ABSTRACTS FROM THE SYMPOSIUM
ON HARMFUL MARINE ALGAE IN THE U.S.
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ABSTRACTS OF ORAL PRESENTATIONS

ECOHAB: FLORIDA OVERVIEW – THE ENVIRONMENT

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ECOHAB: Florida is a five year federally funded (NOAA and EPA jointly) and state-supplemented program to study *Gymnodinium breve* blooms on the west Florida shelf (WFS). The goal is to develop a coupled physical-biological model to predict the development and landfall of this type of harmful algal bloom or red tide. Marine animal mortality, human respiratory irritation, and toxic shellfish (Neurotoxic Shellfish Poisoning) can occur in coastal and nearshore waters once the bloom has developed and intensified as it moves shoreward. Not all blooms are transported inshore. The circulation patterns that cause upwelling can move a bloom further offshore whereas coastal downwelling can drive it onshore. Blooms typically last several months but can last up to 18 months with offshore waters reinoculating inshore areas. A large bloom can occupy thousands of km^2. In 1999, there were three simultaneous *G. breve* blooms between Pensacola and Jacksonville. These phytoplankton blooms start offshore on the mid-shelf and can develop to fish-killing proportions in about two to four weeks. Seventy percent of historical red tides have initiated between September and December. There are four sequential phases of red tide development on the WFS, 1) initiation in oligotrophic waters from resting or vegetative cells, 2) growth (vegetative growth exceeds losses), 3) concentration (physical mechanisms), and 4) dissipation or termination (where red tide can be entrained and advected by a current or a prevailing wind system). In other areas of the Gulf of Mexico where *G. breve* blooms occur, the development and progression of blooms may not follow the same pattern.

There are 23 ECOHAB: Florida investigators representing 13 institutions. The goal is being pursued by 1) characterizing and monitoring (monthly) a control volume (11,000 km^2) of water on the WFS between Tampa Bay and Charlotte Harbor using three cross shelf, two along shelf, and one diagonal transect, 2) repeating a portion of the central cross shelf transect monthly, and 3) conducting an annual three week process cruise in and outside of *G. breve* blooms for physiological, behavioral, and toxin distribution studies. The objectives are to 1) model each phase of the bloom and forecast landfall, 2) describe the physical environment, cross shelf transport, and concentrating mechanisms, 3) determine the interactions of cellular, behavioral, life cycle, and community regulation processes with environmental forcing factors during stages of bloom development, 4) determine the source of nutrients that provide growth inshore and offshore and allow persistence, and 5) determine the fate and effect of *G. breve* toxins in the marine environment (including the food web).

Other micro-, meso- and macroscale studies (e.g., HyCODE) on the WFS (funded by the state of Florida, NOAA, NASA, MMS, ONR, USGS, and EPA) are able to use data from the ECOHAB: Florida cruises and moored buoy arrays, and federally available satellite imagery to characterize the control volume area, as well as areas that may influence it. Some of these data are real-time, or near real-time, and will be used for interpretation of remotely sensed data and for modeling shelf processes. Hopefully remote sensing (water and/or satellite sensors) will be used in the future to monitor systems and subsystems to feed biophysical prediction models for harmful algal blooms and their effects. Long-term monitoring commitments for calibration and validation of prediction models or for status and trend analyses require a

dedicated regional or system data and metadata management network and core group, particularly for common use data such as basic environmental data.

ROLES OF ENDOGENOUS CELLULAR RHYTHMS AND LIFE CYCLE STAGE RECRUITMENT IN *GYMNODINIUM BREVE* BLOOM DEVELOPMENT

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Blooms of the Florida red tide dinoflagellate, *Gymnodinium breve*, occur almost every year off the west coast of Florida. As a component of the Florida ECHOAB program, this work investigates cellular level controls involved in *G. breve* bloom dynamics. *Gymnodinium breve* has both asexual and sexual life cycle phases, both of which play roles in the formation of Florida red tides. The sexual cycle has been only partially elucidated through the planozygote stage. Current laboratory studies include experiments to evaluate planozygote longevity and hypnozygote (resting cyst) formation. The repeated occurrence of early stage blooms (e.g., 5000 - 20,000 cells per liter) in isolated spots in the mid-shelf region suggests that bloom initiation may result from a benthic life cycle stage. The existence of resting cysts of *G. breve* has not yet been confirmed; however, laboratory observations have identified the existence of a benthic palmelloid stage that may alternatively serve as a seed population for *G. breve* blooms. Confirmation of the presence of cysts, palmelloid cells, or other benthic stages of *G. breve* in bloom initiation zones is the focus of current studies. Identification of unusual life cycle stages is problematic in field samples; therefore, we have developed an antibody probe to cell surface proteins of *G. breve* to assist in these identifications.

Once initiated, the development of a *G. breve* bloom proceeds through asexual cell division. In both laboratory and field populations, *G. breve* growth rates are consistently found to average 0.2-0.5 divisions per day. Like most phototrophic dinoflagellates, the cell cycle in *G. breve* appears to be under the control of a circadian rhythm. Consequently, the cell cycle is phased to the diel cycle, such that the portion of the population destined to divide on a given day enters S- phase (DNA synthesis) 6-8 h into the light phase, and enters mitosis 12-15 h later, during the dark. Naturally occurring blooms, observed over four years of ECOHAB cruises, exhibit similar diel cell cycle phasing. In laboratory cultures, the dark/light or "dawn" transition was shown to provide the diel cue that serves to entrain the *G. breve* cell cycle: a forward or backward shift in the timing of this cue results in a concomitant shift in the timing of S-phase entry. We have identified by western blotting and immunolocalization two key components of the cell cycle regulatory complex that are known in higher eukaryotes to control entry into both S-phase and mitosis: cyclin and cyclin dependent kinase (CDK). Inhibition of the activity of this complex with a specific CDK inhibitor, olomoucine, blocks the *G. breve* cell cycle both prior to S-phase and prior to mitosis, and demonstrates a functional role for this complex in cell cycle regulation. Our current work focuses on the mechanisms by which the diel entraining signal acts on these cell cycle regulators to control cell cycle progression.

ECOHAB FLORIDA: FATE AND EFFECTS OF BREVETOXINS IN SELECTED BIOTA, WATER, AND SEDIMENTS ALONG THE WEST FLORIDA SHELF, USA

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Gymnodinium breve red tides have been historically associated with mass mortalities of aquatic organisms. Organisms are potentially exposed to brevetoxins either through ingestion of *G. breve* cells, toxin bioaccumulation, aerosolized transport, water-borne toxin after cell lysis, and sediment sinks. In parallel with other components of the Florida: ECOHAB program, one of the objectives of this study was to determine the fate and effects of brevetoxins in water, sediments, and in selected biota during an intensive bloom.

Samples were collected off the Florida west coast during a non-bloom period and also during three bloom periods. Water and phytoplankton samples were collected at surface, mid, and bottom depths. Benthic pinfish *Lagodon rhomboides*, planktonic thread herring *Opisthonema oglinum*, zooplankton, sediment, and representative benthic invertebrate samples were collected at selected stations. Samples were processed for brevetoxins (PBTx-2 and PbTx-3) by several methods including MEKC-LIF, receptor-binding assay, and HPLC. Selected fish tissues were also evaluated by histopathology and immunocytochemistry. Brevetoxins in the water column were processed by two different methods; one to distinguish toxins associated with suspended particles from dissolved toxins and the other to distinguish intracellular from extracellular toxins. All sediment, water, and biota samples collected during the non-bloom period were negative for brevetoxins.

During a bloom, most of the brevetoxins in the water column were associated with particles (and cells) with very little in a true “dissolved” state. Early stages of the bloom indicated that most of the toxins were intracellular. The extracellular toxins increased relative to intracellular toxins as the bloom progressed. Low concentrations of PbTx2 (<0.5-11.8 ng/g) and PbTx-3 (<0.5-2.9 ng/g) were detected in sediment samples. There was no positive correlation of brevetoxins in sediments and *G. breve* cell numbers in the overlying water column. The persistence of a bloom may have a greater effect on sediment brevetoxin concentrations than transient high *G. breve* concentrations. From a range of benthic animals tested only shrimp, clams, and anemones were positive for brevetoxins. PbTx-2 (7.6-282 ng/g) and PbTx-3 (1.7-71ng/g) were detected in mixed zooplankton collected when *G. breve* cell numbers ranged between 0.74-2.85 x 10^6 cells/L. PbTx-2 (0.62-320 ng/g) and PbTx-3 (0.08-85 ng/g) were detected in both fish species, but only in specific tissues. Planktonic fish had higher brevetoxin concentrations than benthic fish. Brevetoxin-induced pathology was not detected. PbTx-2 and PbTx-3 concentrations in fish tissues and whole zooplankton samples were not correlated with *G. breve* counts, but this does not take into account prior history of exposure or the presence of extracellular toxins in water. Potential trophic linkages between *G. breve*, extracellular brevetoxins, zooplankton, benthic invertebrates, and fish are confirmed.

ECOHAB – FLORIDA: BIO-OPTICS AND PHYSIOLOGY

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The Florida red tide organism, *Gymnodinium breve,* blooms in shallow continental shelf waters principally along the coast of the Gulf of Mexico. Dense blooms are often found at the water surface where irradiance exposure, including ultraviolet, is very intense. Laboratory cultures of *G. breve* are slow to acclimated to high irradiance, seeming to do best at low irradiance. The acclimation capability of this

dinoflagellate is critical to modeling its presence at high cell concentrations in surface waters where it is transported by wind forcing.

A field study with laboratory culture, acclimated to moderate irradiance levels, showed high absorption cross sections in the early part of the diurnal cycle followed by a dramatic reduction at midday. Quantum yield was strongly depressed at midday and recovery was not evident until near sunset. A subsequent field experiment with laboratory culture that had been more extensively acclimated to moderately high irradiance exhibited similar diurnal patterns to those just described. The extent of midday depression of the quantum yield was directly correlated to peak irradiance for each of the three experimental days. Elimination of ultraviolet from the irradiance spectrum did not produce a detectable change in response. Photoprotective pigments were elevated in the higher-light acclimated cultures. Additionally, on days of high peak irradiance the ratios of the photoprotective pigments diadinoxanthin and diatoxanthin indicated strong protective activity.

These findings will be detailed and available results from cruises currently underway will be incorporated as appropriate. This characterization of the photoacclimation capability of *Gymnodinium breve* will provide guidelines for the Florida red tide system modelers addressing this issue in their comprehensive model.

HYDROGRAPHY AND NUTRIENT CHARACTERISTICS WITHIN THE ECOHAB: FLORIDA CONTROL VOLUME ON THE WEST FLORIDA SHELF

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Blooms of *Gymnodinium breve* may re-occur annually in coastal waters of the West Florida Shelf primarily within the area bounded by Tampa Bay on the north and Charlotte Harbor on the south (i.e. the Ecohab:Florida control volume). This region is oligotrophic with typical inorganic nitrate and phosphate concentrations of <0.5 and 0.2 μM, respectively, within 2 to 4 km of the shoreline. *G. breve* is well adapted for life in this oligotrophic environment with a K_s value for nitrate uptake of 0.42, and for growth utilizing phosphate, ammonia and urea on the order of 0.18, 0.47 and 1.07, respectively. However, when blooms with chlorophyll concentrations >10 μg/l persist within limited geographic areas for 2 to 4 months, additional nutrient sources are required for long-term maintenance.

Ongoing monthly quasi-synoptic cruises collect standard hydrographic measurements at approximately 65 locations along three cross-shelf and two along shelf transects to determine the hydrographic features and potential nutrient sources for bloom inception, growth, and maintenance. Several hydrographic features can be related to bloom formation and persistence. Steep cross shelf thermal and salinity gradients occur in nearshore waters during the winter and rainy season, respectively. Rainy season outwelling of estuarine waters have a characteristic signal of elevated inorganic and organic nutrients with inorganic molar N:P ratios of ~1.0, and leads to the formation of fronts in coastal waters and chlorophyll signatures at the mouth of each estuary. Vertical thermal stratification of shelf waters from May through October leads to the formation of near bottom chlorophyll maxima, dominated by diatom populations, which are potentially fueled by nitrate originating from offshore intrusions of the Loop Current. Fall and winter months are typified by vertically well-mixed water columns which resuspend PON and DON from decaying summer populations.

Phosphate flux from estuarine and/or sediment sources is sufficient to meet bloom requirements. Although inorganic nitrogen levels are low, DON concentrations within the estuaries and in nearshore waters range from 5.0 to 15.0 μM and may provide support for nearshore blooms. Mid to late summer blooms of the diazotrophic cyanobacterium *Trichodesmium erythraeum* , which often precede *G. breve* blooms, appear to be stimulated by iron input from Saharan dust events, and may also act as a source of organic N via excretion of amino acids or inorganic N via bacterial breakdown of excretory material and particulate matter. Elevated DON levels coincide with *Trichodesmium* cell counts in offshore and mid-

shelf areas. Other potential DON sources include the degradation of POM such as sea grass blades from near-shore and estuarine sources.

Measurements of particulate δ^{15}PON from various locations within the control volume strongly suggest that DON from several potential sources is used by *G. breve*. The δ^{15}PON values for surface phytoplankton populations during May-June, 1998 ranged from -1.6 to +2.8 ^{0}/oo with a mean of 0.3 ^{0}/oo suggesting *Trichodesmium* accounted for most of the phytoplankton biomass. The δ^{15}PON of estuarine derived and sea grass derived material has a similar but slightly heavier range; +1.1 to +2.9 ^{0}/oo while the diatom dominated near bottom chlorophyll maxima found during September, 1998 after nitrate depletion had the δ^{15}N signature of Gulf of Mexico sub-thermocline nitrate; e.g. +6.7 to 8.3^{0}/oo. The December, 1998 red tide with approximately 5 μg chl/l in nearshore waters had a δ^{15}PON of +4.8^{0}/oo which reflects use of a ^{15}N-enriched DON substrate which has been modified by bacterial or herbivore fractionation or mixing with DON derived from near bottom nitrate enriched diatom stocks. Several potential N sources are therefore available in support of *G. breve* blooms on the West Florida Shelf. Ongoing studies are aimed at distinguishing between offshore and coastal sources of utilizable DON.

ECOHAB FLORIDA, PHYSICAL OCEANOGRAPHY

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The circulation of west Florida continental shelf exhibits seasonal variability in both its background currents and the responses of these currents to synoptic scale weather forcing. The seasonal circulation is primarily forced by local momentum and buoyancy inputs. However, it is also modulated by mass and heat exchanges with the adjacent Gulf of Mexico. In situ measurements show summer and winter seasons of predominantly downwelling and upwelling circulations, respectively. This occurs regardless of adjacent offshore Loop Current influence, although such influence does affect the seasonal behavior by modifying the across-shelf density field. The seasonally varying density field, in turn, presents a critical control on the response of the shelf to local weather forcing. Under stratified conditions we find that the inner-shelf responses to upwelling and downwelling favorable winds are rectified such that the upwelling responses are disproportionately larger than the downwelling responses. This behavior is attributed to the relative slopes of the isopycnals and the bottom. Buoyancy torque adds constructively with planetary vorticity tilting by the sheared coastal jet under upwelling favorable winds, whereas it adds destructively under downwelling favorable winds. The end result is that the bottom Ekman layer response is enhanced (suppressed) for upwelling (downwelling), and this bottom Ekman layer asymmetry causes the asymmetry in the inner-shelf responses. Since the biology of the west Florida shelf is strongly influenced by bottom Ekman layer processes, these physical oceanographic findings are potentially very important for ECOHAB.

Along with its in-situ measurements, ECOHAB Florida has a numerical circulation modeling component aimed at simulating the seasonal and synoptic scale variability and supplying three-dimensional circulation fields for use in ecological models. For reasons given above the numerical model results are critically tied to the in-situ density field and to the fluxes of momentum and buoyancy, both locally and at the shelf break. Model simulations are therefore limited by model initializations and forcing functions. Without adequate in-situ information, modeling is of limited use. Nowcast or hindcast model runs tends to be useful for integration times of order one month. Improving upon this requires improving upon both the surface and the offshore boundary condition data and providing adequate interior density data for model assimilation. Initial attempts at combining the physical and ecological models are encouraging.

THE ROLE OF BEHAVIOR IN *GYMNODINIUM BREVE* BLOOM FORMATION

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The prediction of bloom formation by *Gymnodinum breve*, an autotrophic dinoflagellate capable of swimming at a rate of 1 m/h, requires a consideration of cellular motion in the context of ambient water motion. Unfortunately, the rules that *G. breve* follows in choosing its swimming direction are poorly known. Field observations of the distribution patterns of *G. breve* demonstrate that cell aggregations can occur throughout the euphotic zone, but diel vertical migration generally is difficult to discern. Laboratory experiments (Kamykowski et al., 1998 [MEPS 167:105]) in 225 L columns (45 x 150 cm) under nutrient replete conditions demonstrate that the diel vertical migration of *G. breve* is characterized by a surface aggregation during daylight that diffuses through the available water column during the night. The strength of the surface aggregation depends on the time since last division and the biochemical composition of the cells. Based on an experiment with a quantized culture, parent cells apparently divide into unequal daughter cells. Daughter cells that contain smaller lipid reserves preferentially aggregate at the surface during the day, while those with larger lipid reserves tend to remain distributed throughout the available water column during the day. As both daughter cells age, the strength of the surface aggregation decreases until the sequence is re-initialized by another cell division (Kamykowski et al., 1998 [JPR 20:1781]). A numerical model of *G. breve* biology tuned to its known physiology and behavior provides output that most closely resembles the patterns of laboratory diel vertical migration when behavior is a based on positive phototaxis during the day and weakened negative geotaxis (that is, an increased tendency to descend compared to other times) at dusk, a cell division that yields unequal daughter cells, and a swimming orientation that is influenced by inhibitory light intensity (descent) and by the state of the cellular carbon and nitrogen pools (Liu et al., 2000 [MEPS, In Press]). This biological numerical model is modified for field application (deeper water columns, but with cell behavior as the sole component of vertical motion) by allowing cells to sense the nutrient gradient in the water column. When this modified model is applied to different scenarios of vertical nutrient sources representative of the West Florida Shelf including near-surface *Trichodesmium* blooms, near bottom upwelling plumes, and/or near-surface outwelling from terrestrial sources, cell aggregations occur at different locations in the euphotic zone depending on nutrient availability. The vertical distribution patterns are reminiscent of the field observations. The transition zones where different nutrient sources compete for cells generate model output characterized by complex vertical distribution patterns of cell number and of cell quality in terms of carbon and nitrogen reserves.

These different studies support a working hypothesis that *G. breve* applies its motility in response to a variety of environmental and internal cellular cues that can interact in complex patterns. Under conditions where cell motility is effective in determining vertical location, field aggregations may occur where the balance of these conditions provide an optimal water column location for cell survival and/or growth. Under conditions where vertical water motion is more significant, a gradient of disruption can be envisioned as cells strive for optimal locations that they may never attain. In both instances, the vertical location of the cells determines exposure to horizontal currents and subsequent movement across the shelf and along the coast.

COUPLED NUMERICAL MODELS OF FLORIDA RED TIDES OF *GYMNODINIUM BREVE*

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Successful ecological models are data-driven, distilling qualitative hypotheses and aliased field observations into simple analogues of the real world in a continuing cycle of model testing and revision. Prediction of the origin, transport and fate of *Gymnodinium breve* blooms on the West Florida shelf is the goal of the ECOHAB: Florida project - based on 1) shipboard and remote sensing surveys of hydrography, nutrients, DOM, species composition, pigments, and zooplankton 2) experimental cruises and laboratory studies describing their physiology, life cycles, optical properties, and toxin transfer, 3) arrays of current meters, 4) circulation submodels, 5) cell metabolism and migration submodels, and 6) coupled bio-optical models. The last component utilizes these submodels and assimilated observations in a complex, numerical food web to describe the consequences of phytoplankton competition in terms of

signals seen by satellite, aircraft, and moored sensors. We have used one-dimensional models to specify the rules of engagement between *G. breve* and other functional groups of phytoplankton, two-dimensional models to explore the consequences of their interaction with the microbial food web, and three-dimensional models to predict their transport, landfall, and residence time at the surface of the sea.

Following competition theory, our present models of the limiting resources of light, nitrate, ammonium, DON, phosphate, DOP, iron, and silicate should allow the coexistence of eight functional groups of phytoplankton, without differential grazing pressure on chromatically-adapted phytoplankton. In our analogue of the West Florida shelf, CO_2 and N_2 are state variables, but they are considered to be in excess of algal needs. From our simulation analyses thus far, we find that 1) diatoms win when estuarine and shelf-break supplies of nitrate are made available to a model community of small and large diatoms, coccoid cyanophytes and *Trichodesmium*, non-toxic and red-tide dinoflagellates, microflagellates, and coccolithophores, 2) a numerical recipe for large red tides of G. breve instead requires DON supplies, mediated by iron-starved, nitrogen-fixers in response to Saharan dust events, while their small blooms may persist on sediment sources of DON, 3) selective grazing must still be exerted on the other non-toxic dinoflagellates by copepods, 4) bacteria drive the outer shelf food web into P-limitation, until coastal supplies of low N/P ratio of ~1 favor nitrogen-fixers, 5) light-cued vertical migration of *G. breve* in relation to seasonal changes of summer downwelling and fall/winter upwelling flow fields determines both their duration within the first optical depth as a remotely-sensed signal and the intensity of red tide landfalls along the barrier islands and beaches of West Florida, and 6) termination of G. breve blooms is likely to result from cumulative, biomass-dependent losses in the form of UV-B irradiation, microbial-induced lysis, and unselective grazing pressure from protozoans and heterotrophic dinoflagellates.

ABSTRACTS OF POSTERS

IDENTIFICATION OF CELL CYCLE REGULATORS IN THE FLORIDA RED TIDE DINOFLAGELLATE, *GYMNODINIUM BREVE*

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The diel cycle is a key regulator of the cell cycle in the Florida red tide dinoflagellate, *Gymnodinium breve*, and may play a rate limiting role in bloom formation. Both laboratory cultures and field populations of *G. breve* exhibit phased cell division in which approximately one-third of the population divides each day. The dark/light "dawn" transition provides the diel cue that serves to entrain the *G. breve* cell cycle, with S-phase beginning 6-8 h into the light phase, and mitosis following 12-14 h later. This research is aimed at identifying the molecular targets of this diel cue. Here we identify in *G. breve* the two components of cell cycle regulatory complex, cyclin and cyclin dependent kinase (CDK), which are the molecular basis of cell cycle regulation in eukaryotes. The presence of CDK was identified by western blotting and by cell cycle inhibition with the specific CDK inhibitor, olomoucine. Cyclin was identified in *G. breve* by western blotting using two antibodies: the first one raised against cyclin B of the yeast *Schizosaccharomyces pombe*, the second one raised against the cyclin box of the sea-urchin cyclin. Both antibodies recognize a ~64 kD antigen in *G. breve,* and cross- react specifically with the control peptide cyclin B1, but not with cyclin A. This suggests that the *G. breve* cyclin identified is a cyclin B homologue.

The expression of the cyclin B homologue in *G. bre*ve was followed by western blotting at different time points during cell cycle. Unlike cyclin B in higher eukaryotes, which is expressed only late in the cell cycle, the cyclin B homologue in *G. breve* was expressed at similar levels throughout cell cycle. This unusual behavior was confirmed by immunohistochemistry. Preliminary results show that cyclin B was present in the cytoplasm throughout the cell cycle, where it appeared to be localized to the centrosomes. Unlike higher eukaryotes, cyclin B did not appear to be translocated to the nucleus prior to mitosis; this may reflect the fact that the nucleus remains intact during mitosis in dinoflagellates. However, it was localized to the mitotic spindles in mitotic telophase, similar to observations in mammalian cells. The unusual constitutive expression of dinoflagellate cyclin has been previously demonstrated in *Crypthecodinium cohnii* (Barbier et al., 1995), and has also been shown in the myxomycete, *Physarium polycephalum*.

PHOTOPHYSIOLOGICAL RESPONSES OF THE RED-TIDE DINOFLAGELLATE *GYMNODINIUM BREVE* (DINOPHYCEAE) UNDER NATURAL SUNLIGHT

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Little is known concerning the physiological mechanisms by which microalgae, particularly bloom-forming taxa, tolerate large and variable amounts of photosynthetically available radiation (400-700 nm) and ultraviolet radiation (295-400 nm). Because *Gymnodinium breve* Davis often accumulates at or near the air-water interface, the diurnal, photophysiological responses of this red-tide dinoflagellate were investigated. Laboratory cultures of *G. breve* were incubated outdoors, and allowed to acclimate to attenuated natural irradiance. Aliquots of photoacclimated cultures were exposed to PAR+UV or PAR-only irradiances, incubated within Sarasota Bay, Florida (USA), and assessed for diurnal responses of *in vivo* fluorescence and *in vitro* pigmentation, lipid, carbohydrate, and protein contents over three distinct photoperiods varying from overcast to partly cloudy to extremely sunny conditions. The maximum quantum yield for stable charge separation at photosystem II exhibited midday depressions (roughly) symmetric about solar noon on the overcast and partly cloudy days, but exhibited a pronounced hysteresis on the sunny day. The induction and relaxation of the xanthophyll cycle over the course of the photoperiod during the partly cloudy and sunny days resulted in stoichiometrically inverse cellular accumulation of the photoprotective pigments, diadinoxanthin and diatoxanthin. Only minor adjustments in the cellular chlorophyll *a* and fucoxanthin contents occurred during any photoperiod. No differences in the epoxidation state of the xanthophyll-cycle pigments or in the maximum quantum yields occurred between cultures exposed to PAR-only or PAR+UV treatments. However, the observed differences in the oxygen production rates and other biochemical parameters between cultures exposed to PAR-only or PAR+UV treatments were not directly attributable to UV-induced effects. These findings indicate that *G. breve* possesses an inherent UV resistance and a robust photosynthetic capability, thereby allowing cells to acclimate to variable irradiance regimes over relatively short time scales.

THE RELATIONSHIP BETWEEN THE OCCUPATIONAL EXPOSURE TO *GYMNODINIUM BREVE* (DINOPHYCEAE) TOXIN AND PULMONARY FUNCTION

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The Western Coast of Florida frequently experiences a harmful algal bloom caused by the dinoflagellate, *Gymnodinium breve*, Davis (*G.breve*). *G. breve* releases a toxin when the cells lyse, and this toxin becomes part of the marine aerosol. When humans are exposed to *G. breve* toxin in marine aerosol, upper respiratory symptoms such as runny nose, nasal congestion, cough, and sore throat are commonly reported. Since the estimated size of the particle is 7 - 10 μm most toxin should be filtered by the upper airway. If the red tide toxin impacts the lower airway, bronchoconstriction of the smooth muscle may occur. The common method to detect bronchoconstriction is the measurement of the Forced Vital Capacity (FVC) and the forced vital capacity exhaled in 1 second (FeV_1). A pilot study in 1999, conducted on the Florida ECOHAB cruise revealed that most scientists studied did not have a significant change in pulmonary function when conducting research in a *G. breve* bloom. However, 2 of the 17 did have a change and these two scientists were nonsmokers, young, with no known pulmonary history. Further investigation was indicated.

For the September 2000 Florida ECOHAB cruise, similar procedures from 1999 were followed. The primary purpose of this cruise was to conduct experiments in a red tide bloom in the Gulf of Mexico. Volunteer scientists were instructed on the correct method to perform the FVC maneuver. They were also

asked to complete a Health History Questionnaire (©Hollister Inc, 1980). The volunteers then performed the forced vital capacity maneuver at varying times of the cruises and also documented any respiratory symptoms. Variation in FVC and FeV_1 in correlation to surface cell counts, wind speed, and scientist subjective symptoms were analyzed. Findings from the cruise will be reported.

SEARCHING FOR VIRUSES INFECTIVE FOR *GYMNODINIUM BREVE*

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A project has been initiated to find lytic and temperate viruses infective for *Gymnodinium breve*. During a research cruise into the Gulf of Mexico in early 1999 a *G. breve* bloom near Ft. Myers was sampled for viruses infective for this red tide organism. A viral concentrate was made by vortex flow ultrafiltration of 20 l of the water to yield a 50 ml retentate. One ml aliquots of this concentrate caused 25 ml *G. breve* Davis Piney Island clone cultures to lyse ("crash") within 3 to 5 days. The "lytic agent" was propagated by inoculation into fresh cultures of *G. breve* and "serially passaged" over 12 times before lytic activity was lost. The lytic agent passed a 0.2 µm filter but was retained on a 0.02 µm filter and was inactivated by heating to 60^{o} for 10 min. Viral particles increased in cultures exposed to the lytic agent as detected by epifluorescence microscopy of SYBR Gold-stained culture filtrates. A dominant tailed Siphoviridae was observed by TEM in such preparations. However, no viral particles were observed in thin-sectioned preparations of bursting *G. breve* cells. It is hypothesized that the virus observed was infective for a bacterium that either was necessary for *G. breve* growth or that the lysis of bacteria in the culture cause *G. breve* mortality. Attempts to induce prophage by mitomycin C resulted in certain instances in *G. breve* lysis and viral production, yet this may have been the result of induction of bacterial lysogens in these cultures.

INTRA-CELLULAR, PARTICULATE AND DISSOLVED BREVETOXIN DISTRIBUTION DURING G*YMNODINIUM BREVE* BLOOMS IN THE GULF OF MEXICO, USA

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The dinoflagellate, *Gymnodinium breve*, produces several neurotoxins causing neurotoxic shellfish poisoning (NSP), massive fish kills and respiratory irritation in marine mammals and humans. The common method for public health advisories is enumeration of live cells in the water. Evidence for neurotoxins outside of cells indicates that contamination could result from water masses carrying suspended/dissolved neurotoxins in the absence of viable *G. breve* cells. Therefore, reliance on cell counts only for public health protection may be insufficient.

This study was undertaken as part of the EOCHAB-FL program to assess the concentration of toxins associated with *G. breve* cells (intra-cellular) relative to the amount of brevetoxins outside the cells (extra-cellular) either dissolved or associated with suspended particulate matter. These samples were collected during 1998 and 1999 ECOHAB-FL cruises in the Gulf of Mexico, USA. The water samples were processed by two different methods, one to distinguish toxins associated with suspended particles from dissolved toxins and the other to distinguish intra-cellular from extra-cellular toxins. The dissolved vs particulate method used gentle filtering under vacuum (<100 mm Hg) through a series of filters, a 0.7 µm porosity GF/F glass fiber filter followed by a 0.2 µm polycarbonate membrane. Toxins were separated into three fractions including those associated with cells and particles (> 0.7 µm), toxins associated with small particles (0.7>0.2 µm), and dissolved toxins (<0.2 µm). The intra- and extra-cellular toxin method used a stirred ultra-filtration cell concentrator for separating viable *G. breve* cells from seawater. Water samples were filtered through a 0.8 µm porosity polycarbonate membrane in the stirred cell at 5 psi to gently collect the viable *G. breve* cells on the membrane, allowing the "extra-cellular" toxins to pass through with the filtrate.

Dissolved toxins in the filtrate from the 0.2 µm polycarbonate filters were recovered by filtering through a C-18 bonded phase disc with subsequent elution in acetonitrile. The particulate toxins remaining on the GF/F and 0.2 µm filters were extracted by sonication in methanol. These samples were prepared for analysis by capillary elecrophoresis with laser-induced fluorescence detection according to the method of Shea, 1997. Extra-cellular brevetoxins were recovered from the cell concentrator filtrate by passing through a C-18 bonded-phase extraction disc with subsequent elution of the toxins in methanol. The toxins associated with the concentrated *G. breve* cells were released by osmotic shock with distilled water, recovered on a C-18 bonded phase disc and eluted with methanol as above. These samples were prepared for HPLC-UV analysis according to the procedure of Pierce et al, 1992.

Results showed that most of the toxins in the water column were associated with particles (and cells) with very little in a true "dissolved" state. Early stages of the bloom indicated that most of the toxins were intra-cellular. The extra-cellular toxins increased relative to intra-cellular as the bloom progressed, indicating cell lysis and toxin retention in the water column. Further studies are underway to collect fractions more closely representing toxins with in *G. breve* cells relative to toxins in association with suspended particulates and in the dissolved fraction. Consideration also is given to the persistence of brevetoxins in the different fractions within the water column.

ESTABLISHING AND MAINTAINING CLONAL CULTURES OF *GYMNODINIUM BREVE*

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The toxic dinoflagellate *Gymnodinium breve* has been grown and studied in the laboratory for almost five decades. Most of the early physiological studies were performed using the Wilson clone (isolated by W.B. Wilson from John Pass, Florida in 1953, CCMP718). Renewed interest in the physiology and genetics of *G. breve* has emphasized the need for the establishment and long term maintenance of clonal cultures from a broad geographic range. Until recently, the former successes in establishing cultures for study had not been widely reproduced. One of the primary reasons is that *G. breve* cells are fragile and are easily stressed. The method used in our laboratory is presented as a simple combination of established isolation and culture methodologies. The techniques have been used to establish and maintain 20 different clonal isolates of *G. breve* from the Gulf of Mexico and Atlantic waters for as long as 4 years.

Growth medium is prepared using oligotrophic seawater collected by lowering a submersible pump (all plastic components) over the side of a research vessel. The water is stored, prior to use, in the dark at 5°C in 20 liter polycarbonate carboys. The seawater is 0.45 µm filtered and then sterilized by autoclaving in 1.5 liter teflon bottles. Established cultures are maintained in growth medium prepared by adding sterile filtered nutrients as in "L1" (Guillard and Hargraves, 1993) except for the vitamin additions, which are added as in NH-15 (Gates and Wilson, 1960).

Samples for isolation are usually received as whole water samples shipped to the laboratory in plastic bottles or bags. Individual cells are drawn up into sterile micro-capillary borosilicate glass pipets (~300 µm diameter). Individual cells are then repeatedly washed by transferring them by micro-pipet to drops of sterile filtered oligotrophic seawater on a glass slide spot plate. All mouth pipeting is done on an inverted microscope (40X) under a laminar flow hood. After several washes, individual cells are placed in polystyrene tissue culture well plates containing 1 mL of growth media. The growth media used to establish the single cells in culture uses the medium described above at $1/10^{th}$ concentration in sterile filtered oligotrophic water. This media may be diluted 50% with GF/F filtered water from an established culture in an effort to decrease the shock that newly isolated single cells might experience when placed in water not "conditioned" by *G. breve* cells and their associated bacteria. The isolate cultures are allowed to reach densities of at least 25 cells/mL before additional media is added to the well. Additional media is added 2-3 times per week in small amounts, not exceeding 10% of the existing culture volume. This minimizes the stress to the growing cells while still meeting their needs. The isolates are transferred to successively larger flasks until they are finally moved to 13 liter Pyrex glass carboys. The isolates are maintained in these carboys in volumes of 3-8 liters at S=34, 23°C, and under a 12:12 L:D cycle at 60

$\mu Em^{-2}sec^{-1}$. Transfers of established cultures in carboys are made every 3-4 weeks using cultures with densities of approximately 10^7 cells/L to establish new cultures with densities of approximately 10^5 cells/L. Isolate cultures are maintained at growth rates between 0.2 and 0.3 divisions da^{-1}. Both "grandparent" and "parent" cultures are kept as a safeguard against unexpected culture mortality. The combination of basic culture methods described above, could be easily replicated and improved upon, resulting in the establishment and maintenance of additional *G. breve* or other *Gymnodinium* clonal isolates for use in taxonomic, physiological, and genetic investigations.

Gates, J.A. and W.B. Wilson. 1960. The toxicity of *Gonyaulax monilata* Howell to *Mugil cephalis*. *Limnol. Oceanogr*. 5, 171-174.
Guillard, R.R.L. and P.E. Hargraves. 1993. *Stichochrysis immobilis* is a diatom, not a chrysophyte. *Phycologia*, 32, 234-236.

DEVELOPMENT OF THE VOLUNTEER OFFSHORE RED TIDE MONITORING PROGRAM FOR THE GULF COAST OF FLORIDA

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Gymnodinium breve red tides originate offshore on the west Florida shelf and then can be transported to inshore areas by currents and winds. In the past, detection of blooms before they reached inshore waters was mostly by research cruises or the occasional report of discolored water or dead fish by boaters, commercial fishermen or airplane pilots. The rationale for this program was to have regular sampling in areas offshore of areas where red tides have traditionally come inshore, Tampa Bay to Charlotte Harbor, and where shellfish harvesting is economically important.

Developing a plan for the Volunteer Offshore Monitoring Program involved determining where, when and how the sampling should take place as well as who should be targeted for involvement.
Since red tides may affect any portion of Florida's Gulf coast, a few key areas were selected for volunteer recruitment. These areas included Pensacola, Panama City, Apalachicola, Cedar Key, St. Petersburg, Charlotte Harbor, Naples and the Lower Keys. The coastal nature of these areas, with easy access to the Gulf of Mexico, make them ideal commercial charter fishing operations.

Utilizing FMRI contacts, Internet web-sites, and general networking, charter captains in these areas were contacted about participating in the program. Most commercial charter captains understand that monitoring for red tide is in their own best interest, however, sampling may differ from scheduled times based on weather, business, and other reasons. It was important for us to understand that these volunteer captains were sampling on their paying customer's time. The sampling procedure and return shipping process needed to be quick and user friendly. All the sampling bottles and shipping materials were pre-labeled and any instructions were made as concise and unambiguous as possible. The volunteer captains were asked to sample at offshore distances of 1, 5, 10, 20, and 30 miles approximately twice a month. When possible, additional volunteers in the same area were recruited. This reduced the possibility of sampler "burnout" by any one captain as well as providing backup coverage during mechanical troubles, vacations, etc.

Possibly the most important key to obtaining sampling stability is regular contact with the volunteer captains and providing them with some type of visual feedback on their work. Maps displaying the red tide cell counts from each sample are relayed to the volunteers, allowing them to serve as monitors of their local waters. It is crucial that the volunteers feel as though they are a part of a meaningful program and that their efforts are recognized. Current activities involve developing appropriate rewards and enlisting captains to collect bottom water samples. At present only surface water samples are collected.

OBSERVATIONS OF SEA SURFACE TEMPERATURE AND WINDS ASSOCIATED WITH FLORIDA, USA, RED TIDES (*GYMNODINIUM BREVE* BLOOMS)

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Blooms of *Gymnodinium breve* on the west coast of Florida USA are commonly initiated during the summer but rarely in the winter, with greatest frequency of occurrence at the coast in the fall. The seasonal changes in bloom occurrence correlates to seasonal variations in the wind and sea surface temperature. Blooms typically initiate offshore in the summer, when the winds are weakest. However, they are maintained at the coast during the fall, a period of strong winds blowing offshore. During January and February, satellite imagery shows cool water spreading from the shore towards offshore, raising the possibility that conditions may be less favorable for bloom initiation and maintenance. Also, the winds weaken during the same time. While providing these climatological relationships, our data set cannot resolve the circumstances around a particular bloom events, including major winter blooms, probably because of lack of data on the offshore initiation and presence of the bloom.