SUMMARY

ECOHAB: Florida - EPA

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Note: This proposal is one of two components of ECOHAB: Florida. Please see the full proposal submitted to the NOAA ECOHAB Office, December 1997, for the complete science package.

Harmful algal blooms of the toxic dinoflagellate *Gymnodinium breve* have caused massive fish kills in the Gulf of Mexico since the 1500s, with most occurrences on the west coast of Florida. In 1996, the list of states that have experienced natural resource, public health, and economic impacts related to this organism expanded, with the addition of Alabama, Mississippi, and Louisiana, to include all of the Gulf Coast states and North Carolina. Estimates of economic impacts to Florida and North Carolina from two moderate-intensity blooms ranged from 15 to 25 million dollars, respectively. The harmful impacts caused by *Gymnodinium breve* only occur when cell concentrations increase significantly above low background concentrations that are present year-round in the eastern Gulf of Mexico. Once a bloom has developed offshore in typically oligotrophic waters, cell concentrations at the 10^5 level can be maintained for months. During 21 of the past 22 years, red tide blooms have been observed within the region between Tampa Bay and Charlotte Harbor.

The key to understanding any harmful algal bloom (HAB) lies in knowing how one algal species has adapted and come to dominate in its particular realm of physical, biological, and chemical conditions. Our ability to predict initiation, maintenance, and dispersal of blooms on the Florida shelf has been severely limited by the lack of a quantitative description, or model, of their population dynamics and the physical, biological, and chemical regime in which they are embedded. The modeling components of this project will incorporate the quantitative description of blooms and their surrounding environment provided by the field and laboratory portions of this project. The field component will employ a set of localized small boat operations and an annual 3-week "process" cruise. Selected laboratory studies will refine the descriptions of those parameters not conducive to quantitation in the field. The major objectives set for this project include:

1. Model the biophysical interactions of *G. breve* red tides on the west Florida shelf at small scales.
2. Determine the interactions of cellular, behavioral, life cycle, and community regulation processes with environmental forcing factors during stages of bloom development.
3. Determine the sources of inorganic and organic nutrients that allow growth and persistence of large *G. breve* populations in coastal waters.
4. Determine the production, occurrence, fate, and effects of brevetoxins in the environment during and after *G. breve* blooms.

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.*, 1998). Red tides have been observed in 21 of the last 22 years within the region between Tampa Bay and Charlotte Harbor (Figure 1), compared to 4 to 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. However, red tides may 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* harmful algal blooms (HABs) be mitigated and/or managed? Is man responsible in any way 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: (1) frontal systems are a key to initiation and transport, (2) growth rates are typically 0.2 to 0.3 division day^{-1} *in natura* and in the laboratory, (3) physiological state affects toxin production, *(4) G. breve* photoadapts, *(5) G. breve* is efficient at utilizing inorganic N and P, and *(6) G. breve* can use organic N and P. We also suspect that: (1) there is 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/modeling phase of ECOHAB:Florida will enable us over 4 years to dissect bloom dynamics so we can predict bloom occurrence, distribution, movement, toxic effects, and (hopefully) dissipation. Our project encompasses five focal groups to obtain these goals of prediction. This proposal, seeking EPA support, includes the following three focal groups: (1) ecological modeling (Kamykowski, Janowitz), (2) biological oceanography (Kirkpatrick, Steidinger, Redalje, Lohrenz, Scofield, Tomas, Millie, Van Dolah, and Fahnenstiel), and (3) fate and effects of toxins (Landsberg, Pierce, Fournie, Tester). The focal groups represent 15 senior investigators from 8 institutions representing 3 universities (USM, NCSU, Rutgers) and 1 state (FDEP), 1 private (MML), and 5 federal laboratories [NOAA (3), EPA, USDA].

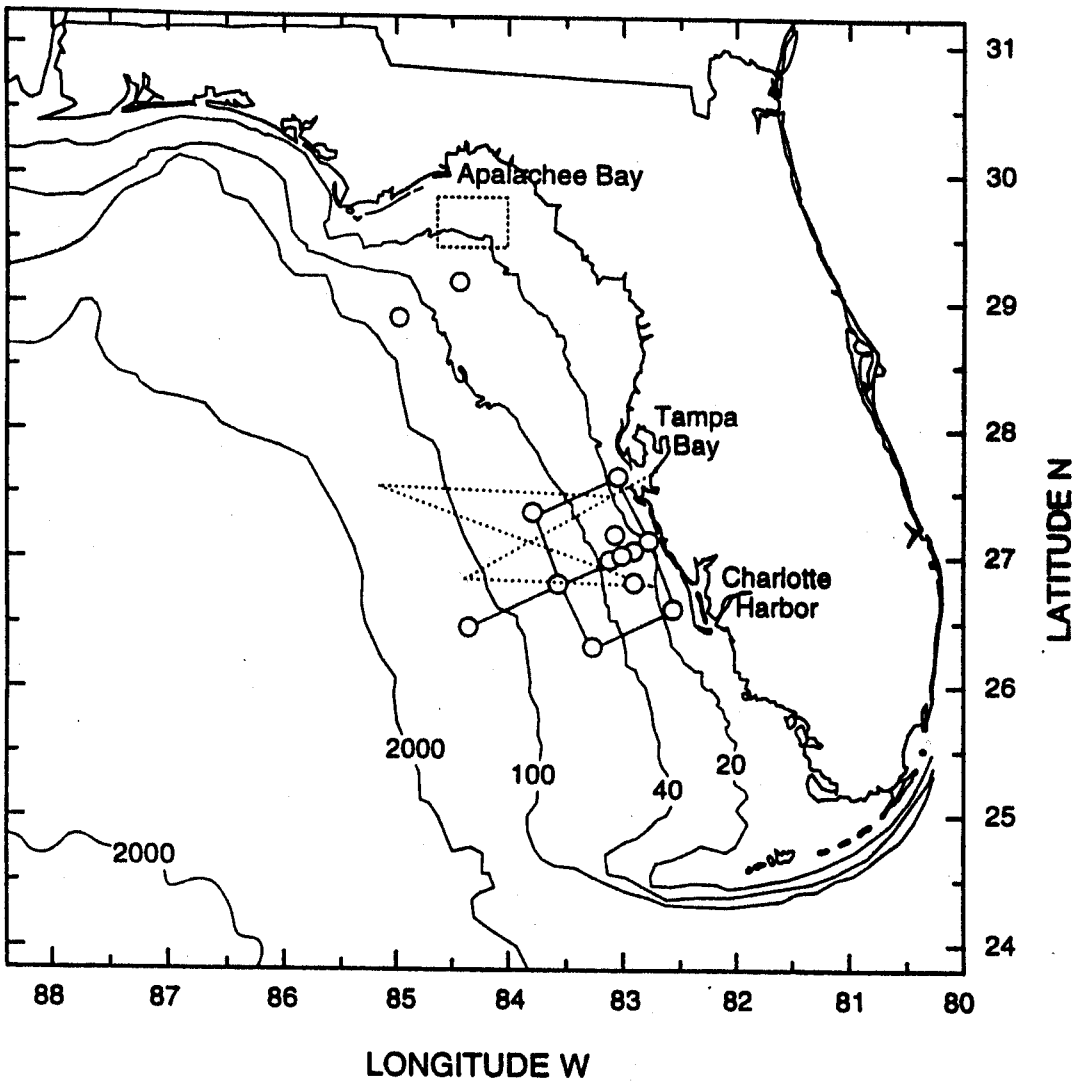


Figure 1. Sampling locations on the west Florida Shelf.

Based on our existing support from the State of Florida, NOAA, EPA, and The Selby Foundation, we have structured a 4-year ECOHAB: Florida field/modeling 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 FDEP, USF, Mote, NOAA, and NCSU. This award was mainly for the 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 FDEP was made 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 to 74 km offshore of central Florida at depths of 12 to 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- to 5-month red tides off Florida caused $15 to $20 million in 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. For example, 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 Howard- Shamblott, 1990) to sustain this population level. However, these concentrations of both inorganic N and P are not found within 2 to 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 (NO_3 + NH4 + NO_2) and phosphate in the Peace River, entering Charlotte Harbor (Fraser and Wilcox, 1981; McPherson *et al.*, 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., 1977*).

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.

**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 MODELING

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. We propose to model the initiation, maintenance, and export of *G. breve* red tides in our ECOHAB: Florida program at several different time and space scales. *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 address space scales of <1 km at the physiological level of *G. breve*'s interaction with its chemical and physical habitat.

Monthly cruises of the R/V Suncoaster during the first 2 years of this study and small boat transect cruises will provide sampling of this offshore section along the center line of moored arrays (Figure 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.

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 modeling effort will combine biological and physical data, especially as collected during the 3-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 modeling 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 photophysiology 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.

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 EPA-funded portion consists of a set of localized small boat operations and an annual 3-week experimental cruise, similar to our pre-ECOHAB study aboard the R/V Anderson.

Small Boat Transects: We will continue a time series of red tide related data collection along an offshore transect using small vessels (30 to 35 ft, >20 kt). The monthly transects, begun in June 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; 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 ECOHAB:

Florida - NOAA and Figure 1), 6 months supported by ECOHAB and 6 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 5 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 3-week process cruise aboard a larger research vessel (R/V Brown requested) is scheduled for October, with Lway on either side to accommodate bloom detection (last week in September and first 2 weeks in October or the last 3 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 and Baden (1991) and modified by Tomas and Baden (1993). A concurrent activity will be to organize the 3-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 a 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 Pelican, 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 (photoacclimation, photosynthetic rates, and production), behavior migration, and toxin production (cellular regulation, cycling, and storage); (3) specify the fate and consequences of byproducts 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. 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 modeling 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, 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 (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 (Baker and 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 and 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, *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 2 years but may be expanded to other bloom stages as dictated by research results and 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 used successfully 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 Biophysical 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) (Kirkpatrick *et al.,* 1990, 1997) fitted with large-volume reservoirs (Reed *et al.*, 1997) will examine physiological and growth processes of mixed phytoplankton communities comprising the various stages of *G. breve* blooms as the assemblages are exposed to vertical motion representative of the biophysical 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 1-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, there will be 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 photoacclimation, 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 (Millie *et al*., 1993), incorporation of ^{14}C into algal protein (DiTullio and Laws, 1986) and cellular-free amino acids (Lohrenz and 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 and 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 used to define carbon fixation states (P_{max}, α, β, I_b) of *G. breve* bloom

communities in conjunction with SUPA manipulation experiments. To extract species-specific production estimates from the community level experiments just described, ^{14}C 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 planozygote stage. More recently, Steidinger *et al.* (1998) 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 recognition antigens to identify vegetative cells, gametes, planozygotes, and hypozygotes if produced experimentally, 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 Protargol) 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 to 20 m and 20 to 38 m), 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 to 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

and 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 and 3, we will track bloom patches in different developmental stages during the 3-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 six 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 to 2 x 10^4 *G. breve* cells copepod^{-1} hr^{-1} with no ill effects (Turner and 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 and 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 and Turner, 1988, 1989, 1990; Turner and Tester, 1989, 1997; Turner *et al.*, 1997) and screened for field verification during bloom tracking process cruises.

Nutrient Dynamic: 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, 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 L^{-1}. Inorganic nitrogen and phosphorus levels rarely exceed 0.5 µg-at L^{-1} in the mixed layer and more commonly are between 0.1 and 2 within 2 to 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 nannomolar levels of nitrogen by conversion to NO_2 via a chemiluminescent method of Bramen and Hendrix (1989). Low-level phosphorus assay (Karl and Tien, 1992) will be utilized for uptake and growth studies. Uptake of P^{32}-labeled substrates also will 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) also will be measured by methods of Burges and Harrison (1995), Eppley (1978), and Slawyk and Rodier (1986), respectively. Growth at various substrate concentrations will be monitored by cell counts (Coulter Counter) and *in vivo* chlorophyll *a* fluorescence. Organic substrates also will be tested for N and P in supporting growth. Cell growth quota for various nutrients and 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 coupled with supply. Results from these assays as well as the laboratory studies will be used to evaluate the nutrient hypothesis.

4. 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 ingestion of toxic animals [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 and Henderson, 1975; Forrester *et al.,* 1977; Roberts *et al.,* 1979; Baden *et al.* 1982; Fowler and Tester, 1989; Geraci, 1989; Pierce *et al.,* 1990; Summerson and Peterson, 1990; O'Shea *et al.*, 1991; Landsberg and 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 and Bunkley-Williams (1990) may have been attributable to biotoxin transfer through dietary exposure (Landsberg, 1995). Also, the worldwide 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 sublethal effects at all trophic levels, leading to impaired feeding, avoidance behavior, physiological dysfunction, impaired 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 6-month period from July through December of 1998 and 1999, four 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 50 km. During two additional months (pre-cruise and post-cruise months), samples will be collected from three stations (see below). These transects will provide a monthly assessment of water quality and, in the event of a bloom, *G. breve* abundance and brevetoxin concentrations. For the four monthly samples, 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 from three stations (pre-cruise transect during one month) in preparation for the first 3-week process cruise. In addition, a post-cruise transect (one month) will be conducted, and samples will be collected as outlined for the pre-cruise transect. Samples will be taken at near-surface, mid-depth, and above bottom (0.5 m). For all samples, water will be collected by Niskin samplers and analyzed for *G. breve* cell abundance and toxins. Water will be filtered and detritus analyzed 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, surface sediment, and water surface microlayer samples will be determined by HPLC analyses (Pierce *et al.,* 1992), MEKC-LIF (Shey, 1997), and ELISA (Tomas and Baden, 1993).

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 and 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 3-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 and Baden, 1993), receptor-binding assay (Van Dolah *et al.,* 1994), and HPLC analysis (Pierce *et al.,* 1992). 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 three designated transects will be revisited and resampled after the bloom has terminated.

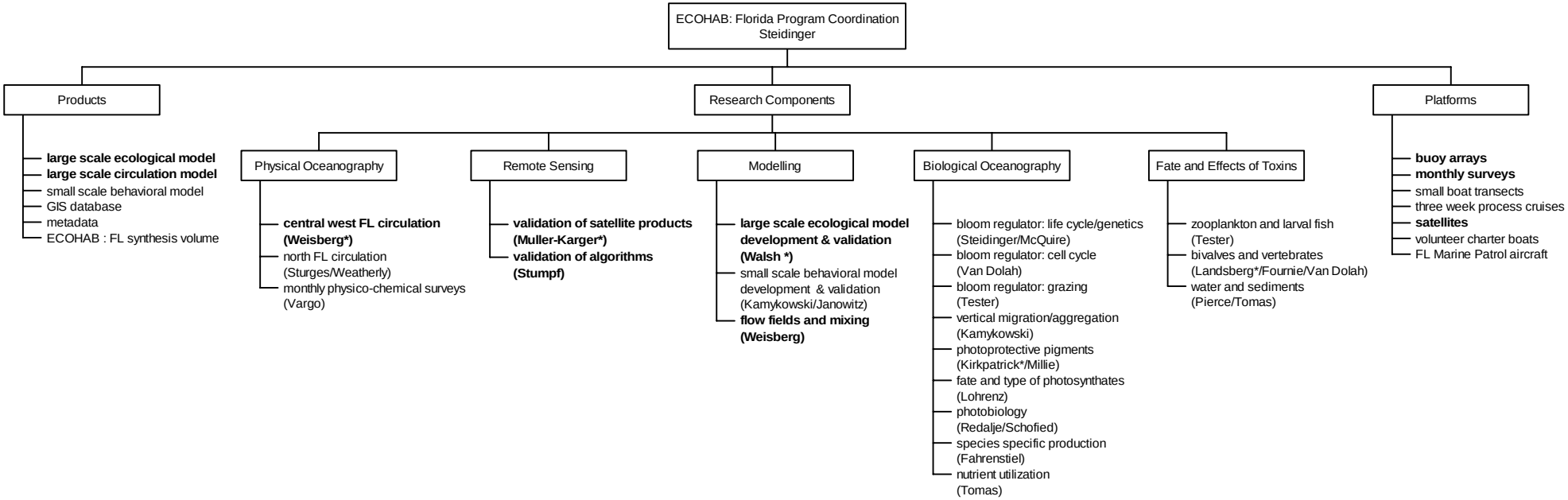
Additional animal material will be measured for baseline brevetoxins. Samples of freshly dead or live animals that may have been exposed to the bloom will be collected and archived as conditions permit. Collaborators will be available to aid in the collection, processing, and analyses of tissues from 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 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 2 represents a programmatic task chart and names the Group Principal Investigators for the five research components: Ecological Modeling, 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 the Florida Marine Research Institute (FMRI) in St. Petersburg for 2 days to review and discuss results, identify and resolve problems, and evaluate the next year's component of the program. During the year, day-to-day activities 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 FMRI's 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



- Group Leaders: Weisberg, Muller-Karger, Walsh, Kirkpatrick, Landsberg
- **Bold Functions** supported by ECOHAB: Florida - NOAA

Figure 2. Organizational Flow Chart for ECHOHAB: Florida

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 Database Manager will be responsible for the integration, co-registration, and distribution to the National Oceanographic Data Center (NODC) of all geographically referenced databases within 2 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, backup, and archiving are accomplished with a variety of devices from tape cartridges to CD-ROMs, and a 28-gigabyte optical jukebox. Federal Geospatial Data Committee (FGDC) compliant metadata will be created for all relevent databases and served through the internet. The ECOHAB:Florida homepage (www.fmri.usf.edu/ecohab) 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 ensure data quality and 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 in a GIS format within 2 years of collection; and (4) metadata will be kept according to 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} (EPA and NOAA funded); 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, FDEP 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. FDEP'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, FDEP 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, FDEP 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.

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