ECOHAB: Florida

Coordinating PI Karen A. Steidinger_____
Florida Marine Research Institute
Florida Department of Environmental Protection
100 Eighth Avenue Southeast
St. Petersburg, FL 33701
(813) 896-8626
(fax) (813) 823-0166
steidinger_k@sellers.dep.state.fl.us

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Organization Representatives

Edwin J. Conklin
Director
Division of Marine Resources
Florida Dept. of Environmental Protection
3900 Commonwealth Blvd.
Tallahassee, FL 32399
(904) 488-6058

Kumar Mahadevan
Executive Director
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FL 34236
(941) 388-4441

Priscilla Pope
Acting Director
Division of Sponsored Programs
University of South Florida
140 Seventh Avenue South
St. Petersburg, FL 33701

Ronald G. Hodson
Interim Director
N.C. Sea Grant College
Box 8605
N.C. State University
Raleigh, NC 27695
(919) 515-2454

Susan D. Allen
Vice President for Research
Florida State University
Tallahassee, FL 32306

INTRODUCTION

The first written report of seasonal fish kills and discolored water (red tide) off the west coast of Florida was published in 1542 by A.N. Cabeza de Vaca. The causative agent, the toxic dinoflagellate *Gymnodinium breve,* was not described until 1948 by C .C. Davis. It was later cultured by Wilson and Collier (1955) and determined to be toxic to fish. Over the last century, the duration of red tides off west Florida has varied from none to 18 months, with 70% of the blooms occurring in late summer-fall (Steidinger et al., 1997). Red tides have been observed in 21 of the last 22 years within the region between Tampa Bay and Charlotte Harbor (Fig. 1), compared to 4-10 outbreaks north and south of that region. This region is thus defined as the epicenter of *G. breve* abundance along the west Florida coast and constitutes a focused study area for ECOHAB:Florida. Red tides may, however, initiate and be confined to other areas north and south of our suggested epicenter between Tampa Bay and Charlotte Harbor - indeed, our pre-ECOHAB study aboard the R/V Anderson involved a red-tide in Apalachee Bay during August 1997 that was still present in October 1997 in offshore waters. Therefore, these other regions will be included in periodic surveys for bloom detection.

Our ability to predict initiation, maintenance, and dispersal of red tides on the Florida shelf is severely limited by the lack of a quantitative description, or model, of their population dynamics. If the occurrence, distribution, and effects of *G. breve* blooms are properly understood and predictable in time-space, at different scales, then questions like the following can be answered: Can *G. breve* HABs (Harmful Algal Blooms) be mitigated and/or managed? Is man responsible in anyway for the occurrence, intensity, or duration of *G. breve* HABs, and are *G. breve* HABs important to the ecological integrity and productivity of the west Florida shelf? Answers can provide risk management strategies to natural resource and public health managers, and critical information to local government.

Of course, the flaws of any model are in the details - those assumptions involved in selection of parameter values. We know the following: frontal systems are a key to initiation and transport, growth rates are typically 0.2 to 0.3 divisions day^{-1} in natura and in the laboratory, physiological state affects toxin production, *G.breve* photoadapts, *G. breve* is efficient at utilizing inorganic N and P, and *G.breve* can use organic N and P. We also suspect the following: that there is 1) a bacteria-dinoflagellate association, 2) new nutrient sources may sustain *G.breve* blooms inshore, 3) long residence times as a result of the seasonal reversal of the longshore flow (Weisberg et al., 1996) may trap nutrients recycled from spring diatom blooms, driven by estuarine nutrient sources (Gilbes et al., 1996), and 4) endogenous cell-cycle feedback loops cue cell senescence and death of *G.breve*.

Many of the above observations were made from laboratory studies that need to be verified in the field. Funding of this proposal for the second field/modelling phase of ECOHAB:Florida will enable us over four years to dissect bloom dynamics - so we can predict bloom occurrence, distribution, movement, toxic effects, and (hopefully) dissipation. Our proposal encompasses five focal groups to obtain these goals of prediction: 1) Ecological modelling [Walsh, Kamykowski, Janowitz], 2) Physical oceanography [Weisberg, Sturges, Weatherly, Vargo], 3) Remote sensing [Muller-Karger, Stumpf], 4) Biological oceanography [Kirkpatrick, Steidinger, Redalje, Lohrenz, Scofield, Tomas, Millie, Van Dolah, and Fahnenstiel], and 5) Fate and effects of toxins [Landsberg, Pierce, Fournie, Tester]. The focal groups represent

22 senior investigators from 11 institutions representing 5 universities [USF, FSU, USM, NCSU, Rutgers] and 1 state [FDEP], 1 private [MML], and 6 federal laboratories [NOAA (3), EPA, USDA, USGS].

Based on our existing support from the State of Florida, NOAA, EPA, MMS, NASA, ONR, USGS, and The Selby Foundation we have structured a four-year ECOHAB: Florida field/modelling program to develop and validate models to address the questions asked in this proposal. On September 1, 1997, a 7-month award of $467,000 was made by NOAA to DEP, USF, Mote, NOAA, and NCSU for mainly purchase of field equipment, in anticipation of a second phase of an ECOHAB:Florida program now proposed, with a first cruise during May 1998. In addition, a January 1, 1998 award of $33,000 from EPA to DEP is expected for refinement of toxin in seawater and sediment methods and for zooplankton exposure studies.

BACKGROUND

Harmful algal blooms have caused massive fish kills in the Gulf of Mexico since the 1500s. In the 1800s, for example, red water or "poisoned water" off Florida's coast was associated with fish, invertebrate, and bird kills, toxic shellfish, and a human respiratory irritant (Rounsefell and Nelson, 1966). By 1996, however, all states in the Gulf of Mexico had experienced *G. breve* blooms that impacted natural resources and public health. Although Texas had recorded *G.breve* red tides in 1935, 1955, 1974, 1986, and 1996 (Buskey 1996), 1996 was the first record for Alabama, Mississippi, and Louisiana.

Blooms of toxic *G. breve* originate 18-74 km offshore of central Florida at depths of 12-40 m (Steidinger and Haddad, 1981), yielding surface stocks of as much as >90 µg chl a^{-1} (Carder and Stewart, 1985), and carbon fixation rates of 1.9 g C m^{-2} day^{-1} (Vargo et al., 1987). They are apparently not subjected to much grazing pressure, are not found in salinities <24 psu, persist from 1 to 18 months, and force closure of shellfish beds if they are transported inshore. Two 3-5 month red tides off Florida caused 15 to $20 million revenue losses to local communities in the 1970s (Habas and Gilbert 1974, 1975). In 1987 when a Florida red tide was transported to North Carolina waters by the Gulf Stream, shellfish closures alone caused an economic loss of $25 million (Tester and Fowler, 1990).

Gymnodinium breve is common in the Gulf of Mexico all year long at cell concentrations of $<1 \times 10^3$ l^{-1} (Geesey and Tester, 1993) which is considered the background level. Most HAB events are recognized and documented by their impacts. These impacts can depend on different cell concentrations: e.g ., at $>5 \times 10^3$ cells l^{-1} , *G. breve* can cause closure of shellfish beds due to the potential of Neurotoxic Shellfish Poisoning (NSP), at $>1 \times 10^5$, it can cause fish kills and manatee mortalities, at 10^5 in surface waters, chlorophyll can be detected by satellite sensors but it isn't until 1×10^6 that the human eye can detect discolored surface water (Tester et al., in press). Cell concentrations have been recorded as high as 1×10^8 l^{-1} in Texas waters (Buskey, 1996). Once a bloom has developed offshore in typically oligotrophic waters, cell concentrations at the 10^5 level can be maintained for months. What nutrient levels and sources support blooms, offshore and inshore?

At intermediate red tide levels of 1×10^6 cells l^{-1}, or ~13 µg chl l^{-1}, initial nutrient stocks of 8.0 µg-at NO_3 l^{-1} and 0.5 µg-at PO_4 l^{-1} would be required (Wilson, 1966; Vargo and Shamblott, 1990) to sustain this population level. However, these concentrations of both inorganic N and P are not found within 2-4 km of the Florida coast (<0.2 µg- at l^{-1}; Dragovich et al., 1961, 1963;

Vargo and Shanley, 1985). Furthermore, the atomic ratios of dissolved inorganic nitrogen (NO3 + NH4 + NO2) and phosphate in the Peace River, entering Charlotte Harbor (Fraser and Wilcox, 1981; McPherson, 1990), and in the Alafia River, entering Tampa Bay, are usually <2; they both drain the phosphate-rich Hawthorne formation of central West Florida (Dragovich et al., 1968).

Nutrient fields are part of the growth component of population dynamic models. For most phytoplankton species and *G. breve* in particular, there is more known about the growth processes than the loss processes (Walsh, 1983) of grazing, lysis, and advection that control population dynamics. Moreover, *G. breve* blooms may not always dissipate nearshore in Florida coastal waters. Blooms have been entrained in the Gulf Stream System and transported around the Florida Keys and up to as far as North Carolina (Murphy et al., 1975; Tester et al., 1991; Tester and Steidinger, 1997). Drift bottle return data from Gulf of Mexico releases in the 1960s mirrors the path, timing, and landfall of *G. breve* blooms transported to the east coast (Williams et al.).

The key to understanding any HAB lies in knowing how one algal species has adapted and come to dominate in its particular ecosystem. HAB species exploit their physical and biogeochemical environment, but what physiological and behavioral adaptations represent successful survival and dispersal strategies in a fluctuating environment? Have red-tides increased in the Gulf of Mexico over the last 400 years, as part of a global epidemic (Smayda, 1990), or has our observational network grown instead? Is there a resident population of *G. breve* at high concentrations somewhere on the west Florida shelf all year long that may or may not inoculate inshore waters?

PROGRAM OBJECTIVES

**Model the initiation, maintenance, and export of *G. breve* red tides on the west Florida shelf at different time and space scales to predict landfall.

**Describe the physical habitat that effects transport and concentration of *G. breve*.

**Determine the sources of inorganic and organic nutrients that allow growth and persistence of large *G. breve* populations in coastal waters.

**Determine the interactions of cellular, behavioral, life cycle, and community regulation processes with environmental forcing factors during stages of bloom development.

**Determine the production, occurrence, fate, and effects of brevetoxins in the environment during and after *G. breve* blooms.

IMPLEMENTATION

1. ECOLOGICAL MODELLING

The ECOHAB:Florida program is designed to describe both the large scale setting of the HABs, so that variations within a more limited near-coast control volume can be specified, and the smaller features such as fronts and patches that may be critical to successful predictive models of bloom dynamics. The first objectives of our fully three-dimensional models are to define why certain shelf regions appear to be important for different stages of bloom development of *G. breve* and how these regions communicate with others in terms of changing population dynamics. Our second set of objectives involves forecasts of the future locations of red tides, such that mitigation policies may be utilized. Forecast models require increased resolution at the scales of coupled biological-physical interactions, however, such that we will nest both higher resolution (1-km) regional models within the low resolution (~10-km) shelf-wide models and patch scale models of very high resolution (<1km).

We propose to model the initiation, maintenance, and export of *G. breve* red-tides in our ECOHAB: Florida Program at different time and space scales. As indicated in the ECOHAB agenda (Anderson, 1995), such models of red tide population dynamics take the form of coupled partial differential equations, with the amount of phytoplankton described at single geographic location, or model grid point, by

(1) $\partial P/\partial t = \partial/\partial x(Kx\, \partial P/\partial x) + \partial/\partial y(Ky\, \partial P/\partial y) + \partial/\partial z(Kz\, \partial P/\partial z) - \partial/\partial x(uP) - \partial/\partial y(vP) - \partial/\partial z(wP) + gP - \varepsilon P - Z \pm \partial/\partial z(wpP)$

where the first term on the left side of eq. (1) is the local change of carbon biomass, P, of the red tides over time, t. The chlorophyll equivalent of this term will be measured by both the satellite and shipboard surveys; in the latter, C/chl ratios will also be determined.

On the right side of eq. (1), the first 3 terms are eddy mixing of phytoplankton at turbulent length scales over the three spatial dimensions (x, y, z), while the next three are the advective field of red-tide transport. Tracer studies on the west Florida shelf (Wanninkhof et al., 1997), using the inert gases SF6 and helium-3, confirm Fickian estimates of eddy diffusivity that increase with time and mixing length scale. This situation makes a nested series of 1) shelf-wide predictive models, 2) control volume budgets, and 3) frontal-scale interactions of vertical migrating HABs not only a goal, but such a modelling approach is a first-order necessity.

For example, we do not know the source stock of the HAB population. Are there dormant resting stages in sediments on the mid shelf, or is there a motile quiescent stage in the water column? Florida HABs appear to start from different areas; there have been outbreaks off Tampa Bay, Charlotte Harbor, Sarasota/Venice, and Tarpon Springs. Are these the results of vagaries of the physical circulation patterns or does this mean there are source areas with different genetic strains for phenotypic expressions, like toxin production? Moored arrays deployed over the west Florida Shelf (Fig. 1) and circulation models at 1-10 km grid resolution will provide the advective terms for this scale of questions.

On the other hand, *G. breve* undergoes diel vertical migrations (Heil 1986) at swimming speeds of ca. 1 m hr^{-1}. When cultured in the laboratory, cells accumulate in dense surface concentrations during the day and fall from the surface due to bioconvection. Conceivably, such behavior allows positioning of individual cells (and by extension, a local population) in the water

column for optimal growth and/or light harvesting conditions (Kamykowski et al. 1997), which in conjunction with horizontal transport mechanisms, creates the locus of a HAB event.
Thus, our models will also address space scales of <1 km at the physiological level of *G. breve*'s interaction with its chemical and physical habitat.

The last 4 biological terms of eq. (1) are the net growth [gross photosynthesis - respiration - excretion, which are respectively ~20% and 10% - Shanley and Vargo, 1993], cell lysis, grazing loss to herbivores, Z, and diurnal migration/sinking of the dinoflagellates. The specific biological rates (in units of t-1) of g, ε , , and wp expand to non-linear, time-dependent expressions (Walsh and Dieterle, 1994). Of these 10 terms, growth is usually known with greater accuracy than any of the others, with great detail available on the saturation metabolic responses of *G. breve* to some nutrients (Vargo and Shamblott, 1990; Steidinger et al. 1997), light (Shanely and Vargo, 1993), and temperature (Eng-Wilmot et al., 1977); but unfortunately all of the terms contribute to spatial gradients of P/ t and their associated brevetoxins along the west Florida coast.

Based on the boundary condition and internal validation requirements of the coupled biological -physical models of eq. (1), we will thus start sampling at the coast within the control volume of the epicenter between Tampa Bay and Charlotte Harbor, where the strongest red-tide signal and greatest impact on humans occur. We will then work outwards towards the presumed initiation areas at both the western boundary of the control volume along the 50-m isobath, and to the north, where the FSU arrays will be moored. One section and the models will extend farther offshore to the shelf-break.

Monthly cruises of the R/V Suncoaster during the first two years of this study and small boat transect cruises will provide sampling of this offshore section along the center line of moored arrays (Fig. 1) at 7-day intervals. Then, an annual 3-week experimental cruise will follow a red-tide, located by a volunteer network of private vessels. This network, including volunteers from Solutions to Avoid Red Tide (START), will provide more frequent samples before and after the patch studies. We will also sample the northern sites for abundance of *G. breve* at the 3-month intervals of the FSU mooring maintenance cruises on R/V Seminole (whose ship-time will be mainly paid by MMS). Finally, a present NOAA/COP-funded seasonal study of the southern west Florida shelf (Lee et al., 1992; 1994) also provides the ECOHAB:Florida program with the opportunity to study the southward export of *G. breve*, in collaboration with Tom Lee and Gary Hitchcock at the University of Miami and Peter Ortner and Rik Wanninkhof at NOAA-AOML.

The underway maps of chlorophyll stocks and red-tide levels in the northern, central, and southern regions of the west Florida shelf will also be used as ground truth for the SeaWiFS satellite color data, received by our USF remote sensing center (Gilbes et al., 1996). When the lack of thermal contrast of surface waters precludes use of AVHRR imagery in the Gulf of Mexico (Muller-Karger et al., 1991), we will use the satellite color fields to both interpolate between observations of P/ t on the ECOHAB:Florida surveys/ cruises and to delineate flow features.

At the boundaries of the control volume and in the northern far-field, we will use ADCP (acoustic Doppler current profiler) arrays and tide gauges to measure u, v and sea level, in addition to monthly hydrographic observations for delineation of the baroclinic currents. At the intermediate scale, the horizontal flow field in the interior of the control volume, as well as the vertical velocity, w, and the eddy coefficients, Kx, y, z , of eq. (1) will then be computed from our existing 3-d, primitive equation circulation model (Blumberg and Mellor, 1987), whose

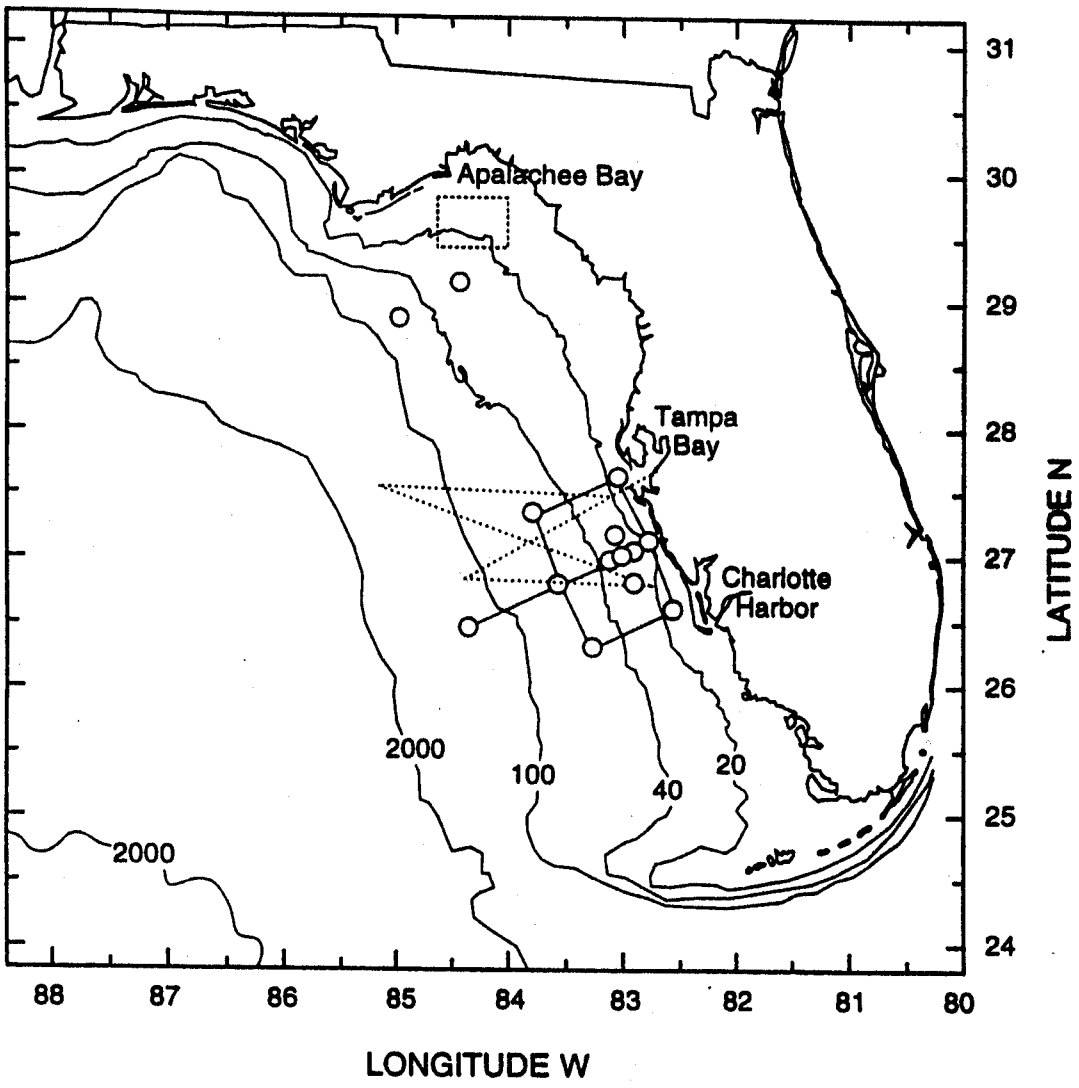


Figure 1. Sampling locations on the west Florida Shelf.

preliminary results are shown in the physical oceanography section. At these boundaries we will also measure the inorganic and organic concentrations of dissolved nitrogen and phosphorus.

At the smaller scale of experimental patch studies, both population growth and behavioral preferences, as affected by physical conditions and water flow, influence local aggregations of dinoflagellates (Kamykowski, 1995). A small-scale modelling effort will combine biological and physical data, especially as collected during the three week process cruises, into a comprehensive, coherent model focused on all identified factors that significantly influence *G. breve*'s life cycle at the encountered stage of the bloom. This effort will act as a bridge between the process cruises and the larger scale modelling effort.

We will utilize predicted currents from the Princeton Ocean Model simulation as input to the biophysical model discussed in Janowitz and Kamykowski (1991), Kamykowski,et.al.(1996) and Liu, et.al.(1996). The present model includes the effects of advection, vertical mixing, and a temporally and spatially varying light field on time-dependent carbon fixation. The model can be readily extended to include the *G. breve* population dynamics as influenced by the intraspecific effects including vertical migration and cell division and by the interspecific effects including cooperation, competition, predator/prey interactions and epidemic losses. As knowledge of *G. breve*'s photo-physiology is gained, a presently coded spectral radiative transfer model, based on the discrete ordinate approach, will be coupled to an enhanced biophysical model, to better predict bloom dynamics on the smallest scales, especially under near-surface bloom conditions.

Once these sets of models are confronted with the first three field seasons of data from the "real world", we will begin forecasts of the future trajectories of red tides along the west Florida coast in the fourth year. Validation data will consist of the continuing experimental studies, possible ONR optical arrays, the volunteer network, and SeaWiFS overflights. We should then be able to specify what monitoring program must be employed, for a reduced data set, to improve accuracy of the predicted land-falls of *G. breve*. An outcome of the ECOHAB: Florida program will thus be an assessment of the data constraints for continued prediction of red tides, after completion of the second four-year phase proposed here.

2. EXPERIMENTAL DESIGN

Our present state of knowledge on red-tide initiation, transport, and dissipation is woefully inadequate for an a priori design of a limited monitoring program - hence the necessity for the comprehensive field and modeling study of the ECOHAB:Florida program. The centerpieces of the field program (Fig. 1) are thus a set of 11 moored arrays (4 purchased and 3 refurbished by ECOHAB funds, 3 provided by the State of Florida monitoring program, and 1 as part of the PORTS study), a set of monthly hydrographic cruises, a set of more localized small boat operations and an annual 3-week experimental cruise, similar to our pre-ECOHAB study aboard the R/V Anderson - see appendix for the cruise report.

Control Volume (Fig.1)*:* The control volume of the intermediate model boundaries will extend from the southern edge of Tampa Bay to the northern edge of Charlotte Harbor and from the shore to the 50-m isobath. The intermediate model and sampling grids are thus approximately 90 km wide by 120 km long, with an area of ~1.1 x 10^4 km^2. The size of this grid is not only determined by the known biology and physics of red tide formation, but it must be of a size which can be sampled in a quasi-synoptic manner at monthly intervals to 1) specify both the initial and post conditions of our experimental cruises, and 2) provide time series of interannual variations of red tide duration off west Florida during the ECOHAB program. The control volume thus

encompasses the inshore region where the greatest frequency of red-tides occur on the West Florida shelf and includes the region identified as the initiation zone at 20-75 km offshore. We must also sample seaward of this zone to identify the properties of pre-bloom waters.

Monthly Surveys: Monthly mapping of currents, surface temperature, salinity, chlorophyll-a (in vivo fluorescence), CDOM (fluorescence), and particle abundance (transmissometry) along four transects (three cross shelf, one along-shelf) within the control volume study area (Fig. 1) will occur aboard the R/V Suncoaster. Information on currents will be acquired using a hull-mounted ADCP while a continuous flow-through deck mounted instrument package will be used for surface mapping. Discrete samples will be acquired during CTD vertical profiles at ca. 18 km intervals along each of the four transects. The cruises will therefore provide the vertical and horizontal distributions of the core parameters required for formulation of the coupled biological-physical models. Each monthly cruise is scheduled for a 5-day duration in the first three years. We anticipate a reduced duration (3 days) and frequency (6 to 9 months) in the last field year to collect a database to be used in model simulation. Two of the monthly surveys will straddle our annual experimental patch study that will be conducted on a second larger vessel (R/V Brown).

Chlorophyll-a concentration (Holm-Hansen and Reimann, 1978 and Welshmeyer filter to correct for chlorophyll-b) and *G. breve* cell counts will be determined in near surface and near bottom samples at each station along each transect. Chlorophyll-a will be used to calibrate the surface maps of in vivo fluorescence and satellite color data. Additional sampling depths will be determined by the vertical fluorescence profile. Biomass as particulate carbon, nitrogen, and phosphorus, HPLC pigments, dissolved inorganic and organic nitrogen and phosphorus concentrations will be determined at select stations and depths. This sampling regime will provide boundary conditions and validation data for the intermediate scale model of the control volume nested within the larger ECOHAB grid and ground truth for satellite observations at a similar 1 km resolution (Gilbes et al., 1996).

Small boat transects: We will continue a time series of red tide related data collection along an offshore transect using small vessels (30-35', >20 kt). The monthly transects, begun in June of 1996 with private funding, extends from Sarasota (MML) to 55 km offshore. This location coincides closely with the USF instrument array at the south end of Longboat Key. Every 9.3 km, water column data will be collected, including profiles of salinity, temperature, dissolved oxygen, chlorophyll fluorescence, turbidity (NTU) and PAR, and discrete measurements of water clarity (secchi), water depth, chlorophyll *a*, inorganic N, P, and dissolved silicate levels, marine bacteria composition and abundance (Buck and Pierce 1989), and *G. breve* counts. In addition, we will attempt to collect continuous surface records of salinity, temperature, dissolved oxygen, chlorophyll fluorescence, turbidity (NTU) and PAR between the 9.3 km stations. Monthly transects will be interlaced with the monthly quasi-synoptic cruises (see above), six months supported by ECOHAB and six months supplemented with private funding. These transects will be augmented with volunteer sampling out to 55 to 80 km, depending on boat availability and weather, including profiles of salinity, temperature, dissolved oxygen, chlorophyll fluorescence, turbidity (NTU) and PAR, and discrete surface and bottom *G. breve* counts. Volunteer sampling has been successful in the past for detecting the early *G. breve* bloom stages. More recently, two transects utilizing charter vessels and five transects by MML in 1996 were successful in identifying a *G. breve* bloom that remained offshore for more than five months. In the event of a red tide bloom, small boats will be used for more intensive monitoring of the bloom.

Ship-board Experimental Studies (Process Cruise): A three-week process cruise aboard a larger research vessel (R/V Brown requested) is scheduled for October with leeway on either side to accommodate bloom detection (last week September and first two weeks October or the last three weeks in October). After the synoptic cruises and/or small boat transects have identified the bloom area, the bloom will be tracked by satellite, fluorometers, *G. breve* counts and toxin analyses. The toxin analyses will be done with an ELISA technique developed by Trainer & Baden (1991) and modified by Tomas & Baden (1993). A concurrent activity will be to organize the three week cruise, the main process-oriented research activity to model the biogeochemical and behavior aspects of HAB dynamics. It will include in-water experiments with the Self-contained Underwater Photosynthetic Appartus (SUPA), small mesocosms, and deck (primary production) and laboratory studies (cell cycles, toxicity). Scientific teams will board or deboard at prearranged schedules.

During the field studies (R/V Suncoaster, R/V Brown, or small boat platforms), we will 1) follow sequential developmental stages of *G. breve*, 2) study their vital processes such as life cycles (excystment, encystment), cell cycles (e.g. growth, senescence, and death), photobiology (photo-acclimation, photosynthetic rates, and production), behavior migration, and toxin production (cellular regulation, cycling, and storage), 3) specify the fate and consequences of by-products in the food web, and 4) define the properties of their competitive exclusion (inhibition of other microalgae and grazing) to allow HAB formation. These components of bloom dynamics will be quantified for the development of our HAB ecological models in relation to the physical and meteorological forcing functions of the above circulation models. Such modeling must similarly include different scales of variability.

3. PHYSICAL OCEANOGRAPHY

The west Florida continental shelf is regularly subjected to periods of upwelling and downwelling, as a result of the orientation of the Florida peninsular in relation to the passage of synoptic scale weather systems. This assertion is well known from coastal sea level records that show coherent variations in response to synoptic scale wind forcing (e.g., Mitchum and Sturges. 1982; Marmorino, 1983a, b). Under the appropriate hydrographic setting, it follows that the onshore and offshore transports associated with these upwellings and downwellings can initiate blooms by the convergence of both seed populations and their required nutrients. Our past analysis of an upwelling event for which we have in situ data, satellite AVHRR thermal imagery and a numerical model simulation (Weisberg et al., 1997) provides a basis, along with the historical records of DEP red-tide logs between Tampa Bay and Charlotte Harbor, for our experimental design, i.e., a test of our hypothesis that red-tides are focussed along the central west Florida coast in response to transport by the prevailing currents.

The present physical circulation model at shelf-wide and control volume scales is an adaptation of the Princeton Ocean Model (Blumberg and Mellor, 1987) that employs a topography-following sigma coordinate system in the vertical, an orthogonal curvilinear coordinate system in the horizontal (e.g., Walsh et al., 1988a), and an embedded turbulence closure submodel for determining vertical mixing (e.g., Walsh and Dieterle, 1994). The model domain, as presently applied to the west Florida shelf is shown in Figure 2 (Li and Weisberg, 1997a,b), which also gives the location of the wind, coastal sea level, mid-shelf currents and temperature data that are presented in Figure 3. The wind data are from a NOAA buoy located at mid-shelf; the currents and temperature data are from a surface buoy-mounted ADCP deployed by

USF (Weisberg et al., 1996); and the sea level data are from our Tampa Bay PORTS system in St. Petersburg, Florida - see the section on contributory programs in Florida waters.

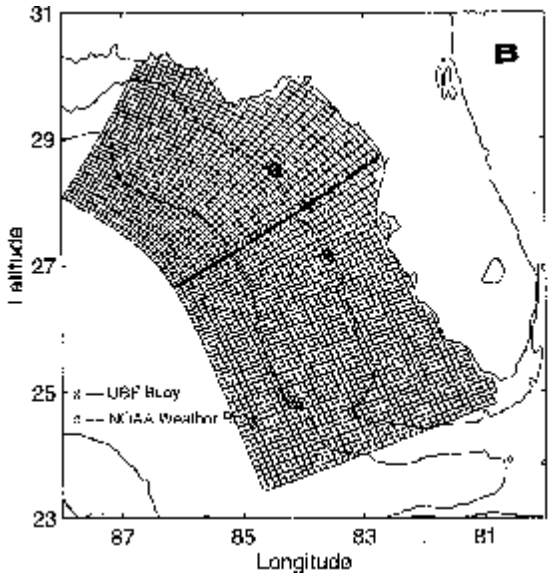


Figure 2. The shelf-wide domain of the coupled biological-physical models at ~9 km grid resolution. Positions of the NOAA weather buoy for local winds and the USF ADCP for local currents are shown.

This particular upwelling event occurred during May 1994. For several days prior to this event the winds were light, thereby providing an opportunity to witness the evolution of the shelf's response to a wind impulse. Beginning on 19 May, the wind stress rapidly increased, peaking in magnitude by noon on 20 May. Sea level at this time was falling at its maximum rate, reaching its lowest value the following day. Coincident with this period of most rapidly falling sea level, the near surface currents were directed offshore at an approximate 45° angle to the right of the wind stress, as predicted by the classic Ekman theory. These are all of the physical ingredients needed to both concentrate water properties and planktonic organisms and distribute them along the coast.

The manifestation of the above in situ observations (and theory) during this period is shown by satellite AVHRR imagery for 22 May 1994 (Fig. 3a), acquired at our USF remote sensing facility. A band of cold water was observed as a continuous feature from Tarpon Springs to the Florida Keys, centered on approximately the 20 m isobath. Note that the coldest waters were found between Tampa Bay and Charlotte Harbor, with a local maximum just offshore from Tampa Bay. This distribution of sea surface temperature, in response to coastal upwelling, can account for the frontal patterns that have been observed in association with red tide blooms (Steidinger and Haddad, 1981).

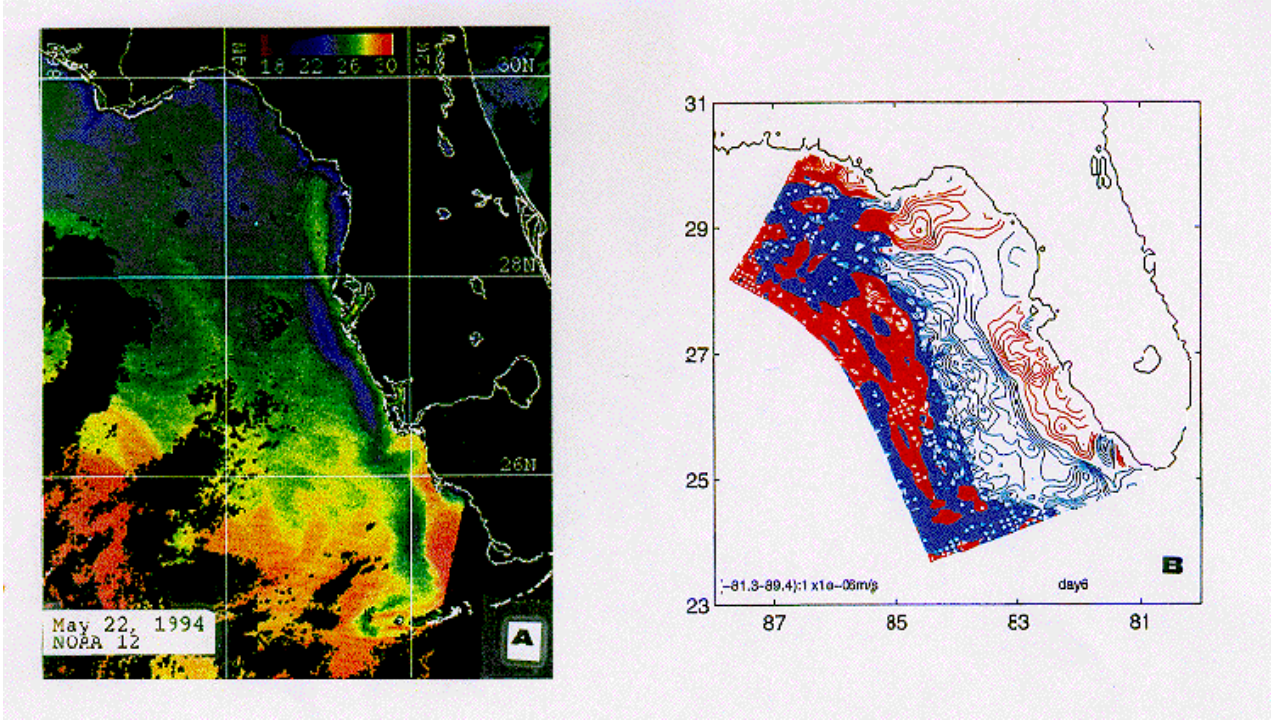


Figure 3. The a) sea surface temperature, recorder at the USF remote sensing facility, from an AVHRR overflight on 22 May 1994 in relation to b) the vertical velocity field near the surface of the circulation model on day 6. The red isopleths denote areas of upwelling and the blue ones show regions of downwelling - note the correspondence of the near-shore upwelling zone with the cold temperatures of the AVHRR image.

A numerical experiment (Weisberg et al., 1997), in which the shelf circulation is forced by a wind impulse, advances the basis for understanding this observed sea surface temperature pattern. The results of the model indicate that it is the geometry of the west Florida coastline combined with shelf bathymetry that makes the region between Tampa Bay and Charlotte Harbor so special. It is here that the wedge of near-bottom, across-isobath flow is the largest, such that the upwelling distribution (Fig. 3b) is in excellent agreement with the observed (Fig. 3a) satellite image.

The winds, of course, are generally more complicated than those just described. They vary seasonally, synoptically and interannually, as does the hydrography of the west Florida continental shelf. Thus, it is the coalescence of both the coastal ocean physics and the offshore distributions of seed populations of *G. breve* that may result in conditions conducive to bloom initiation, maintenance and demise. What is required then is a coordinated study that includes both field experiments (shipboard observations and time series) and modeling to develop an improved description and understanding of the necessary conditions for origin and duration of Florida HABs.

The central shelf will be sampled monthly during cruises of the R/V Suncoaster from St. Petersburg and the northwest shelf quarterly, during the mooring deployments of the R/V Seminole from Alligator Harbor to Pensacola.

Each corner and the center of the control volume will be instrumented in a set of three lines of moorings for at least two years:

1) Offshore from Tampa Bay - 1a. 10-m isobath (provided by PORTS): ADCP, T/S, Optics; 1b. 50-m isobath (provided by Coastal Monitoring): ADCP, T/S - see contributory programs.

2) Offshore from Sarasota County at the center of the control volume (all provided by the ECOHAB program)- 2a. 10-m isobath: bottom mounted ADCP and T/S; 2b. 20-m isobath: bottom mounted ADCP and T/S; 2c. 30-m isobath: surface mounted ADCP, several T/S, surface meteorology; 2d. 50-m isobath: surface mounted ADCP, several T/S, surface meteorology; 2e. 150-m isobath: bottom mounted ADCP and T/S.

3) Offshore from Charlotte Harbor - 3a. 10-m isobath (provided by ECOHAB): bottom mounted ADCP and T/S; 3b. 50-m isobath (provided by Coastal Monitoring): ADCP, T/S

Building upon the presently MMS-funded studies of the flow regime of the northwestern Florida shelf, we also propose to maintain two moorings, at the 30-m (provided by ECOHAB) and 50-m (provided by Coastal Monitoring) isobaths of Apalachee Bay (Fig. 1), to monitor the upstream currents to the north of the control volume. Similarly, eight NOAA-funded arrays are now in place on the 30-m isobath south of the Florida Keys and on the 5-15 m isobaths of the southwest Florida shelf in an on-going study by Tom Lee of the University of Miami - if additional ECOHAB funds become available to us (beyond those requested in this proposal), he would install an additional array off Naples.

We note that these current meter installations will have the capability of connecting moored fluorometers and other bio-optical sensors, e.g. transmissometers, which ONR has expressed an interest in providing. Such measurements may prove to be invaluable (Walsh et al., 1988b), when we enter the third phase of monitoring, designed for minimal data assimilation in support of the biological-physical forecast model. In summary, by marshaling resources through partnerships with other state and federal programs, our in situ moored arrays will cover the entire west Florida shelf at minimal cost, with concentration of the ECOHAB resources within the red-tide epicenter of the central west Florida shelf and extending out to the shelf-break.

4. REMOTE SENSING

The University of South Florida's Department of Marine Science and the U. S. Geological Survey's Center for Coastal Geology will contribute remote sensing components to the ECOHAB:Florida program at no cost. A cooperative agreement with the Minerals Management Service (MMS) is in place for the study of ocean color and infrared satellite data of the west Florida Shelf and the NE Gulf of Mexico. The resultant analyses (satellite image products) will be made available to ECOHAB:Florida investigators as well as the public through the Department of Marine Sciences web site which will be linked to the ECOHAB:Florida web site.

USF has been collecting Sea-Viewing Wide Field-of-View Sensor (SeaWiFS) data since mid-September 1997. We continue to monitor the eastern GOM using ocean color data from SeaWiFS and Advanced Very High Resolution Radiometer (AVHRR). Starting in late 1998, data will be collected from the Moderate-Resolution Imaging Spectroradiometer (MODIS on EOS AM-1). These data will help detect phytoplankton blooms on the west Florida shelf or blooms approaching the shelf from offshore or the NW coasts of the GOM, and examine transport of pigment patches within the ECOHAB:Florida study area.

ECOHAB programs provide a unique opportunity for validation of the satellite products. If trained personnel are available for the monthly cruises or Sarasota transects, bio-optical observations can be taken to include subsurface profiles or spectral upwelling radiance,

downwelling radiance, diffuse attenuation coefficient, and photosynthetically active radiation using existing equipment. Frank Muller-Karger's laboratory has been selected by NASA to participate in the Sensors Intercomparison and Merger for Biological and Interdisciplinary Oceanic Studies (SIMBIOS) ocean color product validation. Analyses of the reflectance data, combined with the spectral particulate and dissolved light absorption measurements will help validate SeaWiFS data collected concurrently. Analyses of the hyperspectral reflectance field data will address the question of whether there is a specific signature for the dominant species present within a bloom. Above- and underwater reflectance measurements may help address G. Mitchell's (pers. comm.) hypothesis that red tides have a specific signature at short wavelengths. R. Stumpf is funded by NOAA COP for development of generic ocean color algorithms for coastal waters, which will provide a framework for incorporation of new data that may be applied to red tides. SeaWiFS algorithms for chlorophyll in coastal waters will result from this other project.

5. BIOLOGICAL OCEANOGRAPHY

Delineating microalgal responses (all life cycle stages) to environmental cues is central to understanding the occurrence and competitive dominance of red-tide dinoflagellates (Anderson 1995). The distinct responses of individual taxa to specific environmental conditions determine the degree to which a species will grow and ultimately, bloom. However, most important phytoplankton processes occur at the cell or species-level, yet very few rate measurements have been made at this level for natural populations. Community or even group-specific rates often lack the necessary discrimination for elucidating the factors controlling population dynamics, as individual species rates can be very different than their respective group or even community rates. As such, delineating species-specific differences are critical for understanding, predicting, and modelling ecosystem dynamics (Fahnenstiel et al. 1995). To resolve the dynamics of natural populations, it is clear that rate measurements need to be made on the appropriate scale.

Gymnodinium breve exhibits variable distribution patterns in the water column, presumably due to the interaction of a population's behavior, its physiological state, and the environmental conditions it experiences. For example, negative geotactic behavior often concentrates cells at the air-sea interface to abundances of 10^6 to 10^8 cells l^{-1}. *G. breve* undergoes diel vertical migrations (Heil 1986) at swimming speeds of ca. 1 m h^{-1}, and if directed in nocturnal descent, would allow cells to move several meters below the surface. When cultured in the laboratory, cells accumulate in dense surface concentrations during the day and fall from the surface due to bioconvection. Near-surface patches of cells in natural populations often occur over the course of a day, apparently due to an accumulation of cells from below (Kirkpatrick, pers. observ.). Kamykowski (1995) suggested that vertical 'excursions' during the diel cycle may be related to cell cycle stage and to the quota of cell storage products, the exact mechanisms underlying swimming behavior are unknown. However, *G. breve* can occur in high concentrations down to 20 m depth and has been observed down to 40 m depth. *G. breve* blooms are not randomly dispersed and their distribution appears to be influenced by the density structure of the water column (D. Kamykowski, pers. observ.). As such, vertical migration behavior and its interaction with horizontal/vertical transport and forcing factors need to be characterized to better understand the complex mechanisms underlying HAB bloom dynamics.

Populations at the surface must cope with high irradiance, high temperature and low nutrient availability which can limit or damage numerous photosynthetic and biosynthetic pathways (cf. Baker & Bowyer 1994). High irradiance can depress photosynthesis, and

ultimately, cell growth. Low-light acclimated *G. breve* cultures are unable to tolerate step changes to moderate solar irradiance. Because cell absorption reflects the degree to which photopigment contents optimize to ambient conditions (Falkowski & LaRoche 1991), it can be used as a proxy on how well cells acclimate to a stochastic environment. Also, the maximum and operational photosynthetic quantum yields are effective proxy measures for cell physiological state (Prézelin et al. 1994) and are sensitive to ambient environmental conditions known to impact growth (Schofield et al. 1995).

Vertical migration and local aggregation field work . Laboratory studies funded under a separate NSF grant are underway to examine the detailed relationship between behavior and the biochemical status of *G. breve* and other dinoflagellate species (Kamykowski, 1995; Kamykowski et al., 1997). Under nutrient-saturated conditions, the *G. breve* cells that aggregate at the surface of a mesocosm during the day are deficient in chlorophyll a, lipid and protein compared to cells deeper in the water column. Furthermore, the strength of negative geotaxis in cells obtained from about 1 m below the surface over a 24 hour period increases as lipid concentration per cell decreases. These results support the idea that, if *G. breve* is given the opportunity to control its position in the water column, its choice is affected by its biochemical state.

Samples will be collected from *G. breve* populations at different depths in the water column at the same time as some of the behavioral measurements. Emphasis initially will be placed on extremes like surface versus deeper populations or sunset versus sunrise populations. Bulk carbohydrate (Revilla et al. 1986), lipid (Cooksey et al., 1987), and DNA (Klut et al. 1988; 1989) concentration will be determined on size-fractionated samples typically isolating the 15 to 25 m diameter organisms. Past experience suggests that *G. breve* represents a large proportion of biomass in this size category in bloom patches.

The initial field work on *G. breve* behavior will take place on the annual 3-week experimental cruises in the first two years but may be expanded to other bloom stages as dictated by research results and the available resources. The temporal pattern of the vertical distribution of *G. breve* cells will be monitored using fluorescence profiling (CTD package on larger ships or water pumped through a Turner Designs fluorometer on smaller boats) and Coulter counts on samples collected from discrete depths in natural water columns and in mesocosms. Both approaches will be verified with microscope counts from discrete depths. Previous experience with laboratory columns and field populations shows that the vertical movement of cells can be subtle. A taxis based approach conceptually based on (Eggersdorfer and Hader (1991a, 1991b) and (Heil, 1986) will be used to supplement the profiling measurements. This approach has been successfully used in the laboratory (Kamykowski, 1998). Video recordings also will be made of *G. breve* populations in single depth cuvettes, based on the same photo- and geotaxis considerations and built suitable for microscopy, to record swimming speed capability and orientation preferences (Kamykowski et al, 1992) from the population peak at a station . These video recordings will be analyzed using the EXPERTVISION Motion Analysis System.

Photobiology and Bio-physical forcing. During the annual process cruises in the third and fourth years, a series of manipulative field experiments utilizing the Self-contained Underwater Photosynthesis Apparatus (SUPA, Fig. ; Kirkpatrick et al. 1990, 1997) fitted with large-volume reservoirs (Reed et al. 1997) will examine physiological and growth processes of mixed phytoplankton communities making up the various stages of *G. breve* blooms as the assemblages are exposed to vertical motion representative of the bio-physical interactions elucidated in previous studies. SUPA provides an ability to deploy, *in situ*, a phytoplankton culture or wild

bloom sample in an instrument that monitors spectral irradiance, temperature and photosynthesis (O_2 and CO_2 as a f(pH)) on a one minute sampling cycle. This allows for the monitoring of photosynthetic response dynamics in a Lagrangian perspective under natural *in situ* light conditions.

To evaluate the acclimation capacity of bloom patches (surface and vertically distributed) two SUPA pairs will be used. Comparisons will be made between identical *G. breve* bloom communities as one pair of samples, contained within a pair of SUPA, is held stationary at the source depth while the other sample pair is made to migrate toward the surface at a speed appropriate for *G. breve* (Heil 1986, Kamykowski, pers. observ.) and the vertical currents it may be entrained in. Within one SUPA of each pair there will be an ambient nutrient condition and within the other SUPA a nutrient-enhanced condition. These nutrient treatments are incorporated to evaluate the role of nutrient status in community photoacclimation capability, not to determine *G. breve* nutrient requirements *per se.* To evaluate UV impacts and photo-acclimation, another experiment will employ the SUPAs as described above, but with identical nutrient conditions and one of each SUPA pair's quartz sample chambers shielded with UV-opaque polycarbonate film. To determine underlying processes responsible for the observed responses, periodic aliquots will be withdrawn from the reservoirs attached to the SUPAs to determine sample conditions, bio-optical characteristics, pigment content , P-I relationships, quantum yield and shifts in cellular organic pools. These measurements will include: ^{14}C labeling of chlorophyll *a* and other pigments (Redalje 1993, Pinckney et al. 1996), photosynthetic and photoprotectant pigment dynamics (see Millie et al.1993), incorporation of ^{14}C into algal protein (DiTullio & Laws, 1986) and into cellular free amino acids (Lohrenz & Taylor, 1987) and cell particulate organic carbon, nitrogen and phosphorus concentrations. Spectral absorption of particulate materials, including phytoplankton, will be determined using the filter pad absorption technique (Cleveland & Weidemann 1993, Kishino et al. 1985).

Because SUPA provides time-series photosynthesis measurements, not state (i.e., P-I) measurements, photosynthesis-irradiance curves (photosynthetron, Prezelin et al., 1994) will be used to define carbon fixation states (P_{max}, α, I_k, β, I_b) of *G. breve* bloom communities in conjuction with SUPA manipulation experiments. To extract species-specific production estimates from the community level experiments just described, ^{14}Carbon track autoradiography will be used (Fahnenstiel et al. 1991, Fahnenstiel et al. 1995; McCormick et al. 1996) over 24 hr periods. Utilizing the track autoradiography results and the variable uptake-division model (McCormick et al. 1996) it will be possible to calculate species-specific growth rates. Species-specific quantum yields will be calculated by dividing productivity estimates by the product of the phytoplankton absorption and light measurements.

Cell cyle,life cycle,genetics, and grazing: bloom regulators. We propose to determine the extent to which endogenous cellular mechanisms control reproduction, initiation/accumulation, length of the maintenance phase, and onset of bloom decline by conducting cell and life cycle studies using light and fluorescent microscopy and flow cytometry. This work will be augmented with genetic studies to determine whether *G. breve* has several genetic strains that are geographically separated. Field studies will be conducted during red tide events on the transect and process cruises, and laboratory studies will be conducted using 14 existing clonal cultures representing six geographic areas from Florida to Texas.

Walker (1982) detailed the sexual life cycle of *G. breve* through the planoyzgote stage. More recently, Steidinger et al. (in press) verified that *G. breve*'s sexual cycle is entrained on an

annual endogenous rhythm with induction of gamete production between August and November. If a benthic, diploid cyst is the endproduct, then cysts could function as a seed stock for future blooms. *G. breve*-like cysts have been observed in field populations but were never isolated. The likely area of gamete production, planozygote formation and hypnozygote deposition is offshore in the zone of initiation, probably at a frontal system because blooms do not initiate inshore. Study of the sexual cycle in the laboratory will initially follow the protocol of Walker (1982) and incorporate media and environmental modifications to induce planozygote formation . A homothallic isolate will be used to validate the use of cell surface recogition antigens to identify vegetative cells, gametes, planozygotes, and hypozygotes if produced experimentially,e.g., manipulating photoperiod, growth rates, and turbulence. In the field, water samples from different depths on transect and process cruises will be used to detect timing and amount of gamete and planozygote production using microscopy (phase-contrast, DIC, and fluorescence with DAPI) and surface recognition probes developed by Peter McGuire at the University of Florida (unpubl.). Sediment samples from the same transect and process cruise stations will be collected with a box corer and processed back at the laboratory. The upper cm and floc layer will be sieved for two size fractions (10-20 um and 20-38 um) and dinoflagellate cysts will be isolated into 96- well tissue culture plates for growout in enriched seawater medium with selenite. High biomass *G. breve* water samples from the same stations will be treated to promote cyst production using the method of Anderson et al.(1996).

Growth rates of 0.2-0.5 div day^{-1} observed in laboratory and field populations of *G. breve* are not sufficiently high to account for its dominance in the water column. Thus, it appears that either the "explosive" growth stage has not been previously documented or it does not exist and higher concentrations are due to other interactive physical and biological processes. Diel phasing of cell division may in fact impose a maximum potential growth rate of 1 div day^{-1} in dinoflagellates. In a preliminary study, we found that cell division in a *G. breve* bloom was phased to the diel cycle. The occurrence of an "explosive growth stage" would require the release of *G. breve* cells from mechanisms which regulate this circadian rhythm.

To address this question, *in situ* diel cell cycle phasing, and correlation of cell cycle events with vertical migration will be determined in blooms located by transect cruises during Years 2-3, using the flow cytometry method of Van Dolah and Ramsdell (1996). Growth rate will be calculated by the method of Chang and Carpenter (1988). In Years 2-3, we will track bloom patches in different developmental stages during the three week process cruises. Shipboard flow cytometric analysis of cell cycle phasing and growth rates will provide near real-time analysis of the growth status of the blooms.

The apparent synchrony with which blooms dissipate opens the possibility that endogenous cellular processes may also play a role in determining bloom longevity. Certain unicellular protists are programmed to undergo a finite number of divisions, after which cells enter senescence. Onset of senescence is accompanied by the loss of cyclins, required for cell cycle progression, and expression of "senescence factors", which specifically inhibit cell cycle entry (Smith and Periera-Smith, 1996). In order to determine if such endogenous rhythms play a role in dynamics of *G. breve* blooms, the cell cycle regulatory machinery in *G. breve* must first be identified. Presence of the eukaryotic cell cycle regulator, CDC2 kinase, in dinoflagellates (Rodriguez et al., 1994; Van Dolah *et al*., 1995) suggests they most likely also express cyclins, the regulatory proteins which control CDC2 kinase activity to drive the cell cycle.

Cell extracts will be analyzed by western blotting with an antibody to a conserved sequence (PSTAIRE) found in CDC2 related kinases (Van Dolah et al., 1995). If an immunoreactive protein is identified, its kinase activity and cell cycle dependence of its activity will be determined using *in vitro* kinase assays. Reversible inhibition of cell cycle progression by the specific inhibitor, olomoucine, will provide independent confirmation of CDC2 kinase in the *G. breve*. CDC2 kinase complexes will be purified by affinity chromatography (Rosenblatt et al., 1992,and a modification of Hampson, 1989). Proteins present in CDC2 complexes will be screened for putative cyclins by (1) immunoreactivity to yeast cyclin antibodies and (2) differential expression of proteins in complexes from different cell cycle stages. Isolation of *G. breve* cyclins will be accomplished by the method of Lew et al. (1991a). *G. breve* mRNA will be prepared to generate a cDNA library expressed in a yeast shuttle vector (library generation will be contracted to Invitrogen). Isolates expressing transfected dinoflagellate cyclin will be selected by viability in glucose medium. Viable isolates will be selected for cloning (Sambrook et al., 1989) and PCR amplification (Lew et al., 1991b) for generation of probes.

Total DNA has been isolated from Wilson's 1953 isolate of *G. breve.* Published PCR primers were synthesized at the University of Florida ICBR DNA Synthesis Core and used to amplify portions of the *G. breve* DNA. These PCR products will be cloned into pGEM-T vectors or sequenced directly using automated methods at the DNA Sequencing Core. Following examination of at least six samples from each of 6 geographic isolates, the DNA sequence data will be aligned and analyzed using GCG software. The OLIGO program will be used to choose oligonucleotides and optimal conditions for high stringency PCR amplification. Once the optimal amplification conditions are found for identification of *G. breve* laboratory strains, parameters will be surveyed to allow detection and quantification of *G. breve* genome equivalents *in situ*.

In year 4 we will sequence PCR products using DNA from several current and established geographic isolates of *G. breve*, using primers already developed for hypervariable regions of nuclear ribosomal RNA genes and mitochondrial D-loop. We will develop PCR primers specific for *G. breve* and, if the data obtained above warrant, for individual isolates. These reagents will be adapted for use in later screening of field samples *in situ*, to identify origin (s) and monitor dynamics of blooms.

Another regulator of bloom initiation, growth, maintenance, and dissipation could be grazing and predator-prey interactions. Within the phytoplankton - zooplankton community there are few examples of toxins as grazing deterrents. At least three common copepods (*Acartia tonsa*, *Labidocera aestiva* and *Oncaea venusta*) can ingest ($1\text{-}2\times10^4$ *G. breve* cells copepod^{-1} hr^{-1}) with no ill effects (Turner & Tester 1989). This leads to an interesting finding. Even though *A. tonsa* and *L. aestiva* could eat *G. breve,* when given an abundant, alternate food source (*Skeletonema*), neither did (Turner & Tester 1989). Do copeods actively select against *G. breve* as a food source? What happens to zooplankton numbers and productivity in a near monospecific *G. breve* bloom? Do grazers avoid *G. breve*-dense layers in the water column? Does *G. breve*'s size protect it from micrograzers? The ultimate question is "Does relaxed grazing pressure allow *G. breve* to bloom?"

These questions will be explored in a series of laboratory experiments (Tester & Turner 1988 1989 1990, Turner &Tester 1989 1997, Turner et al. 1997) and screened for field verification during bloom tracking process cruises.

Nutrient Dynamics. This component of ECOHAB addresses the enigma of how *G. breve* populations can initiate and develop in a nutrient impoverished area of the west Florida Shelf and

equally important how if this species can utilize organic substrates to maintain the dense bloom populations. It examines utilization for growth and uptake of inorganic and organic nitrogen and phosphorus.

According to Steidinger and Haddad (1981), *G. breve* blooms originate in the mid Florida shelf region where the loci of initiation occur. These blooms commonly develop to levels exceeding 10^6 cells $\bullet l^{-1}$. Inorganic nitrogen and phosphorus levels rarely exceed 0.5 $\mu g\text{-at}\bullet l^{-1}$ in the mixed layer and more commonly are between 0.1 and 2 within 2-4 km from shore (Dragovitch, 1961, 1963, Tomas, unpubl. data). *G. breve* can grow on inorganic and organic phosphorus and has measurable alkaline phosphatase activity. Nitrate and ammonia have been used to cultivate *G. breve* but there is some indication of the utilization of amino acids as nitrogen sources (Wilson, 1966). However, the exact kinetics for uptake and growth for the various nutrients requires further examination as does the utilization of organic nutrients. To what extent do N and P sources support growth for the development and maintenance of blooms is a major question for understanding the population dynamics of this species and possible implications of the influence of elevated nutrients of coastal waters in maintaining blooms.

This research primarily relies on laboratory studies although some field work is also required. Uptake kinetics for inorganic N as ammonia, nitrate and nitrite and inorganic phosphorus will be examined in cultures recently isolated from Florida coastal waters utilizing a highly sensitive analytical Antek Instrument which measures nanno molar levels of nitrogen by conversion to NO_2 via a chemiluminescent method of Bramen and Hendrix (1989). Low level phosphorus assay (Karl & Tien, 1992) will be utilized for uptake and growth studies. Uptake of P^{32} labeled substrates will also be measured for determining kinetics to supplement information presently available from Vargo and Howard-Shamblott (1990). Nitrogen assimilative enzymes (nitrate reductase, nitrite reductase and glutamine synthetase) will also be measured by methods of Burges and Harrison (1995), Eppley (1978) and Slawyk & Rodier (1986), respectively. Growth at various substrate concentrations will be monitored by cell counts (Coulter Counter) and *in vivo* chlorophyll a fluorescence. Organic substrates will also be tested for N and P in supporting growth. Cell growth quota for various nutrients, uptake kinetics as well as cellular turnover rates calculated from enzyme rates and cellular content will be made to define the dynamics required for absorption and assimilation. Field studies of natural populations of *G. breve* will be conducted during the research cruises where aliquots of bloom waters will be enriched with various nitrogen and phosphorus substrates and tested for growth. These whole population bioassays will be done by addition and exclusion to determine the influence of the nutrient enrichment. Similar assays were used to study blooms in Florida Bay where nutrient cycling and availability are tightly couples with supply. Results from these assays as well as the laboratory studies will be used to evaluate the nutrient hypothesis.

6. FATE AND EFFECTS OF TOXINS

Gymnodinium breve blooms can cause animal mortalities and affect human health. Organisms are exposed to brevetoxins through: ingestion of *G. breve* cells (filter feeders); bioaccumulation by toxic animal ingestion (e.g. birds, humans [NSP]); aerosolized transport (respiratory irritation in humans and potentially in manatees, turtles, birds); water-borne toxin after cell lysis (fish); sediment sinks (benthic organisms); and possibly through consumption of toxic benthic stages (Steidinger et al. 1973, Hemmert 1975, Quick et al. 1975, Forrester et al. 1977, Roberts et al. 1979, Baden et al. 1982, Fowler & Tester 1989, Geraci 1989, Pierce et al.

1990, Summerson & Peterson 1990, O'Shea et al. 1991; Landsberg & Steidinger 1997). Bubble-mediated transport has been shown to be a major factor in concentration of brevetoxin at the sea surface, with subsequent production of toxin-containing marine aerosol (Pierce et al. 1990).

While acute exposure to lethal doses of brevetoxin results in massive animal mortalities, effects from exposure to low level brevetoxins are unknown, nor is it clear how stable brevetoxins are in the environment. Other biotoxins can be transferred through the food web and cause mortality of animals including fish and birds (e.g., White et al. 1989, Work et al. 1993). Numerous unexplained fish kills as reported by Williams & Bunkley-Williams (1990) may have been attributable to biotoxin transfer through dietary exposure (Landsberg 1995). Also, the world-wide distribution of two major types of cancer in shellfish has recently been hypothesized to be related to chronic exposure of bivalves to biotoxins (Landsberg 1996).

Chronic dietary exposure to brevetoxins could exert lethal or sub-lethal effects at all trophic levels, leading to impaired feeding, avoidance behavior, physiological dysfunction, impared immune function, reduced growth and reproduction, pathological effects, or mortality. A newly developed technique, micellar electrokinetic capillary chromatography and laser-induced fluorescence detection (MEKC-LIF) (Shey, 1997) allows measurement of brevetoxins at trace levels critical for tracking toxins through lower trophic food webs. It has been used to assess the transfer of accumulated brevetoxins from *G. breve*-fed copepods to juvenile fish (Tester et al. 1997). Another promising technique is the detection of stress proteins in exposed animals. Chronic effects on clams have been observed as toxin-induced proteins expressed in response to exposure to *G. breve* cultures at levels of 2×10^6 cells l^{-1} (Ramsdell, unpubl.).

The goals of this ECOHAB:Florida segment are to determine the fate and effects of brevetoxins during and after a *G. breve* bloom, the distribution of brevetoxins in water, air, sediments, and biota, and the stability of brevetoxin in marine ecosystems. These goals are based on the following hypotheses. Transfer of brevetoxins through certain pathways in food webs maintains brevetoxins in the ecosystem after the initial bloom has dissipated. Brevetoxins can enter food webs either directly as toxins or cells or indirectly via zooplankton and other filter feeders. An additional mode of transport and exposure for mammals is inhalation of toxin-containing marine aerosol.

The persistence of toxins in the food web will be determined by investigation of brevetoxins prior to, during, and subsequent to the bloom. Toxin transport downward through the water column will be investigated by collection of water, organic detritus, and sediment below a bloom event. Upward transport and aerosolization of toxins will be investigated during the surveys and under controlled laboratory experiments (Pierce et al. 1990;Van Dolah et al. 1994). Zooplankton, fish larvae, and molluscs will be initially targeted to maximize toxin detection at lower trophic levels.

Field studies. During the six-month period from July through December of 1998 and 1999, monthly samples will be collected from a transect from shore through the middle of the controlled volume study area, at five stations distanced 9.3 km apart out to km. These transects will provide a monthly assessment of water quality and, in the event of a bloom, *G. breve* abundance and brevetoxin concentratons. Water samples will be collected from near-surface and near-bottom at each of five transect stations. These transects also will provide the opportunity for other sample collection and specific measurements to be performed. In addition, background concentrations of brevetoxins in aerosol, water, detritus, sediments, and selected biota (e.g., zooplankton and larval fish) will be measured in preparation for the first 3 week process cruise.

Samples will be taken at near- surface, mid-depth, and above bottom (0.5 m). Water will be collected by Niskin samplers and analysed for *G. breve* cell abundance and toxins. Water will be filtered and detritus analysed for brevetoxin. Aerosol will be collected on glass fiber filters on a high volume particulate air sampler according to Pierce et al. (1990). Sediment samples will be obtained by box core or ponar dredge, to provide an undisturbed sample of the top 2.5 cm of sediment. Toxin composition from water, organic detritus (flocculant layer and water), surface sediment, and water surface microlayer samples will be determined by HPLC analyses (Pierce et al. 1992) supplemented with LC-MS (Poli et al. 1997) and MEKC-LIF (Shey, 1997).

Selected organisms from the water (e.g. zooplankton, fish larvae) will be sampled using pumps or plankton nets towed at different depths and small bivalves from the benthos will be sampled by a box core. Adult and juvenile fish also will be collected for brevetoxin analysis as conditions permit. All organisms will be tested for brevetoxins using the MEKC-LIF method (Shea 1997) and visualization of brevetoxins using an immunocytochemical peroxidase assay (developed by D. Baden & G. Bossart, pers.comms.). Individual specimens will be fixed in 10% buffered formalin, processed by routine histology,and evaluated for histopathological effects and brevetoxin visualization by direct light microscopy.

During the three week process cruise and in order to track the bloom (along with chlorophyll biomass and drifters), water will be tested for *G. breve* cell abundance (microscopy and Coulter counter) and toxins by ELISA (Tomas & Baden 1993) and receptor-binding assay (Van Dolah et al. 1994). From these measurements, toxin content per cell and total toxicity per sample will be calculated to look at horizontal, vertical, diurnal, and temporal differences in bloom toxicity. During the first week of the cruise, a small boat will conduct a transect outward from the center of the bloom going from the most dense to the least dense *G. breve* concentrations and then to zero (outlier control). At these stations, water and plankton will be collected from surface, mid-depth (or at the chlorophyll maximum determined by fluorometry), and bottom for *G. breve* counts; additional phytoplankton composition, abundance and toxin content; and zooplankton composition, abundance, and toxin concentration.

On the process cruise, at select stations (every third/fourth day) water, sediments, aerosol, biota, and detritus will be collected and processed as above. One month after the cruise has ended, select station areas sampled during the process cruise will be revisited (positioned by GPS coordinates) and resampled. Sampling strategies and processing techniques will be those used during the cruise. Additionally, stations sampled prior to the bloom along the 3 designated transects will be revisited and resampled after the bloom has terminated.

Additional animal material will be measured for baseline brevetoxins. Samples of freshly dead or live animals that may have been exposed to the bloom will be collected and archived as conditions permit. Collaborators will be available to aid in the collection, processing, and analyses of tissues from marine mammals, turtles, fish, molluscs, macrocrustacea, zooplankton, and tunicates. In addition to above methods for brevetoxins, verification of toxicity will be done by receptor-binding assays on a representative number of samples.

Laboratory studies: Different copepod grazers (*Temora turbinata*, *Labidocera aestiva*, *Acartia tonsa*) and larval or juvenile fish (*Fundulus majalis*, *Leiostomus xanthurus, Brevoortia tyrannus*) will be tested for species-specific differences in susceptibility to brevetoxin uptake, and in the retention time or pathological effect of brevetoxins in different tissues. The sensitivity of brevetoxin detection methods will be tested using the MEKC-LIF and the immunocytochemical peroxidase tests. Sublethal and lethal levels of dietary toxin can be determined, in conjunction

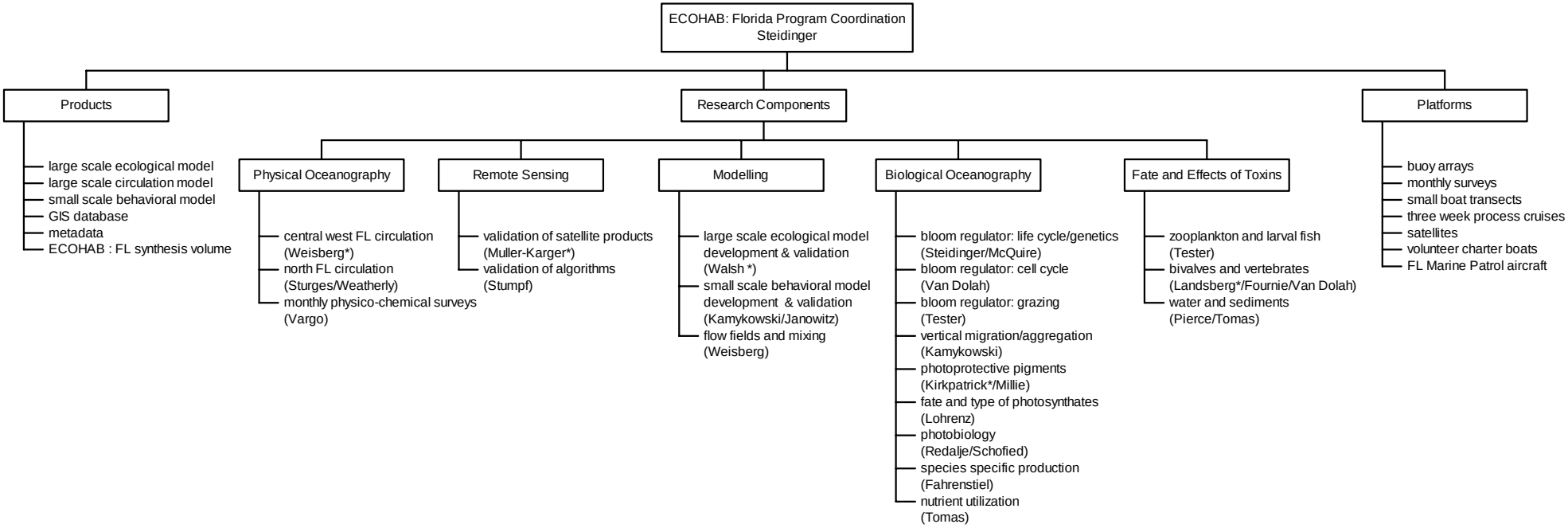
with estimates for depuration of brevetoxins from fish tissues after withdrawal of the dietary toxin.

ECOHAB:FLORIDA ADMINISTRATION

Figure 4. represents a programmatic task chart and names the Group Principal Investigators for the five research components: Ecological Modelling, Physical Oceanography, Remote Sensing, Biological Oceanography and Fate and Effects of Toxins. It is the responsibility of each group coordinator to maintain contact with his/her working group to identify progress, constraints,deadlines, scheduling, and mechanisms for effective communication. Group coordinators will be assisted by the Program Coordinator and associate. Each year a Principal Investigators and Collaborators meeting will be held at FMRI in St. Petersburg for two days to review and discuss results, identify and resolve problems, and evaluate the next years component of the program. During the year, day to day activites will be addressed by individual PIs and if a problem exists and is significant enough to delay the program or affect achieving the program objectives, that PI, group coordinators, and the program coordinator will teleconference to discuss and resolve the issue. Yearly and final reports will be prepared by group coordinators and synthesized by the Program Coordinator for submission to the granting agency.

Each Principal Investigator is responsible for the integrity and verification of his/her data which will be provided in a mutually agreed upon format to the Florida Marine Research Institute's (FMRI) Coastal and Marine Resource Assessment (CAMRA) Program for integration into the Marine Resource Geographic Information System (MRGIS). CAMRA is nationally recognized for its successful application of geoprocessing technologies. In 1994, CAMRA was selected as a finalist in the Innovations in Government Program funded by the Ford Foundation and administered by the John F. Kennedy School of Public Policy at Harvard University. FMRI was awarded a grant to further the development of MRGIS and foster technology transfer to other coastal organizations.

The ECOHAB:Florida Program Coordinator and associate will be responsible for the integration, co-registration, and distribution to the National Oceanographic Data Center (NODC) of all geographically referenced databases within two years of their collection. Physical, chemical, and biological data will be co-registered, rectified to a common earth coordinate system, integrated into the MRGIS, and analyzed using the lastest in raster and vector based technologies. Satellite image products and results of simulation models will be stored separated but be accessible through the ftp site. If warranted they will be intergrated into the database. Software running on Sun workstations and PCs include ARC/INFO, ERDAS Imagine, Oracle, and ARCVIEW. Data storage, back-up and archiving are accomplished with a variety of devices from tape cartridges to CD-ROMS, and a 28 gigabyte optical jutebox. Federal Geospatial Data Committee (FGDC) compliant metadata will be created for all relevent databases and served through the internet. The ECOHAB:Florida homepage (currently under design) and its links to other homepages will be used to provide updated aggregate data information and encourage collaborative exchange. Research data will be available to principal investigators through an ftp site embedded in the homepage and accessible by password. Exchange and dissemination of data will be encouraged by following the spirit of the data policy of the US GLOBEC program, e.g., 1) methods chosen for these ECOHAB studies will be adequate enough to insure data quality and



- Group Leaders: Weisberg, Muller-Karger, Walsh, Kirkpatrick, Landsberg

Figure 4. Organizational Flow Chart for ECHOHAB: Florida

integrity and meet the program objectives, 2) methods, with limits of sensitivity or resolution, will be documented, 3) PIs agree to their data being submitted to NODC within two years of collection in a GIS format, 4) metadata will be kept according to the FGDC guidelines. The ECOHAB GIS database will be kept during the grant period and for up to 2 years following the last collection, but NODC will be the final archive.

CONTRIBUTORY PROGRAMS IN FLORIDA WATERS

It would be impossible to carry out the proposed research of ECOHAB:Florida with a budget of \$900,000 yr^{-1}; we can achieve our objectives, however, by building upon the fiscal resources of other projects supported by universities, private laboratories, federal and state agencies, and the private sector. For example, DEP now monitors shellfish harvesting areas when a *G. breve* bloom occurs and has established a volunteer program for offshore sampling that involves fishermen, boaters, and charter boat captains. DEP's red-tide logs provide a 30-year time series on the occurrence, distribution, and intensity of HABs. As part of a national stranding and salvage network for marine mammals and turtles, DEP also has access to data and tissue samples for study of the impact of *G. breve* blooms on the mortality and stranding of endangered species. Finally, DEP has some funds to examine the role of local biotoxins in acute and chronic mortalities and their potential to induce disease and neoplasia in aquatic organisms.

Mote Marine Laboratory (MOTE) also has a state-supported monitoring program within the control volume. MOTE is conducting brevetoxin aerosol studies to determine the brevetoxin fraction content, concentration, and alteration in laboratory-generated and natural aerosols, as well as developing in situ instrumentation for remote sensing. The NIEHS Marine and Freshwater Biomedial Sciences Center at the University of Miami continues to develop or refine reagents and protocol for the detection of brevetoxins. The National Marine Fisheries Service Charleston Laboratory has a biotoxin program with responsibilities in quantification of brevetoxins in seafood products and endangered and threatened species. The Environmental Protection Agency's National Health and Effects Research Laboratory in Gulf Breeze, Florida is studying the effects of biotoxins on aquatic organisms and evaluating the use of fish as models for biotoxin exposure. A private group in Sarasota, Florida known as Solutions To Avoid Red Tide (START), has funded nutrient studies in coastal waters and may fund exploration of the use of flocculants in managing red tides. START volunteers are a significant component of the volunteer program that will be sampling the Sarasota leg of the monthly cruises on an interim basis.

Present NOAA-, MMS-, USGS-funded programs and a recent USF initiative on "A real-time oceanographic data system for Florida" have been used to purchase the current meter arrays. This state-supported Coastal Monitoring program has provided three of the arrays of the proposed ECOHAB control volume. As part of USF's PORTS (Physical Oceanographic Real-Time System) project, supported by NOAA National Ocean Service, the physical effluxes of nutrients from Tampa Bay would be monitored with another ADCP now moored at the mouth, supplying a fourth array. With funds from phase 1 of ECOHAB:Florida, we have purchased and/or refurbished the other seven current meter arrays. ONR has expressed interest in contributing additional current and optical sensors as part of a satellite validation study.

Similarly, NASA and one MMS project is supporting some of Frank Muller-Karger's research on satellite imagery, such that he requires no ECOHAB funds, while another MMS project is providing most of the ship-time and some of the mooring costs for the deployment of

arrays in the northern shelf area (Fig. 1) by Tony Sturges, Georges Weatherly, and Bob Weisberg. They will make space available on their periodic cruises for biological and chemical sampling. MMS and USGS are funding processing of satellite imagery for the eastern Gulf of Mexico. NOAA is funding Stumpf for general algorithm development for remote sensing of chlorophyll in coastal waters. Recall that in the southern region, Tom Lee is now supported by NOAA for maintenance of some of the arrays on this part of the shelf. Finally, during August 25-31, 1997 on an EPA-supported red tide synoptic survey, pre-ECOHAB measurements were made on the west Florida shelf - see the appendix for the results of this study.

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