estimate as experimental studies show that during the wet season, sediment nutrient fluxes to the water column are low and the sediment may well be a sink of nutrients instead of a source (see Objective 2).

The biggest nutrient source to the Tidal Caloosahatchee is upstream of S-79, with 18%-27% of the nutrients coming from Lake Okeechobee (Table 8.8). However, downstream of S-79, submarine groundwater sources of nutrients cannot be discounted, with loadings equal to those from Lake Okeechobee (25%-27%, Table 8.8). Sediment fluxes of nutrients are low, and depending on the season may be a small sink for nutrients within the CRE (Table 8.8).

Residence times for nutrients were calculated for the dry and wet seasons, as well as on the annual basis (Table 8.9). Total loadings from Table 8.8 and the average nutrient concentrations for the Tidal Caloosahatchee from this study were used to calculate these residence times. Residence times for TN and TP were higher during the dry season (19 and 15 days, respectively) compared with during the wet season (10 and 11 days, respectively). This is in agreement with modeling efforts to determine the residence time of water in the CRE (see Objective 7).

When comparing the hydrologic conditions between 2003 – 2005, 2005 – 2007 and 2008 – 2010 (Table 8.10), the period between 2003 – 2005 (P1) where the area saw the greatest stranding of drift algae, had 69% greater average rainfall and 61% greater average freshwater discharge than the period of this study (2008-2010, P3). These differences were especially evident during the wet season (Table 8.10). During the dry season, these differences were less marked. The percent differences in maximum monthly-averaged rainfall and discharges between P1 and P3 during the wet season were 81% and 50% greater, respectively, while these differences were reversed during the dry season (Table 8.10). These results indicate that during the period between 2003 – 2005, this study area had higher amounts of rainfall (and subsequently higher freshwater discharges, a “wet year”) than during the period between 2008-2010. These higher freshwater inputs mostly brought with it greater input of nutrients that may have triggered blooms of macroalgae of great enough magnitude to cause the massive stranding events seen. Interannual variability in the amounts of rainfall (and freshwater discharges) is sometimes tied to more global climatic events such as El Niño and La Niña. When looking at the Multivariate ENSO Index (MEI), the period between 2003 – 2005 had a positive MEI indicating an El Niño event while the current study period (2008 – 2010) was during a La Niña event (Table 8.10). As these climatic events are fairly predictable and with some advance warning, we can use this index to predict if the coming year will be a “wet” one or a “dry” one.

Objective 9: To summarize data on potential grazers and on past macroalgal events and conduct a limited macrograzing study with relevant information (Coen and Provost).

Summary of Events 2003-2010

All available data concerning discharges/releases (S77, S79), major drift macroalgal events, red tides (significant HABs), local beach closures from *Enterococcus* events, named hurricanes and tropical storms (including directions), are summarized by year and month in Appendix 9.1. An examination of these anecdotal data revealed no obvious seasonal or spatial patterns for drift algal stranding events in the last seven years as gleaned from all sources. Macroalgal events occurred in all seasons throughout the time period.

One possible hypothesis for the appearance of increased algal biomass on the areas' beaches may be an increase in the abundance of available attachment substrates, potentially in the form of artificial offshore reefs. While Lee County keeps records of relief, or vertical height from the seafloor (Table 9.1), there are inadequate records on horizontal cover or algal cover to conclude whether this small area of reefs could have contributed to the above hypothesis. It is our conclusion that the area of enhanced artificial reef substrate is orders of magnitude too low to serve as sources for macroalgal bloom development.

Trawls and In-Water Sampling to Assess Potential Grazers

Qualitative species composition of the trawls conducted in October 2009 and April 2010 indicated a lack of obvious macro- or mesograzers at any of the inshore sites in either Pine Island Sound or San Carlos Bay (Table 9.2, Fig. 9.12). As in other Florida inshore habitats, juvenile and adult pinfish (*Lagodon rhomboides*) dominated most trawls, with the most common invertebrate grazer being the sea urchin, *Lytechinus variegatus* (Table 9.2). Other potential grazers (Table 9.2) included the emerald parrotfish, *Nicholsina usta*; however, individuals were uncommon, and thus not chosen for subsequent caging and grazing experiments. Most of the smaller invertebrates are considered generalist 'feeders' such as spider crabs (*Libinia* spp.), but these were observed in relatively low numbers. Surprisingly, few other herbivores such as majid crabs (e.g., *Mithrax* spp., Coen 1988), or smaller mesograzers (isopods, amphipods, gastropods) were ever collected from the algal quadrat samples live sorted from Objective 5 over the two year effort at any of the 13 stations (see Fig. 5.1). Hence, *Lytechinus* was selected for the subsequent field and (most) lab grazer studies.

Few sea urchins were found in seagrass beds in either Pine Island Sound or San Carlos Bay based on our in-water collections, trawls and other SCCF Marine Lab sampling efforts. This was surprising given that they are often so common elsewhere in *Thalassia* beds inshore throughout Florida and the Caribbean, and act as major algal biomass controllers (e.g., Lawrence 1976, Wanders 1977, Klinger 1982, Valentine and Heck 1991, Heck and Valentine 1995, 2006, Rose et al. 1999, Valentine et al. 2000, Watts et al. 2001, Williams and Heck 2001, Camp et al. 2003, Cobb and Lawrence 2005, K. Heck pers. comm.). In fact, we were quite surprised to find so few

small invertebrates, including amphipods, typically associated with macroalgal and seagrass habitats (e.g., Hooks et al. 1976, Hawkins and Hartnoll 1983, Creese 1988, Heck and Crowder 1991, Pennings et al. 2000, Goecker and Kall 2003).

Macroalgae was common in inshore trawls, with identified species similar at all sites and greater algal biomass collected in the April 2010 sampling (Table 9.3). The majority of species were common red algal species observed previously at the inshore sampling stations (see Objective 5). An extensive literature search indicated that these common algal species do not exhibit any obvious chemical or mechanical defenses typical of many tropical species that could hinder urchin grazing (Table 9.3), demonstrating that they are likely to be palatable (e.g., Randall 1961, 1967, Lawrence 1976, Wanders 1977, Warner 1977, Littler 1980, Littler and Littler 1980, Hawkins and Hartnoll 1983, Shacklock and Doyle 1983, Hay 1984, Steneck and Watling 1985, Targett et al. 1986, Paul and Hay 1986, Coen 1988, Duffy and Hay 1990, Steneck and Dethier 1994, Bolser and Hay 1996, Lotze et al. 1999, Pennings et al. 2000, Valentine et al. 2000, Duffy and Hay 2001, Williams and Heck 2001, Goecker and Kall 2003). Conversely, the encountered algal species are not strongly preferred by the two most common invertebrate grazers encountered in this study, *Lytechinus variegatus* and *Arabacia punctulata*.

Lab Feeding Trials

Analysis of single species feeding trials demonstrated that there was a significant difference between algal species consumed ($p < 0.001$, One-Way ANOVA). Urchins ate significantly less *Gracilaria blodgettii* (offshore/nearshore) and *Hypnea spinella* (inshore species) than the other species (Fig. 9.13).

Trials using multiple algal species revealed that the urchins consumed more of what they happened to come upon first, with no significant difference attributable to algal species differences (Fig. 9.14; $p = 0.361$, One-Way ANOVA). Similar results were obtained for the extended multiple choice trials ($n = 4$; Fig. 9.15). There may have been some preference for *Solieria filiformis* and *Agardhiella subulata* (Fig. 9.16), although the results were not significant ($p = 0.139$, by One-Way ANOVA) and may have been due to first encounter processes once again.

Caging experiments: Urchin-Enclosures

Caged-survivability experiments indicated that urchins survived at many salinity regimes found around Sanibel. The only cage that experienced urchin mortality was the SCCF Shell Point River Estuary Coastal Observing Network (or RECON, see <http://recon.sccf.org/>) unit, which experienced several large fluctuations in salinity in the range of ± 5 psu (Figs. 9.10-9.11). Urchins at all other sites survived and were released upon completion of the month-long trial.

Based on literature assessments and field experiments, urchins such as *Lytechinus* and *Arbacia* may be limited by low salinities, either as adults or more likely as larvae or juveniles (e.g., Goodbody 1961, Klinger 1982, Lawrence 1996, Irlandi et al. 1997, Watts et al. 2001, Böttger et

al. 2002, Heck and Valentine pers. comm.). For example, salinities lower than 18 psu appear to be stressful for *Lytechinus* over an extended period of time, with values < 5 psu causing 100% mortality (e.g., Irlandi et al. 1997). Low to variable salinities may be part of the reason that urchins are rare inshore except for near passes to the Gulf of Mexico (e.g., Goodbody 1961, Lawrence 1996, Irlandi et al. 1997, Böttger et al. 2002). Salinities on the Atlantic coast of Florida undergoing similar cyclical salinity reductions from freshwater releases as through S-79 have been shown to cause significant mortalities (e.g., Irlandi et al. 1997). Likewise, tropical rainfall events and runoff from watersheds can cause near 100% mortalities (e.g., Goodbody 1961, Böttger et al. 2002). Our local, extended salinities may explain the absence of echinoderms throughout many of the local seagrass beds.

Caging experiments: Urchin-Exclosure Experiments

Further analysis of data obtained in Objective 5 (biomass and distribution) indicated that there are several sites with an absence of algae throughout the two years of sampling that have some commonalities. While these sites were recorded as having adequate attachment sites for macroalgae growth, they also had high numbers of urchins. High urchin densities at these sites may contribute to the apparent lack of algae growth. Macroalgae recruitment and growth to specific areas has been controlled by grazing pressure, or “top down” pressure in several varied areas and studies. Seasonality, nutrient availability, and grazer type may also contribute to suites of macroalgae assemblages (e.g., Anderson and Underwood 1997, Lotze and Worm 2001, Smith et al. 2001). This study utilized sites in an area that is generally classified as “nutrient-enriched”, or eutrophic. However, characteristics of several of these sites seem to be oligotrophic in nature, in that bottom cover was sparse, and invertebrate grazer density was variable. The exclosure experiments were designed to effectively control the bulk of grazing pressure by the most prevalent macrograzing invertebrates from our varied efforts, the variegated sea urchin (*Lytechinus variegatus*).

The nearshore stations chosen for the exclusion experiments exhibited significantly larger numbers of urchins per 100 m² than inshore or offshore sites (Figs. 9.17 to 9.19). These sites typically lacked large algal accumulations as well. GOM10 proved to be a troublesome site, experiencing continued problems with strong currents and scouring. After one month of deployment, one cage was completely missing, most likely due to local bottom currents. The cage was replaced, however each time this site was visited, cages bottoms were above the sediment interface. Cages became severely fouled at the Redfish Pass site in August 2010, and were pulled in September 2010 (Fig. 9.20). At most other sites, cages remained intact throughout the three months, with one cage replaced at the site closest to the Sanibel Lighthouse.

Only two sites had macroalgae present, Redfish RECON and GOM04. At GOM04, the cages were covered 1% in algae in July and September 2010, and had a range of 25% to 100% in October 2010 (Table 9.4). The dominant algae found outside the cages was *Gracilaria mammillaris* in September 2010, and *Halymenia pseudofloresia* in July 2010. Algae inside the

cage was limited to *Halymenia pseudofloresia* in both time periods. Similarly, in October 2010 the dominant algal species were the same both inside and outside of the cages: *Agardhiella subulata* and *Botryocladia occidentalis* (Table 9.4). However percent (%) cover outside of cages in October 2010 at GOM04 was lower than that inside the cages (Fig. 9.21, mean of 18.5 % cover inside cages, 4.55% outside cages). At this site, urchins were found sporadically (see Table 9.4, Figs. 9.11 and 9.12), as well as pen shells. Both species of sea urchins were found (*Lytechinus variegatus* and *Arabacia punctulata*). However, when the cages were pulled (October 13, 2010), only *Arbacia punctulata* individuals were observed. In fact, one very small individual urchin was observed to be entering the cage. Sediments were quite “shelly” (Table 9.4, see also Objective 4) also at this site. At this site, cages also seemed to be “fish attracting” structures, with large schools of Atlantic spadefish (*Chaetodipterus faber*) seen on two sampling periods (July and October).

At the Redfish RECON site, algal cover was low in the cages (5% in one, 0% in the other two). The dominant species found inside and outside the cages was *Gracilaria mammillaris*, with higher density outside the cage (3 quadrats, 15, 10 and 10% cover, respectively; Table 9.4). Observations in July 2010 indicated that at most sites, small (<3.15 cm) *Lytechinus* were observed inside cages. Observations in the following months indicate that several urchins inside had grown sufficiently so that they had to be removed from the inside of the cages by hand (Figs. 9.22-9.25 for examples of several sites). No macroalgae was observed outside of any of the cages during the three month experiment at the other sites, although urchins were found at several dates, and no algae were recorded either inside or outside of any cages.

While these experiments were limited, trends indicated that these areas may be more controlled by nutrient availability than by excluding grazers. For the three months that cages were deployed, algae was found at only two of the eight sites. The Redfish RECON site had similar densities of algae outside the cages. Conversely, results from GOM04 demonstrated that grazer control resulted in more growth inside of the cages than in unprotected area outside of cages. However, the presence of algae, and of similar species composition, inside and outside the cages indicates that while reducing grazer pressure (i.e. juvenile urchins found inside cages with some algae) allowed for larger percent cover found inside the cages, other factors most likely contributed to the presence of algae at this site (e.g., the presence of pen shells and parchment worm tubes for algal attachment; see Objectives 4 and 5).

Grazers, once algae are ashore may play their most important role, feeding directly or indirectly on material coming ashore. These species under normal biomass levels decompose rapidly through grazer interactions, along with natural physical and chemical breakdown processes (e.g., Behbehani and Croker 1982, Bedford and Moore 1984, Llewellyn and Shackley 1996, Colombini et al. 2000, Dungan et al. 2003, Dawes 2004, Catenazzi and Donnelly 2007). Sea urchins in particular have been studied in areas of significant seagrass loss, and have been implicated for large-scale biomass loss in areas of high densities (e.g., Valentine and Heck 1991, Heck and

Valentine 1995, Rose et al. 1999, Valentine et al. 2000). Unfortunately, often the emphasis in our region with regard to macroalgal blooms has stressed only coastal eutrophication (e.g., Lapointe and Bedford 2007), rather than a relaxation of grazing pressure. Such “top down” mechanisms should be further studied to determine their significance in controlling algal biomass.

As discussed in Objective #5, we need more focused experimental or mesocosm efforts to tease apart those specific factors that might trigger and allow macroalgae to accumulate in such vast volumes, along nutrient supply, and wind and current patterns that may all have to be in sync to trigger the “perfect storm” of events to cause a massive outbreak of macroalgae on local beaches observed previously, but not during our study period (e.g., Dawes 2004, Jacoby et al. 2004, Lapointe et al. 2005, Bartleson et al. 2006, Krause-Jensen et al. 2007, Lapointe and Bedford 2007). A great deal of information was gleaned from the other Objectives conducted during this study. As we integrate this diverse information and follow events as they occur in the future (e.g., mapping and diving, spatial sampling related to species observed on the beaches vs. in the surrounding waters), we may be better able to understand what causes these bloom events.

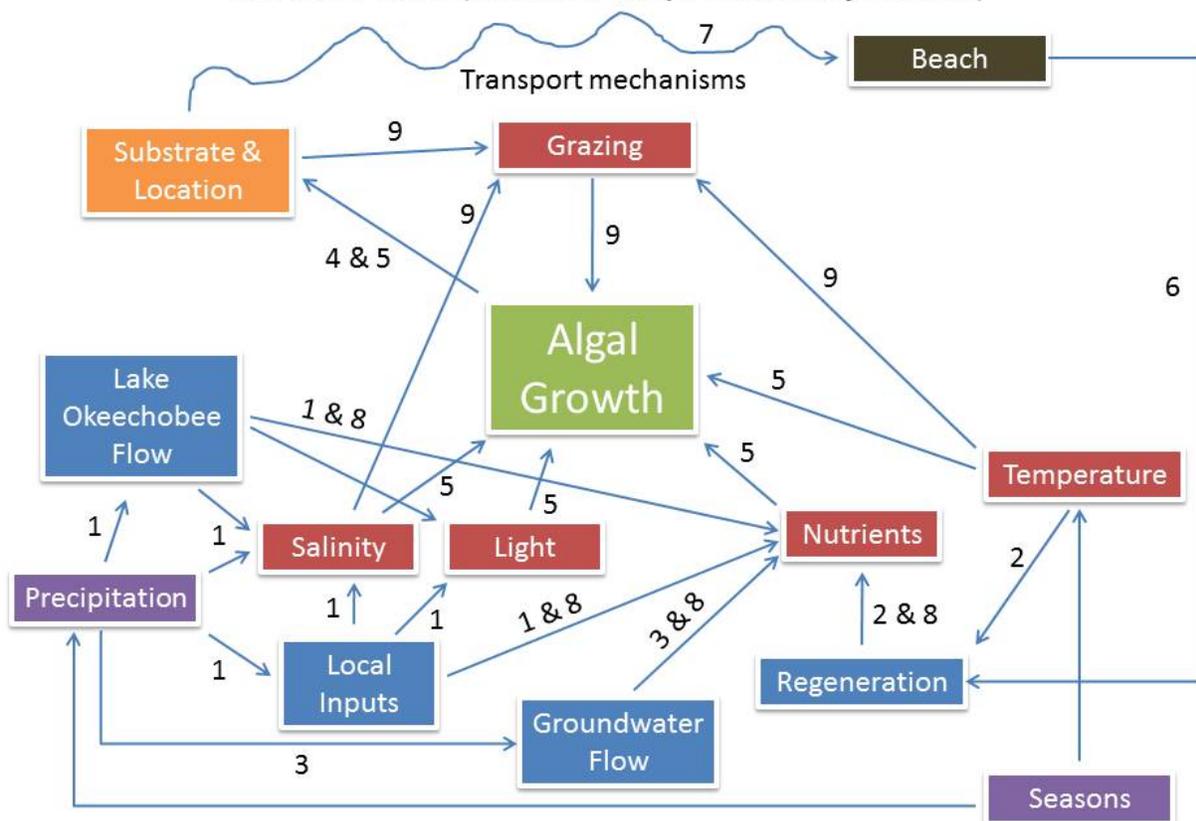
Often drift algae may serve as another critical habitat for estuarine and marine species (e.g., Hooks et al. 1976, Heck and Wetstone 1977, Coen et al. 1981, Virnstein and Carbonara 1985, Zieman et al. 1989, Heck and Crowder 1991, Sogard and Able 1991, Kingsford 1995, Norkko et al. 2000, Epifanio et al. 2003), as these accumulations are often important nursery habitats for ecologically and economically-important species. We should not lose sight of the value of algal habitats under normal, non-bloom conditions.

Synthesis: Conceptual Model of Algal Growth

Background

The accompanying flowchart depicts a conceptual model of the factors that 1) influence algal growth, 2) determine when and where algae will grow, and 3) result in algal biomass being deposited on our area beaches. Each of the primary factors (i.e., those directly influencing the metabolic processes involved in algal growth) is shown as a red box. The secondary factors (i.e., those that influence the primary factors) are shown as blue boxes. Two tertiary factors (precipitation and seasons) are shown in purple, and represent regional/global processes (e.g., ENSO) that influence the secondary factors (as well as season affecting precipitation). Substrate is treated separately as an orange box as it represents a geospatial aspect where macroalgae are likely to grow and/or accumulate in our coastal/estuarine waters. Lastly, the beach box (brown) represents the condition where drift algae is deposited on area beaches. The arrows indicate how each box relates to one another, where the numbers associated with each arrow represent one or more of the nine objectives of this study to determine the conditions that lead to drift algae accumulations on area beaches. The data and literature supporting the model components presented below are presented in the respective objectives associated with each arrow.

Conceptual Model of Algal Accumulation and Transport to the Shoreline (numbers represent objectives)



Below is a summary of our results as they relate to each factor, thereby providing an overall synthesis of our findings on what we learned about the conditions conducive for algal growth and accumulation.

Primary Factors (red boxes)

Algal growth is dependent primarily on adequate amounts of light and nutrients. Temperature and salinity affect algal physiology (metabolism), including how well the algae utilize the available light and nutrients. Our results regarding these four factors are summarized as follows:

Nutrients

1. Nitrogen is the limiting nutrient. This means that algae will be stimulated most by additional nitrogen inputs, but not as much by adding more phosphorus. This finding also suggests that nitrogen reduction strategies can be effective in reducing algal biomass.
2. Organic nitrogen may be a nutrient source. Plants and algae typically utilize inorganic forms of nitrogen such as nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+). Organic nitrogen is generally thought of as inert, i.e., unutilized by plants and algae. These organic compounds may be broken down into utilizable inorganic compounds by microbial (or photolytic) processes. Our results suggest that inorganic nutrients by themselves were not abundant enough to support the concentrations of microalgae encountered in this study. Therefore, it is logical to assume that the organic compounds could be a source of inorganic nutrients. The importance of this finding is that there are far greater amounts of organic nitrogen versus inorganic nitrogen in the Caloosahatchee River and Estuary. Therefore, a greater pool of nitrogen is present than if inorganic nitrogen sources were considered alone. Organic nitrogen sources should also be addressed in reduction measures. The first step in this process would be to determine what the significant sources of organic nitrogen are, and then the implementation of Best Management Practices to reduce the inputs as appropriate and feasible.
3. Nutrient concentrations generally decrease from Lake Okeechobee to the Gulf of Mexico. These results suggest that Lake Okeechobee is a significant source of nutrients to the CRE, which in turn provides a significant amount of nutrients to the coastal waters in the Gulf of Mexico. The nutrients decrease through a combination of conservative (i.e., tidal mixing with nutrient-poor Gulf waters) and non-conservative (uptake of nutrients by algae) processes.
4. Submarine groundwater discharges (SGD) are a significant source of nitrogen. These inputs vary seasonally, with nitrogen loads being over two times higher during the dry season than the wet season. The factors influencing the hydraulic head (i.e., delay between precipitation and subsequent discharge) remain unknown.

5. On an annual basis (averaged between 1995 – 2005), Lake Okeechobee provides 27% of the total nitrogen and 18% of the total phosphorus reaching the lower estuary. SGD account for 25% of the total nitrogen and 27% of the total phosphorus reaching the lower estuary. The West Caloosahatchee sub-basin (between S-78 and S-79) provides 16% of the total nitrogen and 20% of the total phosphorus reaching the lower estuary. Therefore, there are multiple, significant sources of nutrients influencing estuarine/coastal processes (i.e., Lake Okeechobee should not be the only source of concern).
6. More total nitrogen (76%) and more total phosphorus (63%) enter the lower estuary during an average wet season versus an average dry season. On average, 60% of the total nitrogen and phosphorus enter the lower estuary during the wet season.
7. Sediments do not appear to be a significant source of phosphate over the course of an annual cycle. Nitrogen (primarily as ammonium), however, is regenerated out of the sediments during the wet season (thereby becoming a nitrogen source) and absorbed into the sediments during the dry season (thereby becoming a nitrogen sink).
8. Decomposing algae do not appear to release a significant amount of nitrogen back into coastal ecosystem (e.g., the swash zone and shoreline longshore troughs).

Algae-nutrient relationships

1. Microalgal biomass (phytoplankton and benthic microalgae) decreases from Lake Okeechobee to the Gulf of Mexico, reflecting the decrease in available nutrient concentrations from Lake Okeechobee down to the Gulf of Mexico, or dilution with nutrient-poor Gulf of Mexico water.
2. Macroalgal (seaweed) tissue nitrogen content is higher inshore and during the wet season. These results may indicate that the algae are sequestering the nitrogen during the wet season when inputs are higher, or that algae are growing more slowly in the wet season and are therefore storing rather than utilizing the available nitrogen.
3. $\delta^{15}\text{N}$ ratios from algal tissues were significantly higher during the dry season. These results suggest that inshore (and/or during the dry season), algae either rely more heavily on regenerated nitrogen, or that SGD-derived nitrogen has a heavier $\delta^{15}\text{N}$ signature than surface water nitrogen sources. SGD $\delta^{15}\text{N}$ signatures should be determined in a future study.
4. Macroalgal tissue phosphate content was significantly higher in the dry season, suggesting that phosphate may be more available (or more sequestered) during the dry season.

Temperature

1. Quantum yields were significantly, negatively correlated with temperature, suggesting that algae will grow best at temperatures below 25°C.

Salinity

1. Quantum yields were significantly, positively correlated with salinity, suggesting that algae will grow better as salinity increases (especially >30 ppt).

Light

1. Quantum yields were negatively correlated with I_z (light intensity at depth). The results suggest that algae might be light limited at low intensities (<20 $\mu\text{E}/\text{m}^2/\text{s}$), and photoinhibited at higher values (>40 $\mu\text{E}/\text{m}^2/\text{s}$).

Grazing

1. Our results suggest that inshore (away from passes), algal growth may be controlled more by nutrient availability than by grazer activity (at least during the course of this study).
2. Urchin grazing appears to be a controlling factor in coastal and offshore locations (based on their abundances at these sites).

Secondary Factors (blue boxes)

The secondary factors (Lake Okeechobee flow, local run-off, SGD, and sediment regeneration) were discussed above in various bullets, but will be further summarized below incorporating their influences not only on nutrients, but light and salinity as well.

Lake Okeechobee Flow

1. Lake Okeechobee provides 27% of the total nitrogen and 18% of the total phosphorus reaching the lower estuary.
2. As flows out of the lake are highly managed, they do not necessarily follow wet and dry seasonal cycles.
3. Flows through S-77 affect salinity and light attenuation; salinity is reduced as flows increase, and light attenuation increases with increasing flow. These processes have been studied elsewhere (e.g., Bissett et al. 2005; Milbrandt et al. in prep.).

Local Inputs

1. On an annual basis, local inputs account for 48% of the total nitrogen and 62% of the total phosphorus reaching the lower estuary.
2. The West Caloosahatchee sub-basin (just upstream of S-79) is the largest local contributor, providing 16% of the total nitrogen and 20% of the total phosphorus reaching the lower estuary.

3. Local inputs likely affect total suspended solids and salinity, but will vary with precipitation and according to land use (i.e., erosion). Previous modeling efforts have examined the influence of local tributary discharges (see FDEP 2008).

Submarine Groundwater Discharge (SGD)

1. SGD accounts for 25% of the total nitrogen and 27% of the total phosphorus reaching the lower estuary.
2. SGD inputs are greater during the dry versus wet season, suggesting a considerable lag (>60 days) between precipitation and subsequent discharges. This relationship should be examined further.

Sediment Regeneration

1. Sediment fluxes in the tidal Caloosahatchee (between S-79 and Shell Point) account for 5% and 2% of total nitrogen and phosphorus inputs to the lower estuary respectively.
2. Sediment fluxes in San Carlos Bay account for 2% and -2% of total nitrogen and phosphorus inputs to the lower estuary respectively. The negative flux for total phosphorus indicates the sediment in this area is a sink rather than a source.
3. Sediment fluxes in the tidal Caloosahatchee are consistent between the wet and dry seasons, whereas they are positive during dry season (i.e., a source) and negative during the wet season (i.e., a sink) in San Carlos Bay.

Tertiary Factors (purple boxes)

The two tertiary factors of the conceptual model are precipitation and seasons. As season influences precipitation, it can also be considered a quaternary factor, but will be treated here for simplicity's sake.

Seasons

1. Inshore, algae reach maximum biomass in early spring. Offshore, maximum algal biomasses occur in mid to late summer.
2. Algal growth is more favorable inshore, especially during the dry season.
3. Therefore, the conditions most suitable for algal growth would be moderate temperatures (<25C), high salinities (>35), and low/moderate light intensities (15 – 35 $\mu\text{E}/\text{m}^2/\text{s}$). If the peaks in algal biomass in the spring (inshore) and late summer (offshore) reflect higher growth, then it suggests that these optimal conditions may be more common in inshore waters during the spring and offshore during the late summer.

Precipitation

1. Local/regional precipitation will influence local nutrient inputs and likely will affect SGD.

Substrate and Location (orange box)

We have learned several important facts regarding where algae grow and/or accumulate, and the substrates most conducive for growth and accumulation.

1. Geographic location and depth were more important than substrate type for determining the overall macroalgal community structure (i.e., sites that are closer to each other and/or occur at the same depths will have more similar assemblages).
2. Inshore algal assemblages differ from offshore assemblages.
3. Inshore (and nearshore) habitats favorable to algal growth/accumulation include patchy seagrass beds and dense tube-building polychaetes (e.g., Onuphidae, *Diopatra cuprea*).
4. Offshore habitats favorable to algal growth/accumulation include live/hard bottom substrates and worm tubes of other polychaetes (e.g., parchment worms, *Chaetopterus*) and bivalves (e.g., pen shells, Pinnidae).
5. Although the hydroacoustic and video surveys indicated that unconsolidated, soft substrates were most common (and unsuitable for algal attachment and accumulation), pen shells and parchment worm tubes are often associated with these substrates and could allow algae to grow and accumulate in regions where pen shells and worm tubes are abundant. Therefore, soft sediment environments should be included along with the live/hard bottom areas in future monitoring/survey studies.
6. Inshore quantum yield values are highly correlated to offshore values suggesting regional influences (e.g., temperature and water clarity).
7. Hydroacoustic and video survey results indicated that there are two significant expanses of rough seabed thought to be suitable for macroalgae attachment. These two areas covered a total of 19 km² (7.3 mi²), including a large area of seagrass beds and “live hard-bottom” in the mouth of San Carlos Bay, and a site offshore of Lighthouse Point, where a large sand bar extends from the beach to approximately 6 km offshore. Along the west side of this sandy area is a substantial area of predominately composed of unconsolidated, shelly hash.
8. Areas further offshore in the Gulf of Mexico appear to be predominantly soft sediments, indicating that the open Gulf of Mexico waters around Sanibel-Captiva are probably not a major source of drift macroalgae (but see #5 above).

Transport mechanisms

The above factors provide information on the conditions optimal for algal growth as well as the locations where algae can be expected to grow and/or accumulate. The next step is to determine

the mechanisms involved in detaching and transporting the algae onto the beaches of Sanibel and Fort Myers. A summary of these mechanisms is provided below.

1. Wind is the primary long term (i.e., over weekly time scales) forcing factor of the water compared to tidal action or river discharge outside of the Caloosahatchee River.
2. Northwest winds can transport water (and therefore drifting algae) onto Estero Island (and southward) from within the tidal Caloosahatchee, near the Causeway, and from offshore sites west of Sanibel (e.g., GOM12 off Redfish Pass). The southeastern shore of Sanibel Island can be impacted by these transport vectors as well, primarily from within the tidal Caloosahatchee.
3. Southwest winds can transport water onto south Sanibel beaches from nearshore locations southeast of Sanibel, as well as from San Carlos Bay and the tidal Caloosahatchee during wet season. Fort Myers Beach may also be affected.
4. During the dry season simulation using actual wind, tide, and river flow data from February 1 – March 16, 2008, the southern shore of Sanibel was significantly exposed to water that originated near the Causeway. Small amounts of accumulated drift algae were noted on Sanibel in February and March 2008 (please refer to references 36 and 37 in Appendix 9.1).
5. The largest stranding event that occurred during this study happened in June 2008. The wet season simulation results (based on actual wind, tide, and river flow data from June 22 – August 5, 2008) suggest the algae came from nearshore locales (i.e., near the Causeway) rather than inshore sites (i.e., within San Carlos Bay or Caloosahatchee Estuary).
6. During the dry season, the largest sediment transport events are clearly associated with strong wind speeds, especially when the wind direction is parallel with the orientation of the southern coast of Sanibel.
7. During the wet season, sediment is deposited onto the ebb deltas at the barrier island passes via the ebb dominant currents. These deposits likely represent significant reserves of sand just offshore of the passes which are available for cross shore transport into the passes during storms.
8. When winds from the north coincide with flood currents there is significant longshore sediment transport along the south coast of Sanibel.
9. At a smaller scale, tidal propagation tends to move sediment northwards once outside of Caloosahatchee River. This is supported by the general morphology of Sanibel and Captive islands.

Beach

While no major algal strandings occurred during the course of this study, there were several small events that provided useful information.

1. Beach algae assemblages collected during the winter of 2009-2010 were significantly different from all the artificial and natural reef (hard bottom) samples. These hard bottom sites were likely not the sources of algae for this event.
2. Algae collected from a later stranding (November 2010) were composed of primarily hard-bottom associated species, suggesting that these sites (e.g., GOM12) were the source in this instance. Fig. 7.18 of the hydrodynamic model indicates that it is possible for algae to be transported from these sites to the beach.
3. The species composition of the stranded algae in events occurring from January through March 2010 appeared to match more closely with the composition at the inshore stations, near the causeway. Hydrodynamic modeling results support this scenario, as do the results of the hydroacoustic/video survey.
4. Algae collected during minor stranding events between January and March 2010 were most similar to inshore algal assemblages, again supporting the above scenario.

Conditions Leading to Excessive Algal Growth and Subsequent Stranding Events

While no large-scale stranding events occurred during this study, the results presented above can be used to construct a plausible scenario leading to such an event.

1. Build-up of a significant algal biomass

The results of this study suggest that algae grow best (and accumulate the most biomass) on either side of the wet season (just beforehand offshore; just after inshore). Algal biomass is lowest during the peak of the wet season, typically when nutrients and temperatures are highest, but when salinity and light availability are lowest. It appears that the higher temperatures, coupled with the lower salinity and light levels, hinder algal growth. Algae do not grow, therefore, until the discharges related to local run-off and lake releases drop significantly. While our initial hypothesis was that regenerated nutrients from the sediments would support algal growth at this juncture, our results indicate that such inputs appear to be insignificant. Rather, submarine groundwater discharges (SGD) are extremely significant, especially during the dry season. We are now hypothesizing that SGD are the source of nutrients building up the algal biomass (inshore and offshore) during the dry season and before/after the peak of the wet season. This hypothesis is substantiated by the fact that SGD accounts for 25% of the total nitrogen and 27% of the total phosphorus reaching the lower estuary annually, but 76% and 62% of each respectively during the dry season. Hu et al. (2006) hypothesized a similar scenario for the extensive red tide that initiated in 2005, where SGD related to an active hurricane season in 2004 provided the “nutrient boost” needed to initiate the bloom. A similar scenario may be active for

macroalgae, in which SGD-derived nutrients can build up biomass of macroalgae (nearshore and inshore) prior to a large stranding event. Hydroacoustic/video survey results indicate that this algal biomass is most likely to grow and/or accumulate near the causeway and/or Lighthouse Point. Therefore, these areas should be closely monitored for such a build-up in algal biomass that would (hypothetically) precede a stranding event.

2. *Detachment of algae*

After a significant algal biomass has built up, the next step would be for the algae to detach. Algae can detach for a variety of reasons including nutrient deficiency, extremes in temperature and salinity, wave action, grazing animals, and natural life history processes (Norton and Mathieson, 1983). While we do not know the specific triggers for algal detachment in local waters, algae have been observed to detach when they reach 15 – 20 cm in height in the Indian River Lagoon (Foster, pers. obs.). Such detachment could be part of the natural life cycle, or may be triggered by warming temperatures and falling salinities as wet season conditions intensify into August. As beach strandings have been documented through the year, detachment may be caused by different factors (e.g., wave energy during hurricane season, falling temperatures in winter, low salinities in fall, nutrient limitation in dry season). Further study of potential triggers will be needed to narrow these possible detachment scenarios.

3. *Mass stranding event*

Once a significant amount of algae has been detached, a consistent northwest wind would provide the best mechanism to blow the algae onto the beaches of Sanibel and Fort Myers. Longshore currents could also transport the algae further north (towards Captiva) or south (towards Bonita Beach). A southwest wind will result in a similar, yet smaller stranding, as more water (and drifting algae) will stay off the beaches. Accompanying this report are four scenarios that we believe are most likely in leading to a mass stranding event, further elaborating on the processes presented here.

Why was there no significant stranding event in the past two years?

We offer the following hypotheses for the lack of a stranding event during the course of this study:

1. Lack of significant rainfall. Average monthly precipitation at S-79 was 69% lower in 2008 – 2010 versus 2003 – 2005 (0.13” versus 0.42”). Correspondingly, average monthly flow through S-79 was 61% lower in 2008 – 2010 versus 2003 – 2005 (1,467 cfs versus 3,810 cfs). The differences were greater for wet season-only averages: (0.18” versus 0.74” for a 75% difference; 2,116 cfs versus 5,707 cfs for a 63% difference).
2. Lack of wave action. The lack of significant winter and/or tropical storm activity in the past several years likely caused a reduction in wave energy and/or currents needed to detach algae. Not only is there less algae biomass present because of the lack of rainfall, but the algae that are present are not detaching as frequently because of the lack of energy.

3. Lack of consistent northwesterly winds. An examination of historical wind data has indicated that winds can be quite variable on a daily to weekly basis as cold fronts move through and high pressure cells shift position. Additionally, accurate initiation dates for algal stranding events are not consistent and/or available. This ambiguity, coupled with the variable nature of the winds, prevents us from further examining historical wind data as needed to verify this hypothesis. But if there have been less consistent northwesterly winds since 2008 versus 2003 – 2007, it could result in less algae washing up on local beaches.

What could have caused a reduction in precipitation, storm activity, and northwesterly winds? One possibility is that the time period of 2003 – 2007 was dominated by El Niño conditions, whereas La Niña has been a bigger factor since 2008. This possibility is supported by the fact that La Niña conditions were more prevalent in 2008 – 2010 versus 2003 – 2007 (53% of the time versus 8% of the time; data from the NOAA Earth System Research Laboratory: <http://www.esrl.noaa.gov/psd/people/klaus.wolter/MEI/rank.html>). Simply stated, La Niña conditions can result in lower precipitation and river discharge in south Florida (Schmidt et al. 2001), so this hypothesis seems likely. However, the processes involved are complex, so this statement may not apply everywhere in south Florida, or during all La Niña conditions. Conversely, La Niña conditions tend to stimulate hurricane activity for storms similar in dynamic and track to Donna, Charley, and Wilma (Kossin et al. 2010), so the complexities in climate dynamics are apparent when we consider the lack of local hurricane activity since 2008 (excepting Tropical Storm Fay). ENSO influences on wind patterns in southwest Florida are complex, and may be influenced more by the Atlantic Warm Pool (Wang et al. 2006). It is unclear, therefore, if La Niña conditions could have affected local winds.

Management and Policy Implications

1. Nitrogen-reduction measures should be considered to reduce algal biomass in the CRE.
 - a. Microalgae (and possibly macroalgae) are nitrogen-limited in the CRE. Reducing the levels of nitrogen-based nutrients (e.g., nitrate and ammonium) in the CRE will therefore reduce the amount of algae that can be supported in the ecosystem.
 - b. 18 – 27% of the nutrients entering the tidal portion of the CRE (i.e., below S-79) are derived from Lake Okeechobee, with an equal proportion (25 – 27%) coming from submarine groundwater inputs. The remaining nutrients (40 – 50%) are then estimated to be coming from the local watershed, particularly above S-79 (the East and West Caloosahatchee sub-basins, contributing 24 – 29% of the nutrients). These results indicate that nitrogen-reduction measures should be implemented, beginning with the watershed above S-79, and later expanding to the watershed below S-79 (which contributes 12 – 20% of the nutrients).
 - c. As there are significant differences in nutrient inputs between wet and dry seasons, efforts to reduce nutrient inputs should focus on those that are greatest during the wet season (i.e., non-point sources related to run-off).
 - d. The best approach to reduce nutrient inputs would be through Best Management Practices (e.g., fertilizer ordinances, stormwater treatment areas, required septic system inspections, etc.), as outlined in CRWPP (2009).
2. A program should be established to monitor for greater than average (“significant”) accumulations of macroalgal biomass that might precede a major drift algae stranding event.
 - a. Areas conducive for macroalgal growth and accumulation have been identified.
 - i. The first is the large area of seagrass beds and live hard-bottom near the mouth of San Carlos Bay, where large amounts of drift algae are often variably present (e.g., during the April-May 2009 surveys).
 - ii. The second is an area of coarse “shellhash” offshore of Lighthouse Point, located near a large sand bar that extends from the beach to approximately 6 km offshore.
 - iii. As no major stranding events occurred during the course of this study, we cannot confirm that algae associated with past stranding events did in fact come from these regions. However, the most common macroalgae reported from the 2003/2004 stranding events were species that we found

commonly at inshore and nearshore locations from 2008-2010 (e.g., *Solieria filiformis* and *Hypnea spinella*).

- iv. While nearshore artificial reefs and natural ledges do not appear to be a significant source of algae in the quantities necessary to cause a significant stranding event, algae that have washed-up on area beaches (e.g., Sanibel in November 2010) are also known inhabitants of these nearshore reef (hardbottom) environments. Therefore, regularly monitoring of these sites, especially if algae above and beyond average algal biomass would expand the spatial and environmental coverage of the future program.
- b. “Sentinel” sites should be chosen for such a monitoring program (e.g., time series with quarterly monitoring of algal abundance and species composition).
 - i. The recommended sites include: the mouth of San Carlos Bay, the sides of the sand bar located off the Sanibel Lighthouse, one or two natural ledges (e.g., GOM12 and 53 Ledge), and one or two of the artificial reef sites (e.g., Edison Reef and GH Reef).
 - ii. These sites can be monitored by City/County personnel, the Volunteer Scientific Research Team (VSRT), or contracted scientists as funds allow (e.g., from SCCF or FGCU).
 - c. Standard measurements and collections should be made at these sites.
 - i. Methods should be standardized to determine absolute algal abundance (e.g., video surveys, dives, snorkeling, or quadrats/transects as needed or as manpower is available).
 - ii. Algal samples should be collected to determine species composition and to collect tissues (if possible) for stable isotope analysis and nutrient composition (to determine changes in nutrient conditions and sources over time).
 1. Inshore algal communities (e.g., in and around San Carlos Bay) are often composed of different species than nearshore and offshore communities based on this study.
 2. By monitoring the species composition of macroalgae at the “sentinel” sites, and comparing these assemblages to those found at a “stranding” event, we can potentially determine the source of drift macroalgae washing ashore.

3. Standard collection and analysis methods should be agreed upon with County and City staffs.
 - d. A monitoring program of this nature will likely require additional resources.
 - i. A permanent funding mechanism to annually contribute to the program needs be identified.
3. Efforts should continue to find ways to proactively respond to a build-up of algal biomass prior to beach deposition.
 - a. Details of location, timing, and extent of future large-scale stranding events should be recorded in a complete and standardized fashion (see #4 below).
 - b. After details of such events are recorded, continued modeling efforts could be focused on these events to better test the hypothesis of the interaction between seasonal nutrient advection and wind forced transport.
 - c. The presence of large amounts of algae at the identified “sentinel” sites, coupled with a better knowledge of the mechanisms that transport the algae to area beaches may allow for a proactive response to any impending event.
 - d. The best proactive response at this time (without an event to characterize) would be to reduce algal biomass through nutrient reduction measures as outlined in #1 above.
 - e. A second option would be to further study the role of grazers, and the impacts caused by regulated releases (e.g., drastic salinity changes). A healthy grazer population can potentially help keep the local algal populations in check.
 - f. A third option would be to somehow intercept the algae prior to deposition.
4. Determination of and Response to Beach Stranding Event(s)
 - a. Event Determination. A mechanism needs to be put in place to monitor for, and assess whether an above average stranding event is taking place
 - i. A decision tree should be constructed to determine the level of response needed for various categories of events.
 1. Categories should be defined and standardized based on clearly defined parameters including (but not limited to) size of the deposition (length, width, and height), condition of the algae (fresh or degraded), odor, and citizen reaction to the deposition.

2. First responders should provide an initial assessment (including photographs, GPS coordinates, etc.) to the appropriate staff/personnel (second responders - to be determined).
 3. The second responders should determine what additional assessments/actions are needed.
- ii. These assessments/actions should follow the Event Response guidelines outlined below.
- b. Event Response. Once an above average stranding event has been identified, several courses of action must be implemented. Each “event” should be documented using standard procedures and measures. A list of considerations includes the following:
- i. One has to ascertain what constitutes a baseline (acceptable) level of algae on local beaches, and what amount would represent an above average stranding event.
 - ii. The timing of the event should be accurately recorded (i.e., when did it start, how long was algae being deposited).
 - iii. The spatial extent of the event should be ascertained (i.e., is it a small (and likely temporary) event or large? How is a large event categorized? What are the parameters to be quantified? Can the mass of algae be estimated?).
 - iv. If the event is of large scale, aerial reconnaissance is recommended to estimate biomass and the probability that the event will last days or months.
 - v. GIS and photos with GPS information can aid in assessing and quantifying the extent of the event.
 - vi. Other factors such as tourist perception and odor should also be considered.
 - vii. The composition of the algae must be determined. For example, do the algae appear fresh (firm and pigmented) or degraded (soft and bleached)? Are holdfasts present? What species are present? At what abundances are the species present (absolute and relative)?
 - viii. Algal samples must be collected and kept on ice for species identification and tissue analysis (e.g., stable isotopes, carbon and nitrogen content).

- ix. Collected samples can be identified and processed with help from seaweed experts (e.g., SCCF or FGCU scientists).
- x. A decision tree should be developed to determine if and when the deposited algae should be mechanically removed from the beach, allowed to be removed by natural (tidal) processes, or allowed to be left in place for natural degradation. Factors that need to be considered include:
 - 1. the cost for removal;
 - 2. the potential for tides and wave action to remove the deposition;
 - 3. the negative impact on the recreational use of the beach; and
 - 4. possible positive ecological implications of the deposition (e.g., stabilization of beach sands and enrichment of beach biotic communities).
 - 5. Objective 6 outlines such considerations that will be useful in constructing the decision tree.

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