1. INTRODUCTION

The Gulf of Maine (GoM) is a continental shelf sea that supports productive shellfisheries that are frequently impacted by *Alexandrium fundyense* blooms and outbreaks of paralytic shellfish poisoning (PSP). Nearshore resources are monitored by state agencies, whereas most offshore stocks have little or no routine monitoring. As a result, large areas are permanently closed or underexploited because of the threat from PSP toxins (PSTs) and the lack of scientific understanding and management tools.

PSP is a relatively new phenomenon in the northeast (Anderson, 1997). Toxicity was restricted to far-eastern Maine (ME) until 1972, when a massive, visible red tide of *A. fundyense* stretched from ME to Massachusetts (MA), causing toxicity in southern areas for the first time. Virtually every year since 1972, western ME has experienced PSP outbreaks, and for the first 20 years of that interval, MA did as well. That pattern was a direct result of *A. fundyense* cysts being retained in western GoM waters after the 1972 bloom and subsequent events (Anderson and Wall, 1978). Between 1994 and 2004, toxicity was infrequent in MA and the southern GoM, but in 2005, another massive bloom occurred (Anderson et al., 2005a), closing shellfish beds from ME to southern MA and 40,000 km^2 of offshore federal waters as well. Economic losses are now estimated to be $50 million for the MA shellfish industry alone.

There are striking similarities between the 2005 & 1972 blooms: both extended into southern MA and both caused extremely high toxicity as well as toxicity in areas with no prior history of PSP. *If the past is a prologue, southern New England may experience a significant change in the pattern of PSP in the coming years, as occurred following the 1972 event (Anderson et al., 2005a).*

The 2005 bloom highlighted how little we know about *A. fundyense* bloom dynamics in the southern GoM and the waters south and east of Cape Cod (hereafter referred to as "GoM and southern New England shelf waters") and in particular, the link between blooms in surface waters and toxicity in deep offshore shellfish. A huge area of offshore shellfishing grounds was closed and then partially reopened during the 2005 bloom, while a much larger zone (~80,000 km^2) that includes Georges Bank has been closed since 1990 after high levels of toxicity were detected (Fig. 1). Pressures are mounting from industry to open these areas and to develop management strategies so that surfclam (*Spisula solidissima*), ocean quahog (*Arctica islandica*), and roe-on sea scallop (*Placopecten magellanicus*) fisheries can be developed and sustained (see Wallace support letter).

Industry interest is motivated in part by problems with the $75M mid-Atlantic Bight surfclam and ocean quahog fishery, where surveys reveal decreasing stocks that are considered too low to sustain the fishery (D. Wallace, P. Jensen, pers. comm.). Over the past several years, FDA and state regulatory agencies have been approached by the industry with requests to open the closed areas in Fig. 1 and to establish the regulations and procedures that would allow offshore fisheries to develop there and in adjacent waters. Using NMFS survey results (SARC 37, 2003) and current landed values, our industry partners estimate the total value of the Georges Bank ocean quahog and surfclam resources to be $1.5 billion and $0.5 billion respectively (D. Wallace, pers. comm.). As there has not been any harvest of surfclams since 1990 and no quahog harvest ever, both populations are near carrying capacity. Surfclams and quahogs worth up to $50 million could be harvested annually on a sustained basis if the areas reopen. This is truly a significant, unexploited resource.

One response to industry interest is an FDA/NMFS-sponsored workshop in February that will "*examine the establishment of a dockside testing program that would enable the safe harvest of molluscan shellfish from federal waters in the northeast that have been closed because of PSP*" (P. Distefano, FDA, pers. comm.). Another recent regional workshop explored the feasibility of "*a new, multi-million dollar fishery to meet ongoing demand for whole and roe-on scallops*" (see http://seascallop.com/). In the U.S., only the scallop adductor muscle is sold, whereas elsewhere, the

roe (gonads) are left attached and the "roe-on" scallop is marketed. A related product is the whole, in-shell scallop. In both cases, value is increased because of added weight and higher value per unit weight. The estimated value of a roe-on scallop fishery in the northeast is $60 -70 million annually, based on demand from Europe (Montfort, 2002), Asia, and US ethnic communities and white-tablecloth trade (G. Day, pers. comm.) Due to biotoxin regulations and the lack of knowledge on the patterns and distribution of PSTs in sea scallop viscera and roe, no US industry exists at present.

In response to clear scientific unknowns and societal needs, here we propose GOMTOX - a regional observation and modeling program that will investigate the patterns and mechanisms underlying A. fundyense *blooms and the resulting toxicity in shellfish in the southern GoM and its adjacent New England shelf waters, with special emphasis on the delivery pathways, mechanisms, and dynamics of offshore shellfish toxicity.*

2. BACKGROUND AND PRIOR WORK

A. fundyense blooms were the subject of intense investigation through ECOHAB-GoM– a regional program designed to characterize *A. fundyense* blooms in the northern GoM. More than 30 papers were published from this research, many in a special journal issue (Anderson et al., 2005d). A synthesis of the results of many of those studies was provided by Anderson et al. (2005c) and McGillicuddy et al. (2005a) in the form of conceptual models of *A. fundyense* bloom dynamics. Central to these formulations and to this proposal are major transport pathways in the GoM (Fig. 1). These include the Maine Coastal Current system (MCC), a composite of seven segments with multiple branch points (Lynch et al., 1997). The upstream, eastern segment (EMCC) extends from the Bay of Fundy (BoF) to Penobscot Bay. The EMCC often veers offshore near Penobscot Bay, a critical branch point. Some EMCC water continues offshore, and some returns shoreward to form the western segment of the MCC (WMCC), which is augmented by river outflow. Near Cape Ann, another branch point occurs where some WMCC water enters Massachusetts Bay, a basin bounded on the east by Stellwagen Bank, while some bypasses the bay, traveling along the eastern flank of Stellwagen Bank toward Georges Bank. Downstream, the Stellwagen segment

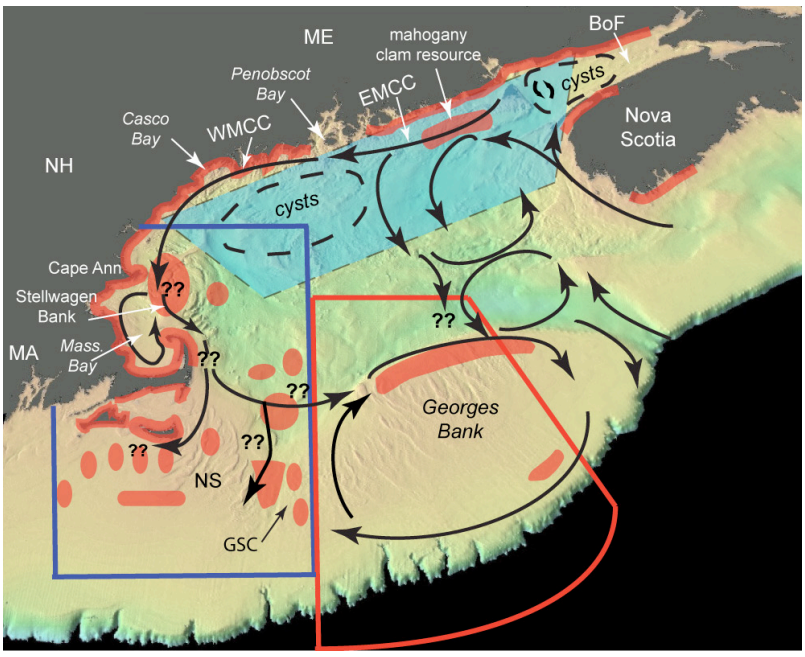


Figure 1. The Gulf of Maine showing major currents, transport pathways, and branch points (arrows), areas with known PSP toxicity (red shading), and offshore zones closed in 2005 & 1989 (outlined in blue and red respectively). Due to limited sampling, the red shaded zones represent a small fraction of the offshore area that may experience PSP outbreaks. Also shown: *A. fundyense* cyst seedbeds (dashed lines), ECOHAB-GoM domain (blue shaded area), EMCC – eastern segment of the Maine Coastal Current; WMCC – western segment; NS – Nantucket Shoals; BoF – Bay of Fundy; GSC – Great South Channel. Question marks denote areas where we do not understand hydrographic forcings of *A. fundyense* dynamics.

bifurcates into a Nantucket segment, which exits the GoM via the Great South Channel, and a Georges Bank segment that travels to and around the bank.

Key features in the ECOHAB-GoM conceptual models are two large cyst "seedbeds"- one in the BoF, and the other offshore of Casco and Penobscot Bays (Fig. 1; Anderson et al., 2005c). Cysts germinate from the BoF seedbed, causing recurrent *A. fundyense* blooms that are self-seeding with respect to future outbreaks in that area, as well as "propagatory" - i.e. some cells escape the retention zone near Grand Manan Island and enter the EMCC where they bloom. Some of these cells are entrained into the WMCC, while others deposit cysts in the Penobscot/Casco Bay seedbed. In subsequent years, these cysts (combined with cells from the EMCC) inoculate WMCC blooms that cause toxicity in western ME, NH, and MA before being advected to offshore waters along Stellwagen, Georges Bank, and the Great South Channel. It is possible, though not yet confirmed, for EMCC cells to travel to Georges Bank across the central GoM, (the "short-circuit" pathway; Fig. 1).

***A. fundyense* blooms and toxicity in southern waters.** From the early '70s through the early '90s, PSP episodes within Massachusetts Bay were more sporadic than those in ME (Franks and Anderson, 1992). Then, from 1994 to 2004, there was no toxicity in Massachusetts Bay, possibly reflecting the observed diminution of the Penobscot/Casco Bay cyst seedbed (Anderson et al., 2005c). This pattern was broken in 2005 with a massive *A. fundyense* bloom that stretched from ME to southern MA (Anderson et al., 2005a). Due to this unprecedented event, the NMFS, at the request of the FDA, closed ~40,000 km^2 of offshore, federal waters (Fig. 1) to harvesting of all molluscan shellfish except scallop adductor muscle. Through an FDA/industry collaboration similar to that proposed here, fishermen collected and processed shellfish weekly from 6 stations south of Nantucket and shipped meats to the FDA for analysis. Ocean quahog toxicity was below the action level (80 µg STX eq $100g^{-1}$ tissue) and lower than for surfclams (max. 526 µg STX eq $100g^{-1}$). Toxicity varied with depth. By August, surfclam toxicity was below the action level so the southern portion was reopened to harvesting (with the exception of whole and roe-on scallops) on 9 September. There were insufficient data, however, to support reopening of the northern portion, which remains closed until June 2006, and perhaps longer.

This same sequence happened to the 80,000 km^2 area that was closed in 1990 and remains closed to this day for surfclams and ocean quahogs. In 1989, PSTs were found in scallops from the Canadian sector of Georges Bank and in surfclams from the American sector (Nassif and Timperi, 1991). In August of that year, the NMFS issued a 90-day emergency closure of the American sector for surfclam harvesting, but in May, 1990, 8 fishermen were severely poisoned after eating mussels from Cultivator Shoal on Georges Bank. As a result, the harvesting closure was extended. That closure remains in effect not because of proven and persistent toxicity in the resource, but because federal regulators have not been able to reopen the area due to a lack of toxicity data and an ignorance of the pathways through which the shellfish become toxic. No offshore toxin measurements were made from 1990 - 2004 in US GoM waters. Only recently have scattered analyses been made (hence the "measles" pattern in Fig. 1), motivated by the 2005 bloom and by fishermen and managers interested in opening these areas (see RSA 008 at www.seascallop.com).

Another offshore resource of interest is the mahogany clam or ocean quahog (also *Arctica islandica*) in eastern Maine (Fig. 1). This species is regularly contaminated with PSTs and only a small portion of the resource is typically open. Here again, information on the manner in which offshore shellfish become toxic would be of great help to resource managers (see Couture letter).

Given insufficient federal or state resources to monitor PSTs in offshore shellfish and the extensive shellfish stocks that are consequently unavailable for harvest, there is a clear need for a study to document the time/space variability of toxicity in offshore plankton and shellfish and to

provide information on the relationship between *Alexandrium* blooms and shellfish toxicity at depth. Such a study would be unique in HAB science, as there are virtually no data on the mechanisms underlying deep-water shellfish toxicity. Armed with maps of toxicity, numerical models, shipboard testing kits, and an understanding of transport pathways and mechanisms of toxin delivery to the benthos, harvesting could be allowed in areas or times likely to be safe, with onboard testing of the shellfish (i.e., the Jellett Rapid Test) providing the assurance that product could be taken to shore for confirmatory dockside testing. *A viable fishery could be maintained despite sporadic outbreaks of PSP toxin contamination, just as state-run monitoring programs in nearshore waters sustain healthy shellfish industries in areas subject to recurrent PSP events*. In this regard, ultimate responsibility for offshore closures, re-openings, and regulations falls to the FDA and NMFS, and their interest and need for the type of information to be generated by GOMTOX is evidenced by the strong participation of both agencies in this program (see section 5).

A final note is that much of the foregoing has focused on offshore shellfish, but the 2005 bloom also highlighted the manner in which cells and toxins can "turn the corner" near the elbow of Cape Cod and proceed south and west towards Nantucket and Martha's Vineyard. Drifters suggest that cells in these transport pathways could reach RI, LI, and even NJ (Anderson et al., 2005a). These pathways will also be a subject of investigation in GOMTOX.

Vertical flux of cysts. An on-going 2004-06 field program (Pilskaln, Anderson, Keafer, co-PIs) is mapping *A. fundyense* cysts in the bottom sediments and benthic nepheloid layer (BNL) of the major basins of the northern GoM. Of particular relevance to GOMTOX are the time series data of *A. fundyense* cyst flux near Penobscot Bay and Jordan Basin. Time series sediment traps were deployed at these sites in April 2005. Preliminary results show extremely elevated particle resuspension fluxes on the order of 10 g m^{-2} day^{-1}. The April-Oct. samples from above and within the BNL at the 2 sites are dominated by copepod fecal pellets. A previous 1995-1997 sediment trap deployment in Jordan Basin documented delivery of 10^4 cysts to the trap over a 2-week period, and revealed particle fluxes rich in zooplankton fecal pellets (Pilskaln et al., 1998; Pilskaln, unpubl.).

Modeling and forecast capabilities for *A. fundyense* blooms and toxicity. Prior modeling work in ECOHAB-GoM utilized a hierarchy of hydrodynamic and physical-biological models. Early studies focused on the spatial disconnect between inshore *A. fundyense* blooms and offshore cyst beds (McGillicuddy et al., 2003; Hetland et al., 2002). Models were used to elucidate a mechanism for offshore initiation of blooms through the joint effects of organism behavior and the wind-driven response of a buoyant plume of fresh water originating from rivers. During upwelling-favorable winds, the plume thins and extends offshore; downwelling winds thicken the plume and confine it to the nearshore region. In the western GoM, the offshore extent of the plume during upwelling is sufficient to entrain upward-swimming *A. fundyense* cells germinated from offshore cyst beds.

Bloom dynamics are of course strongly affected by growth and mortality. Stock et al. (2005) describe a detailed population dynamics model and its evaluation with a maximum likelihood approach using observations from the western GoM in 1993. The baseline biological model, which parameterizes growth as a function of only temperature, salinity, and light, severely over-estimates observed *A. fundyense* abundance and is thus rejected with > 99% confidence in favor of models that include mortality or nutrient dependence. The model solutions suggest that germination from the Penobscot and Casco Bay seedbed provides the majority of cells inoculating spring *A. fundyense* populations in the WMCC. Input of EMCC cells becomes increasingly important later in the spring.

As observations from ECOHAB-GoM became available, larger aspects of the bloom became apparent: distributions of cells are gulf-wide in geographic scope, the distributions are associated

with the MCC, and the center of mass of the distribution shifts from west to east during the bloom season (Townsend et al., 2001). This latter aspect is notable since the MCC flows in the opposite direction (Fig. 1). A model that includes germination, growth, mortality, and nutrient limitation yields simulations that are qualitatively consistent with the observations (McGillicuddy et al., 2005).

Moving beyond climatology into the realm of hindcasting past bloom events or forecasting future events requires advanced data assimilation techniques. Recent results from our MERHAB and COHH projects illustrate the utility of data-assimilative models for both at-sea forecasting and post-cruise hindcasting (see http://www.whoi.edu/science/AOPE/people/olga/mm_main_page.html and http://www.whoi.edu/science/cohh/whcohh/projects/habs2_abstract.html). For example, He et al. (2005) describe the application of a data-assimilative model to an 11-day MERHAB survey in early summer 2003. The hindcast system consists of both forward and inverse models used to assimilate both coastal sea level and ADCP currents via inversion for unknown sea level open boundary conditions (Lynch and Naimie, 2002). Model skill was evaluated by the divergence of observed and modeled drifter trajectories. The mean divergence rate between actual and modeled drifters was 1.78 km day^{-1}, demonstrating the utility of this approach for predicting transport of materials in the region. This same system was used in real time at sea during the extraordinary *A. fundyense* bloom in 2005 (Anderson et al., 2005a), facilitating adaptive sampling of the bloom as it evolved.

To summarize, we understand and are able to model some key aspects of *A. fundyense* bloom dynamics in the ECOHAB-GoM domain (Fig. 1) – i.e., we are making progress towards efficient monitoring, modeling, and forecasting of bloom and PSP events for nearshore portions of the Gulf. Considerable work remains, however, to expand this domain to include all regions of the GoM and adjacent shelf waters subject to PSP toxicity. Moreover, we face a major challenge to quantify the causes of the extraordinary bloom of 2005, and to understand and forecast the potential implications of a new regime of *A. fundyense* and PSP dynamics in the coming years.

3. RESEARCH PLAN

The GoM and its adjacent southern New England shelf is a vast region with extensive and diverse shellfish resources, large portions of which have the potential to be contaminated with PSTs. There are significant challenges to the management of shellfish in this region – in particular the need to document the major transport pathways for *A. fundyense* and to develop an understanding of the relationship between blooms in surface waters and environmental forcings, as well as linkages to toxicity patterns in nearshore and offshore shellfish. An additional challenge is the need to expand modeling and forecasting capabilities to include the entire region, and to transition these tools to operational, management use through the incorporation of new capabilities such as data assimilation and a submodel that accounts for uptake and loss of toxin by shellfish. Here we propose GOMTOX – a regional observation and modeling program focused on the southern GoM and its adjacent New England shelf waters. **The overall objective of GOMTOX is to establish a comprehensive regional-scale understanding of *A. fundyense* dynamics, transport pathways, and associated shellfish toxicity and to use this information and relevant technologies to assist managers, regulators, and industry to fully exploit nearshore and offshore shellfish resources threatened by PSP, with appropriate safeguards for human health**. This program is consistent with ECOHAB priorities, in particular the need to *"take an ecosystem approach in determining the linkages between HAB species and their environment"*, and *"to contribute to the development of methods for the ... monitoring, prediction, ... and mitigation of HABs and their impacts"*. The proposed work falls under both "Bloom Ecology and Dynamics" and "Food Webs and Fisheries" themes. Furthermore, GOMTOX places significant emphasis on modeling and has a high level of stakeholder involvement. The team includes managers, regulators, and industry representatives who

helped to design the study and will assist in sample collection and analysis, provide guidance at PI meetings, and ultimately work with the tools and information developed here to expand and manage a multi-million dollar fishery currently inactivated by the threat of PSTs.

GOMTOX is a five-year program with multiple subprojects, outlined in Fig. 5 (p. 20). Large-scale field surveys of *A. fundyense* abundance and distribution will be conducted in Years 1,2, and 4, emphasizing not only the surface manifestation of blooms, but their vertical structure and the transport of toxins to bottom waters. Autonomous gliders will extend the range of these surveys and provide high-resolution detail as well. Satellite-tracked drifters will be deployed and, along with satellite imagery, will be used to track blooms, water masses, and validate model simulations. Time-series sediment traps and samplers will be deployed in Years 1-4 at a key location where time series of shellfish toxicity and vertical cell and toxin distribution will also be obtained. Shellfish toxin time-series will also be obtained at three other offshore beds. GOMTOX will benefit from NMFS regional surfclam sampling in Years 2 and 5 and scallop sampling in Years 1-4. These collections will be supplemented by targeted sampling by collaborating fishermen in Years 1-4. A regional cyst-mapping survey will take place in Year 3. All moorings will be recovered in Year 5, leaving the remainder of that year for synthesis activities, workshops, and outreach and transitioning efforts.

A focal point of the field program will be the Stellwagen Bank Marine Sanctuary (SBMS), a relatively shallow area with surfclam, ocean quahog, and sea scallop populations that are on the pathway that *A. fundyense* cells travel from the WMCC region towards the Great South Channel (Fig. 1; Anderson et al., 2005a). SBMS staff are partners in GOMTOX and will collect shellfish using their vessel and staff (including divers), and will issue permits as needed (Haskell support letter).

PSTs are the toxins of concern to this program and the region (Anderson, 1997), but domoic acid (DA) is a potential problem (e.g., Martin et al., 1990), even though no New England states currently monitor for that toxin in shellfish. Since there is a possibility of DA toxicity in offshore shellfish, we will therefore analyze for this toxin in a subset of our offshore shellfish samples, with additional analyses run if justified. In a similar manner, water samples will be preserved for *Pseudo-nitzschia* enumeration, with most being archived. It will simply not be possible for GOMTOX to address bloom dynamics and toxin transfer for both *A. fundyense* and *Pseudo-nitzschia* spp. It is an unfortunate reality that GOMTOX cannot address all potential HABs in the region without dropping program elements that are critical to the PSP problem – an established threat.

OBJECTIVE 1: Investigate *A. fundyense* bloom dynamics and the pathways that link this organism to toxicity in nearshore and offshore shellfish

Rationale: The ECOHAB-GoM program investigated the nearshore waters of the northern GoM but did not include areas to the south and west that contain significant nearshore and offshore shellfish subject to PSP toxicity (Fig. 1). Vast areas of offshore shellfish grounds have been closed since 1990 and industry as well as federal and state managers are requesting information and tools to re-open these areas and manage them effectively.

Subtask 1.1: Conduct region-wide survey cruises for *A. fundyense*

The peak of the western GoM bloom is during May/early June. As noted above, 8 fishermen nearly died in May 1990 due to PSP in mussels from Georges Bank, so a May/June sampling period is appropriate for that region. Therefore, two broad-scale survey cruises (15 days each) are proposed for May & June in Years 1, 2, and 4. In Year 1, we will leverage each of 2 previously scheduled cruises funded by another program by adding 5 days of ship time to each. The cruise plan (Fig. 2) is designed to meet three objectives: (1) survey transport pathways and key branch points of the MCC leading from the BoF; (2) sample the critical offshore excursion of the EMCC towards Georges Bank (the "short-circuit" pathway); and (3) sample the offshore waters of Martha's Vineyard and Nantucket,

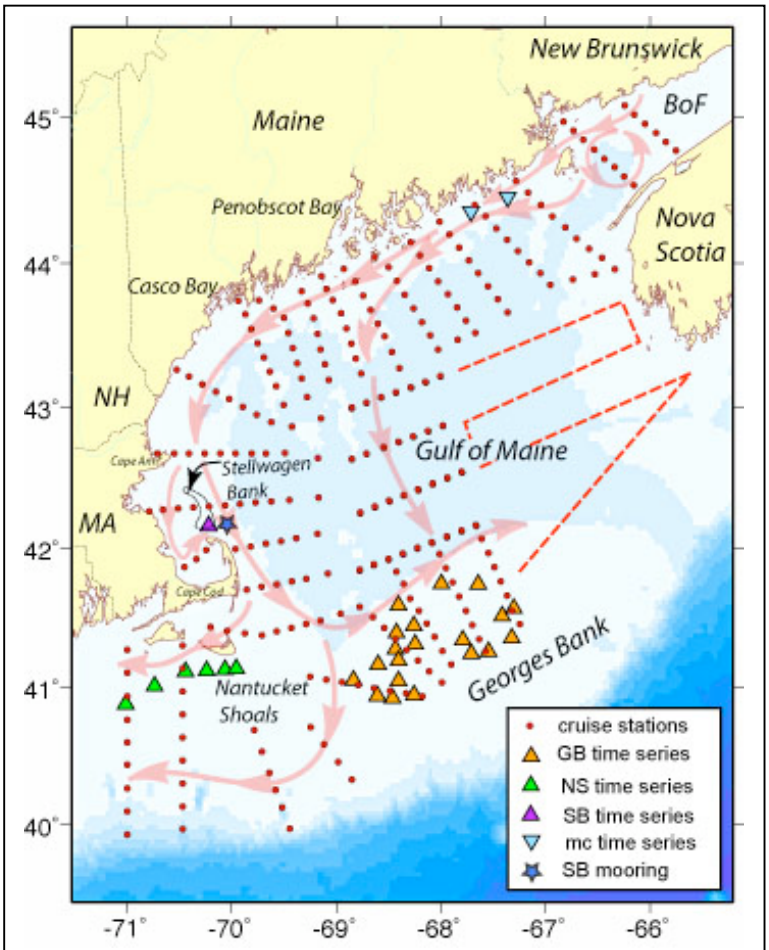


Figure 2. Map showing: stations to be sampled in the large-scale plankton and hydrography surveys, Slocum glider tracks (dashed line), shellfish toxicity time series stations, and sediment and particle filtration trap mooring. Major *Alexandrium* transport pathways are shown with red arrows. Note that dozens of state shellfish monitoring stations are located on the coast from Maine to Massachusetts. GB – Georges Bank; NS – Nantucket Shoals; SB – Stellwagen Bank; mc – mahogany clam.

the southern extent of the 2005 bloom. The domain can be covered with 250 CTD stations (~17 /day). Transects are generally perpendicular to the flow and extend across shellfish sampling sites on Georges Bank (Subtask 2.3). Stations may be relocated at sea based on *A. fundyense* counts and satellite imagery. At each station, T, S, fluorescence and transmittance will be acquired by a CTD/rosette package. Water samples for *A. fundyense,* nutrients, and chlorophyll *a* will be collected in 10-liter Niskin bottles from 1, 10, 20, 30, 40 and 50m. The 40 and 50m samples will be archived for analysis if needed. Additional nutrient samples will be collected at 100, 150, and 200m depths where possible. 20 ml subsamples for nutrients will be filtered though HA filters and immediately frozen for later analysis using a Bran Luebbe Autoanlyzer III, for NO_3+NO_2, NH_4, PO_4 & $Si(OH)_4$.

The surveys will be significantly extended in the eastern GoM (Fig. 2) by data from two Slocum gliders equipped with CTD, fluorescence, and dissolved O_2 sensors. These gliders, owned by the University of Maine and deployed at no cost to GOMTOX, will significantly augment the physical characterization of the GoM and provide important boundary information at the inflow region. (Fig. 1).

Several methods will be used for *A. fundyense* cell enumeration, including sandwich hybridization assay (SHA) and whole cell (WC) microscope counts, (both of which use molecular probes for detection; Anderson et al., 2005b), and "live" on-board light microscope counts. Of these, the SHA is our method of choice as it is accurate, and provides high sample throughput and the capability for near real-time measurements at sea (Anderson et al., 2005b). In the lab, archived SHA samples and a subset of WC samples will be analyzed to confirm SHA results from the field. Sample collection, processing, and analysis protocols for SHA and WC are described in Anderson et al. (2005b). 10L from a replicate 1m Niskin bottle will be sieved for a quick "live" count to validate SHA results. In addition, 500ml of water from each depth will be filtered (5µm) without sieving to capture *Pseudo-nitzschia* and other species. These will be archived and analyzed as needed.

Subtask 1.2: Conduct high-resolution vertical profiles of *A. fundyense* vegetative cells, nutrients, and hydrography in key areas

During the large-scale surveys, high resolution (1m) vertical profiles will be obtained at selected stations within major subdomains and near key MCC branch points. We will relocate on stations that indicate the presence of significant *A. fundyense* populations on the basis of "live" counts at 1m and SHA counts from 1-30m. Profiles will be obtained for *A. fundyense* abundance, phytoplankton chlorophyll *a*, and inorganic nutrients using a submersible pumping system (Townsend et al., 2005). Briefly, the hose intake is attached to the CTD/rosette system, lowered to 50 m, and brought back to the surface at 1-m intervals for sample collection, allowing for hose clearance between depths. If station depth is > 50m, the profile will continue with reduced resolution to near bottom. This technique has revealed subsurface layers of high densities of *Alexandrium* sp. in the GoM (Townsend et al., 2005). To examine the development and seasonal nature of these layers, the hi-resolution profiles will also be performed on Stellwagen Bank to provide a biweekly time series and to complement the size-fractionation profiling (Subtask 2.2).

Subtask 1.3: Release drifters to document transport pathways

Drifters will be built under contract by students at Southern Maine Community College. Surface and drogued units are standard CODE and WOCE designs, respectively. Traditional electronics are replaced by a low-cost GPS transmitter that communicates with GLOBALSTAR low-orbiting satellites and tracks are posted on a public website daily. Three pairs of surface and drogued units will be deployed across shelf with each pair at significantly different depths. Two transect lines per year will be positioned upstream of important branch points. Exact dropsites will be decided based on real-time satellite imagery preceding the ship's arrival. Drifters will also be deployed in *A. fundyense* patches, detected using live counts or on-board SHA.

Subtask 1.4: Use remote sensing data on sea surface temperature and ocean color to assist interpretation of survey cruise and toxin time series data

Satellite imagery will be used to optimize sampling and to better define station locations and delineate oceanographic features prior to drifter deployments and high-resolution profiling. Imagery time series will assist interpretation of hydrographic and *A. fundyense* observations, using both sea surface temperature and ocean color data as appropriate. Data from NOAA and MODIS satellites are received and processed daily at the U. Maine laboratory of PI Thomas using the most recent NOAA and NASA protocols. As in previous ECOHAB/MERHAB operations, data will be downloaded to NMFS in Woods Hole to generate specific products and help in adaptive sampling. Customized images, for example, will be sent to the ship with proposed sampling stations overlaid relative to frontal locations. Objectives of this subtask are to: a) ensure that relevant oceanographic features are adequately sampled in real time; b) interpret the results of water sampling in relation to the proximity to sea surface fronts; and c) assist interpretation of field data by providing enlarged in both time and space, of overall patterns of surface temperature and ocean color.

OBJECTIVE 2: Investigate the vertical structure of *A. fundyense* blooms, vertical toxin flux, and linkage to toxicity in offshore shellfish

Rationale: During the 2005 *A. fundyense* bloom, officials responsible for managing offshore shellfish resources asked what we know about the linkage between blooms in surface waters and toxicity in deep-water shellfish. We answered that we could tell them nothing, as this phenomenon

has not yet been studied. We do not know: 1) the form of the toxin ingested by surfclams, sea scallops, and ocean quahogs; 2) the relationship between cell abundance in the overlying waters and the supply of toxin to depth; 3) whether toxin is supplied episodically or continuously through cysts and materials that accumulate on surface sediments. Furthermore, we have no numerical models that can adequately address this multi-dimensional process of toxin delivery, accumulation, and depuration. Here we describe a program for characterization of planktonic materials using long time series and single-point collections. The material will be analyzed using methods that allow us to characterize the vertical distribution of toxins down to the sea bed, the size categories that contain those toxins, and, where possible, the nature and toxicity of the particle flux. This information can be related to time series measurements of shellfish toxicity at the same locations, which in turn can be related to the distribution and abundance of *A. fundyense* cells in the region.

The relative importance of various toxin inputs to deep-water shellfish remains unknown. Although bivalves can ingest cysts (Shumway et al., 1987), the possibility that PSTs can be accumulated from them remains speculative due to their resistant cell walls. Since sea scallops ingest particulates up to at least 350 μm, we hypothesize that off-bottom resuspension and/or the vertical flux of sinking vegetative *A. fundyense* cells, organic debris, disaggregated marine snow, and fecal pellets could contribute to the toxin source available to deep-water shellfish. Resuspended material of this type may contribute to shellfish toxicities in nearshore waters during the off-bloom season.

We therefore propose to identify the main seasonal toxin sources available to deep-water bivalves and to obtain new detoxification rate estimates for natural shellfish populations since previous field estimates were based on analysis of shellfish tissues and may be confounded by the continuous input of toxin. This will be accomplished by determining the relationship between toxicity in tissues of surfclams, ocean quahogs, and sea scallops and: a) the toxicity and concentration of vegetative cells in near-surface waters during blooms, b) the flux of toxic particulates in traps, c) total particulate toxin concentration near-bottom (within the nepheloid layer, ≤ 50 cm off-bottom, and d) toxin concentration of cysts near-bottom.

Subtask 2.1: Measurement of vertical fluxes of cells, cysts, fecal pellets and other materials

The time varying vertical and resuspension fluxes of cells, cysts, and particles will be measured at two sites in the SBMS. Vertical flux measurements will be from relatively shallow locations where we expect such delivery to be high based on shellfish toxicity (S. Etheridge, unpub. data) and *A. fundyense* cell transport (Anderson et al., 2005a). Our plan is to: 1) place small tube traps on the bottom at a ~30 m deep station at the southern end of the SMBS (Fig. 2) to collect sinking and resuspended particles at a location where a time series of plankton toxin size-fractionation profiles and shellfish toxicity will be obtained (Subtasks 2.2 and 2.3); 2) place a McLane time series filtered particle/plankton sampler (WTS) on the bottom at the same site to obtain ~daily filtered samples; and 3) deploy high resolution, time series sediment traps and a WTS on a mooring in 80-100 m of water at the SE corner of the SMBS (Fig. 2) immediately adjacent to the shallow time series site to provide inter- and intra-annual toxin data plus cell, cyst, and total particle flux data. We will work closely with SBMS staff to obtain permits and insure the safety of all instrumentation.

At the shellfish toxicity timeseries site on Stellwagen Bank, divers will deploy a set of acrylic tube traps. These will be 8 MultiPIT traps; 7.5 cm x 40 cm tubes with a small baffle insert in the collection end, all mounted to a Plexiglas cross bar frame (Knauer et al., 1979). The trap frame and tubes will be set into interlocked and weighted milk crates placed on the seabed and secured with earth anchors (Butman, 1989). Half the tubes will be pre-poisoned with a density-adjusted, 4% buffered formalin solution and the remainder of the tubes will be filled with a dense 5M acetic acid solution (all covered with biodegradable clear wrap). Similar traps have been used in shallow

systems and on shellfish beds to quantify larvae and particulate delivery over 1-2 wk periods (Butman, 1989; Newell et al., 2005). The tube traps will be deployed and recovered biweekly by SMBS divers from May-Aug, and monthly from mid Aug to May, in conjunction with shellfish sampling (Subtask 2.3). The WTS will be serviced monthly from mid Aug through May and will filter 2 L of water every 1.25 days to collect 24 samples on 20µm Nitex, retaining the >20µm *A. fundyense* fraction. The WTS filter reservoir will be filled with 5% formalin in seawater to preserve filtered material. Particulate material will be analyzed for cysts and vegetative cells following routine protocols (Anderson et al., 2003; 2005b). Supplementary T, S, and current flow data will be obtained with sensors available to the project at no cost and attached directly to the frame of the WTS.

In the SE corner of the SBMS in 80-100 m of water and as close as possible to the tube trap and shellfish time-series site on the bank (Fig. 2), paired, high-resolution, time series sediment traps will be deployed at 2 depths on a bottom-mounted, subsurface mooring. The 13 cup, cone traps (McLane, Inc; 0.25 m^2 collection area) will be placed ~15 m above the bottom and 50 m below the surface. A paired trap configuration will facilitate the collection of: 1) time varying toxin data (toxicity and composition) using 1 set of trap cups pre-filled with a 5M, density-adjusted (40-45PSU) aqueous acetic acid solution, and 2) a coincident time series of preserved sinking particulates (fecal pellets, etc.) using a set of trap cups pre-poisoned with a density-adjusted, 4% buffered formalin solution (Knauer et al., 1984; Lee et al., 1992). PSTs readily diffuse from particulates into the acetic acid solution, are stable in it, and the solution can be concentrated by freeze drying without significantly altering toxin composition (Hall et al., 1980; Hall, 1982). Adjustment of trap cup solutions to 5-10PSU excess density significantly reduces diffusion of dissolved components out of the cup while it is open (JGOFS, 1996). Each 250 ml cup is sealed from the water column after rotating out of the open position. Cup rotation will be 1-3 wks depending on season. Net toxicity and toxin composition in the acetic acid-treated trap cups will be determined in Subtask 2.3. We anticipate no problem measuring toxicity in our collected material using highly sensitive methods. The formalin-preserved trap samples will be analyzed microscopically for quantification of fecal pellets and algal aggregates, and for identification and counts of major phytoplankters and zooplankton. POC content and flux will be determined using a coulometric carbon analyzer. A split of this material will be sonicated and sieved for *A. fundyense* cyst enumeration from which toxin content can be estimated. A CTD/transmissometer placed on one of the McLane trap frames will provide a hydrographic and suspended particle time series for correlation with the time varying particle flux cell and cyst delivery data. Traps will be deployed in April of Year 1 and serviced every 6 mo for 3.5 years. A second mooring at the site will consist of a lighted surface buoy and a WTS sampler placed at ~5 m to collect cells and cysts from near-surface waters. The mooring-mounted WTS will be serviced on the same 6-mo schedule as the moored sediment traps. Filtered samples will be analyzed for *A. fundyense* cell abundance as described above.

Subtask 2.2: Conduct size fractionation studies of plankton material

We will obtain a time series of vertical profiles of size-fractionated levels of PSTs, phytoplankton and zooplankton community composition, and fecal pellets at the tube trap and shellfish toxicity station in the SBMS (Fig. 2). Profiles will be obtained at biweekly intervals from May - mid August, and during large-scale cruises at stations chosen for their relevance to the *A. fundyense* bloom and to the location of shellfish resources. Methodology for the size-fractionated plankton and toxin measurements derives from previous studies (Turner et al., 2000; 2005; Doucette et al., 2005), improved by sampling with quantitative pumping at all depths. This will allow not only characterization of % plankton community composition comprised by various taxa in each size fraction, but also their enumeration as either cells l^{-1} or animals m^{-3}. It will also be possible to

measure toxin per volume of water sampled, in addition to toxin per unit of wet weight. Toxin content of microscopically-sorted fecal pellets will also be determined.

As detailed in Doucette et al. (2005) and Turner et al. (2005), known volumes of water (typically several m^3) will be pumped from specific depths (e.g., surface, 6m, 12m, 18m, and near-bottom at the 30-m deep SBMS site, and surface, 15m, 30m, 45m and near-bottom at deeper sites) using the pumping system described in Subtask 1.2. Water will be pumped into a 20 μm-mesh plankton net, and the collected material divided into subsamples that are screened through 5 size fractions (20-64, 64-100, 100-200, 200-500, and >500 μm). These fractions will be further subdivided into samples fixed in 0.05 N acetic acid for HPLC and receptor binding assay (RBA) for PSP toxin composition and content, respectively. Subsamples will also be preserved in 8% Utermöhl's solution for microscope counts and community composition analyses of dinoflagellates and microplankton (20-64, 64-100 μm samples), and in 5% formalin for dissecting microscope counts and community composition analyses of zooplankton and fecal pellets (100-200, 200-500, > 500 μm samples). Fecal pellets and fecal pellet contents, including *A. fundyense* cells, will be examined using a scanning electron microscope methodology (Turner, 1984, and references therein).

Based on results of Doucette et al. (2005) and Turner et al. (2005), size fractions will be dominated by different taxa and particulates. The 20-64 μm samples will contain *A. fundyense* cells (30-40 μm diameter), other large dinoflagellates, tintinnids and aloricate ciliates, and small fecal pellets. The 64-100 μm samples will contain similar components, but also copepod nauplii and rotifers. The 100-200 μm samples will contain copepod nauplii and copepodites of small copepods and larger fecal pellets. The 200-500 μm samples will contain progressively larger copepodites and adults of small copepods. The >500 μm samples will contain primarily adults of large copepod taxa, that are the likely producers of the larger fecal pellets that will reach the sediment traps.

PSTs were found in all size fractions during previous studies (Doucette et al., 2005). Highest levels were found in the > 500 μm samples (Turner et al., 2000; 2005), dominated by large copepods which feed upon *A. fundyense* in the GoM (Campbell et al., 2005; Turner and Borkman, 2005) and may produce PST-containing fecal pellets that transport toxins to offshore shellfish.

Subtask 2.3. Collect offshore shellfish and analyze for toxicity

Surfclams have a high capacity for PST accumulation (Bricelj and Shumway, 1998), are characterized by slow detoxification rates, and have an unusually high capacity for toxin biotransformation, rapidly converting low potency C toxins to more potent decarbamoyl gonyautoxins (dcGTXs), and, at a slower rate, gonyautoxins to dcGTXs (Bricelj et al., 1996). This capacity for toxin conversion provides a useful tool to hindcast the timing of new PST inputs in the form of *Alexandrium* cells, if their toxin composition is known. Biotransformation by surfclams is of public health significance as it can yield higher toxicity in shellfish compared to the toxin source. The only field study on toxin composition changes in surfclams was that of Cembella and Shumway, (1995), but the toxin source was unknown. Data linking surfclam toxin profiles to those of *Alexandrium* cells during blooms are required to test the value of toxin composition data as a management tool. This is important for offshore populations where phytoplankton sampling is limited and shellfish may provide the most reliable information on bloom timing and duration.

Very limited information is available on PSP toxin kinetics in ocean quahogs (Bricelj and Shumway, 1998). Furthermore, reports on the toxin profile or evidence of toxin biotransformation for this species are lacking and will be generated by this project.

The sea scallop, *P. magellanicus*, is another species capable of high PST accumulation, with relatively slow detoxification (Bricelj and Shumway, 1998). Sea scallops have a limited capacity for biotransformation of PSTs (Cembella et al., 1993). Scallop adductor muscle does not accumulate

significant PST or DA (Douglas et al., 1997), but there is strong interest in the development of high-value markets for roe-on and whole scallops, which can pose a health risk because PST and occasionally DA can exceed the action level in scallop roe. An understanding of anatomical toxin compartmentalization is essential for increased exploitation of the offshore scallop resource.

The scallop gonad undergoes seasonal changes in biomass associated with gametogenesis and spawning. Therefore seasonal reproductive cycles and toxin transfer between the viscera and gonad during gametogenesis will greatly affect both the absolute toxin concentration and the contribution of total toxin by this organ (toxicity × biomass). As with surfclams, there is interest in developing predictive relationships from toxicities of tissue pools. So far these have shown little success (Cembella et al., 1993), but limited consideration has been given to the confounding effects of individual body size and season in establishing predictive relationships.

This subtask has several objectives. The first is to determine temporal changes in surfclam, ocean quahog, and sea scallop PST content and composition and their relationship with *A. fundyense* abundance and toxin composition. This will allow determination of toxin uptake and loss rates in natural populations, and mapping of broad-scale PST distribution in the region. DA distribution in shellfish will be obtained as a secondary objective. Using this information, the second objective is to validate the use of toxin composition profiles in surfclam tissues as a tool to identify (hindcast) the sources and timing of toxin inputs to these populations. We hypothesize that the presence of C toxins in surfclam viscera, a lower ratio of dcSTX/STX, and a greater contribution of viscera than other tissues to total toxin burden will be indicative of recent exposure to *A. fundyense*. The third objective is to determine temporal patterns in the anatomical compartmentalization of PSTs in sea scallops, in relation to the gonadal cycle and the timing of blooms.

There will be 5 components to shellfish collection efforts, focused on stations shown in Fig. 2: 1) NMFS regional survey cruises, 2) a time-series at 6 stations south of Nantucket, 3) a time-series at one station in the SBMS, 4) a time-series of ocean quahog toxicity from eastern ME; 4) low-frequency time series surveys on Georges Bank; and 5) yearly survey cruises for sea scallops and triennial (Years 2, 5) cruises for surfclams will be conducted by the NMFS (see Brown letter). These will provide extensive spatial coverage of the southern GoM and the adjacent southern New England shelf, including Georges Bank. The Nantucket time series will be conducted at the same 6 stations monitored during the 2005 PSP federal waters closure. This time-series will continue throughout Years 1-4 of GOMTOX. Samples will be collected by industry weekly in late spring and summer and monthly thereafter (Wallace letter). Quahogs from offshore waters of eastern ME (Fig. 2) will be collected during monitoring operations by the state (Couture letter). Another time series will be conducted in the southern area of the SBMS in Years 1-4, in conjunction with our study of vertical fluxes of toxin sources (Subtask 2.1). Shellfish collection by SBMS staff will occur biweekly May-Aug, and monthly thereafter. Lastly, survey cruises will be employed using the industry to collect shellfish from Georges Bank (primarily Cultivator Shoal and Little Georges) at 24 stations representing major surfclam and ocean quahog resources (Fig. 2). Two yearly cruises are anticipated in each of Years 1-4, one in early spring and one in the fall. This multifaceted sampling program has the necessary spatial and temporal coverage to address our objectives.

The industry will collect and prepare shellfish for the time-series at stations south of Nantucket as well as on Georges Bank. SBMS staff and/or FDA personnel will collect and prepare samples collected from the SBMS. Samples on NMFS cruises will be collected by FDA personnel on board. Sampling will follow FDA protocols and those followed by industry during monitoring of the 2005 federal closure. Depending on the objective, each shellfish sample will consist of either 1) individuals (selected tissues) or 2) ~12 pooled individuals. Selected samples will be separated into tissue pools: 1) surfclams will be separated into viscera and other tissues (found to be the most

appropriate for modeling of toxin kinetics in this species (Silvert et al., 1998), and 2) sea scallops will be divided into gonad, viscera, and other tissues (gonado-somatic index, the ratio of gonad wet weight to total wet weight, will also be determined to relate gonad toxicity to reproductive condition). Immediately after harvesting, shellfish will be shucked, the liquid drained and discarded, and tissues separated. Samples will be stored frozen and then packed on dry ice and sent overnight to FDA for testing.

Shellfish tissues will be weighed, homogenized, and extracted with cold 0.1 M acetic acid to maintain the toxin profile. Lyophilized extracts will be resuspended in 5mM HCl, a process through which the original toxin profile will be maintained (Hall, 1982). Aliquots of selected homogenized shellfish will also be extracted (Quilliam et al., 1995) and analyzed for DA using HPLC-UV-detection (AOAC, 2000). The RBA will be the primary method for determining net toxicity. Selected samples will also be assayed on shipboard and in the lab using the Jellet Biotek MIST^{TM} Alert Test Kits to validate their use as a pre-screening method to be included in the dockside testing program. PST profiles will be determined for selected samples using HPLC-FD (Oshima, 1995), with some samples analyzed additionally by LC-MS (Negri et al., 2003) for confirmation. FDA toxin standards will be cross-validated with certified toxins from IMB/NRC's CRMP program.

OBJECTIVE # 3: Assess interannual to interdecadal variability in *A. fundyense* abundance and PSP toxicity

Rationale: The extraordinary bloom of 2005 (Anderson, 2005a) raises the question of whether we are on the cusp of a regime change in *A. fundyense* and PSP dynamics similar to the one that followed the 1972 bloom. We propose to examine two hypotheses concerning the mechanisms of interannual to interdecadal variability. The first deals with fluctuations in cyst abundance as a primary control on the source of the blooms. The second deals with long term North Atlantic Oscillation (NAO)-driven changes in hydrography and nutrient stoichiometry that influence competition between dinoflagellates and diatoms. These hypotheses will be evaluated with historical and proposed observations, working closely with shellfish toxicity records provided by ME and MA.

<u>Subtask 3.1:</u> Document cyst seedbed fluctuations and their impact on source populations

In the ECOHAB-GoM program, we considered the cyst seedbed offshore of Casco and Penobscot Bays to be a secondary one, downstream of the major source at the mouth of the BoF (Fig. 3, left). However, a repeat cyst survey in the fall, 2004 revealed that the total number of cysts in the survey region was nearly an order of magnitude higher than in 1997, with much of that change in western Maine (D. M. Anderson, unpub. data; Fig. 3, right). We don't know when this change occurred between the 1997 and 2004 mappings, but it is of note that the high abundance of cysts in the western GoM preceded the massive 2005 *A. fundyense* bloom, leading Anderson et al. (2005a) to hypothesize a linkage between increased cyst abundance and the anomalous bloom. Those authors also suggested that toxicity in the southern GoM could be altered for years to come, as occurred after the 1972 bloom. If 2005 does indeed constitute the beginning of a new regime of *A. fundyense* and PSP dynamics in southern waters, we would expect *A. fundyense* cyst and motile cell abundance and PSP toxicity to be high in the coming years, with a decline thereafter.

It is therefore an especially opportune time to be making additional measurements of *A. fundyense* in the region. In addition to the plankton and hydrographic surveys in Years 1, 2 and 4, a large-scale cyst survey cruise will be conducted in the fall of Year 3. Eleven days of ship time are needed to sample 125 stations (shown in, Fig. 3, right). Cores will be collected, processed, and cysts enumerated as in Anderson et al., 2005c. The cyst abundance map from Year 3 will be compared to maps from 1997, 2004 and 2005, as well as to maps of the vegetative cells observed and modeled in

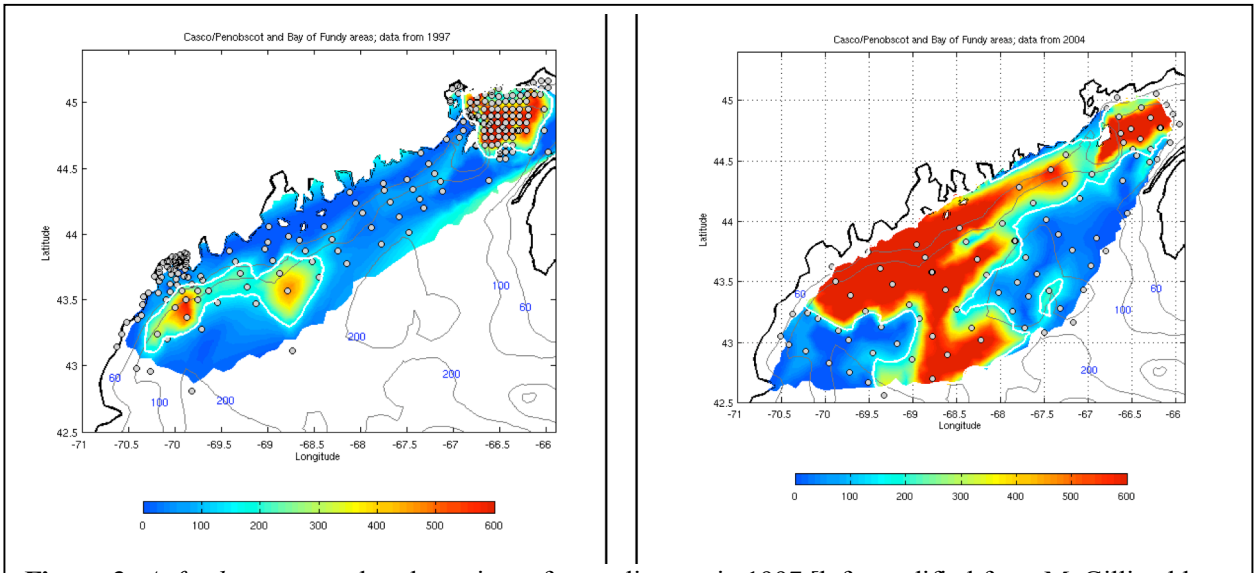


Figure 3: *A. fundyense* cyst abundance in surface sediments in 1997 [left, modified from McGillicuddy et al. (2003)] and 2004 [right, D. M. Anderson, unpublished].

the overlying waters during ECOHAB-GoM and GOMTOX survey years. Cyst maps are a critical element of modeling efforts since each map provides the initial conditions from which the bloom starts each spring. A large-scale, decade-long dataset of cyst and motile cell abundance is unique in HAB science, and would be highly informative, given the importance of cyst stages in many HAB events.

<u>Subtask 3.2:</u> Examine the relationship between hydrography, nutrients, and the North Atlantic Oscillation on growth conditions

One possible explanation for the apparent increased frequency and severity of PSP events in the GoM since the early 1970s may be a change in the hydrographic regime in the Gulf that is linked with the NAO. Prior to the 1970s, the preponderance of NAO Lows resulted in a dominance of Labrador Slope Water influxes to the GoM, a water mass that is cold, fresh and relatively low in nutrients, especially nitrate. Since the early 1970s a predominance of NAO Highs has resulted in influxes of warm Slope Water, which is saltier, warmer, and higher in nitrate and nitrate:silicate ratios (Townsend et al., 2006). Should Townsend et al. (2005) be correct in their assertion that there is a competitive interaction between diatoms and dinoflagellates such as *A. fundyense*, then a shift to predominantly warm Slope Water fluxes into the Gulf would favor *A. fundyense,* as occurred in the 1970s. Our series of survey cruises, combined with historical analyses of shellfish toxicity data, will help to explore this apparent relationship between multidecadal shifts in water mass types in the GOM and variability in suitable growth conditions for *A. fundyense* blooms.

OBJECTIVE 4: Incorporate field observations into a suite of numerical models for hindcasting and forecasting applications

Rationale: Modeling activities are integrated into each element of the proposed research (see Fig. 5, section 5). The diverse spatial and temporal scales of interest, together with the interdisciplinary nature of the problem, necessitate a suite of numerical models. A limited-area data assimilative model run in real time while at sea will yield detailed hydrodynamic fields to be used in siting drifter releases and high-resolution profiles during the large-scale cruises (Subtask 4.1). Large-scale, low

frequency hydrodynamic context for interpretation of cruise data will be provided by a nested regional-scale model (Subtask 4.2). Population dynamics of *A. fundyense* will be coupled to the nested hydrodynamic model to examine seasonal to interannual variability in bloom dynamics (Subtask 4.3). Finally, a toxicity submodel will provide specific predictions of PSTs in shellfish that can be tested with observations (Subtask 4.4). All of these elements are needed to achieve our ultimate goal – a regional modeling capability that can be used in operational HAB management.

Subtask 4.1: Support adaptive sampling activities during large-scale survey cruises with sea data-assimilative nowcasts and forecasts

Although the grid of stations for the large-scale cruises (Fig. 2) is mostly fixed, two important aspects of the sampling program require flexibility: drifter releases at key points in the flow (Subtask 1.3) and high resolution profiling to be carried out across the frontal boundaries of the EMCC (Subtask 1.2). Real-time nowcasts and forecasts of the hydrodynamic environment will be essential to optimal deployment of these efforts. We will utilize the Dartmouth Numerical Methods Laboratory finite element model for this purpose, as we have done successfully in recent MERHAB and COHH cruises (He et al., 2005; Anderson et al., 2005a). Lynch and Naimie (2002) provide the most up-to-date description of the hindcast/forecast system. In this application, we will assimilate velocity measurements from GoMOOS moorings and shipboard ADCP, as well as coastal sea level observations from NOS tide gauges. Initial conditions are specified by blending best prior estimates (climatology) with hydrographic measurements from the initial survey with an optimal interpolation algorithm. Other inputs include atmospheric forcing and a prior estimate of the boundary conditions.

The overall system consists of three models: the forward model (Quoddy); the exact inverse of a linearized, frequency-domain version of the forward model (Truxton); and the exact inverse of the linearized time-domain version of the forward model (Casco). Quoddy, driven by the prior estimate of the boundary conditions and other inputs, makes a prediction and computes mismatch with data. Casco and Truxton constitute an approximate inverse of Quoddy. They are arranged in series, with the mismatch de-tided first by Truxton, and Casco operating on the remaining wind-band mismatch. The two adjustments to the boundary conditions - tidal from Truxton and subtidal from Casco - are merged with the previous boundary condition estimate to produce an improved estimate. This drives another Quoddy prediction, and the process is repeated until either the mismatch or the change in the mismatch from one iteration to the next is within observational error.

Subtask 4.2: Construct seasonal hydrodynamic hindcasts for each field year

The impact of large-scale low-frequency forcing will be examined using an existing nested Regional Ocean Modeling System (ROMS). ROMS is a state-of-the-art, free-surface primitive equation ocean circulation model (Schepetkin and McWilliams, 2005). Its computation kernel uses high-order time-stepping and temporal averaging to guarantee both exact conservation and constancy preservation for tracers, and to yield accurate separation of barotropic and baroclinic modes for stability in shallow coastal waters. Vertical interpolation in the terrain-following coordinate system is based on conservative parabolic splines.

ROMS has been configured for the northeastern US coastal ocean (He, in prep.), consisting of a shelf-scale ROMS and a high-resolution GoM ROMS (Fig. 4, left). The shelf-scale model (3-10 km resolution), encompassing region from Cape Hatteras to Nova Scotia, is embedded within the existing data assimilative North Atlantic Hybrid Coordinate Ocean Model [HyCOM] (Chassignet et al., 2003) via one-way nesting. The method of characteristics (Browning and Kreiss, 1982) is used for barotropic open boundary conditions on velocity and pressure. Boundary relaxation with e-folding times of 0.1 to 10 days (outer to inner grid) are used to relax baroclinic mode temperature,

salinity, pressure and velocity components to the corresponding HYCOM fields. Tidal forcing is imposed with a data-assimilative tidal model (Egbert and Erofeeva, 2002). He (in prep.) demonstrates that the shelf-scale ROMS faithfully reproduces both mean circulation features and synoptic variability when compared with *in situ* observations, lending confidence that it can be used to provide initial and open boundary conditions for the nested GoM ROMS.

The GoM ROMS is embedded within the shelf-scale ROMS with either one- or two-way nesting schemes, and covers the coastal regions from Nantucket Shoals to the BoF at ~1-km resolution. Fresh water input from major rivers is provided by USGS stream gauge data. Satellite scatterometer (QuikScat) winds and NCEP reanalysis are used to specify surface momentum and buoyancy forcing. An example snapshot from a hindcast of 2005 (Fig. 4, right) illustrates how downwelling-favorable wind during a northeaster storm drove strong onshore currents, transporting freshwater and *A. fundyense* cells into Massachusetts Bay (Anderson et al., 2005a).

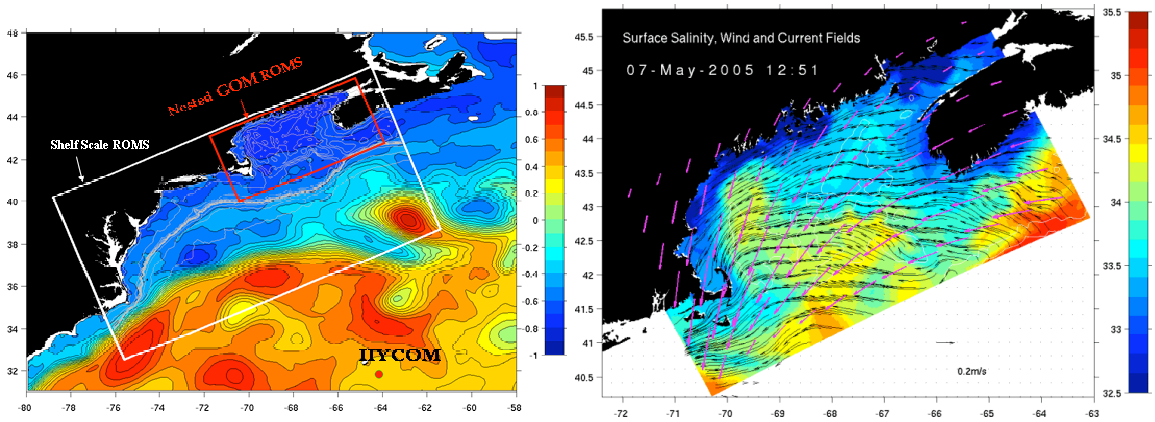


Figure 4. Left panel: the nested model configuration. Shown are sea surface elevation (in color) modeled by the North Atlantic HyCOM, the coastal bathymetry and the nested shelf-wide ROMS domain, and the further-nested high-resolution GoM ROMS domain. Right panel: an example snapshot from the nested high-resolution GOM ROMS domain hindcast for May 7, 2005. Surface salinity, currents, and wind fields are indicated by color, black arrows and red arrows, respectively. Figure from *He (i prep)*.

<u>Subtask 4.3:</u> Construct seasonal *A. fundyense* hindcasts for each field year

The high-resolution GoM ROMS is an ideal framework for a coupled physical-biological model of *A. fundyense* dynamics. Fundamental to this approach is the concept that the ecosystem in which *A. fundyense* resides is not explicitly modeled, since with few exceptions, *A. fundyense* constitutes a very small fraction of the phytoplankton species assemblage in the GoM. As such, it influences neither the ambient nutrient fields nor predator distributions. Therefore, ecosystem effects are parameterized through their influence on the vital rates of *A. fundyense*'s dynamic processes.

Formulation of the population dynamics model follows Stock et al. (2005) and McGillicuddy et al. (2005). Input of cells into the water column results from germination of cysts from the cyst maps, modulated by our functional fit to laboratory data on an endogenous clock that regulates germination (Anderson and Keafer, 1987). The specific rate of germination is further modulated by light and temperature, based on laboratory measurements. The function describing vegetative growth is from Watras et al. (1982). The overall growth rate is based on this function and is modulated by the ambient light field as per Liebig's law of the minimum (Liebig, 1845). Nutrient limitation is represented by the Michaelis-Menten formulation. An upward swimming rate of 10 m day^{-1} and a net "mortality" of 0.1 per day are specified. Mortality is in quotes here because it represents the net effect of all loss processes, which include predation and encystment. Term-by-term diagnosis of the model solutions will facilitate quantification of the effects of germination, growth/grazing dynamics,

behavior, and transport in controlling the observed distributions of vegetative cells.

Subtask 4.4: Compute shellfish toxicity from *A. fundyense* hindcasts and evaluate with observations

Guided by the work of M. Bricelj and others (Blanco et al., 1997; Silvert et al., 1998), we have formulated a toxicity submodel to predict PSP levels in shellfish based on the simulated concentration of *A. fundyense* and first-order uptake and depuration kinetics. The equation governing the concentration of saxitoxin equivalents [*STX*] within a shellfish exposed to *A. fundyense* concentration [*Alex*] of cell toxicity *t* is:

$$\frac{d[STX]}{dt} = \alpha[Alex]t - k_1[STX]$$

The value of *k* was chosen from experimental detoxification values for *M. edulis*, ranging from 0.05-0.15 day^{-1} (Bricelj and Shumway, 1998). Choosing α =1.0 makes the toxicity prediction relative; evaluation of this parameter using available data will facilitate quantitative predictions. Animated results of the toxicity submodel applied to a hindcast of the western GoM in 1993 is available at http://science.whoi.edu/users/mcgillic/ecohab/movies/toxicity_submodel/tox93_2.avi. The existing toxicity submodel for *M. edulis* will need to be adapted for offshore shellfish such as surfclams and sea scallops. The first order uptake and depuration model will be used as a first approximation, but the parameters of the model will have to be adjusted to reflect differing kinetics amongst the various species. M. Bricelj will provide available laboratory data on surf clams for this purpose. In the event that the first-order model is not sufficient, we will experiment with two-component models that treat viscera and other tissues separately. These more sophisticated models can include the effects of water temperature (Bricelj et al., 1998) and animal size on the kinetics of toxin processing.

Clearly there are multiple pathways for intoxication of deep-water shellfish in offshore areas, including sinking flux of PST-containing material in particulates other than intact *A. fundyense* cells. We will formulate a submodel for the vertical flux of toxin to offshore shellfish beds, based on near-surface vegetative cell concentrations, grazing pressure, and particulate sinking rates. Data provided by Turner and Pilskaln in Objective 2 will be especially useful for calibration of the vertical toxin flux submodel. In turn, the toxicity submodel for offshore shellfish beds will include uptake of toxin-bearing particulates. Taken together, these two submodels will be used to evaluate pathways of intoxication in the target offshore shellfish populations.

OBJECTIVE 5: Synthesize results from this study and those that preceded it and disseminate the information and technology widely, emphasizing the need to transition scientific and management tools to the management community for operational use

Rationale: At the completion of this project, considerable information will be available to support robust conceptual and numerical models of *A. fundyense* bloom dynamics and the patterns of shellfish toxicity throughout the study area. We foresee the need to synthesize program data in the form of a special journal issue, and to hold several workshops and public presentations that transfer knowledge and concepts to managers and the general public. An additional activity will explore the steps needed to develop an operational bloom detection and forecasting system.

Subtask 5.1: Convene PI progress and synthesis meetings

PI meetings will be held twice each year that will include all active investigators, other scientists who have been funded separately to work on HABs within the region, as well as members of industry, management, and regulatory communities. The strong working relationships that already

exist between GOMTOX scientists and resource managers and industry officials will be strengthened through frequent communication, workshops, and outreach activities. Indeed, since many of the activities proposed here have been motivated by the needs of managers and industry, it is a natural process to continue to seek their input and feedback throughout the program.

Subtask 5.2: Publish a special journal issue on the program

Papers will be published throughout the program, but we also plan for a coordinated effort during Year 5 that will culminate in a special journal issue on the program. It is important that the PIs have time to synthesize and write without new cruise or data-gathering activities.

Subtask 5.3: Hold regional workshop to transfer information and technologies

In Year 5, a meeting will be convened with PIs, managers, and stakeholders to describe the findings, tools and technologies of the program. Discussion will focus on needs for additional management tools, and the steps to transition GOMTOX products to management. Participants will develop a plan for the immediate and long-term future, working towards a regional observing and modeling capability that would complement ongoing state PSP monitoring. By Year 5, viable surfclam, ocean quahog, and scallop roe-on fisheries should be well on their way to reality, but if not, then impediments to progress will be discussed, and recommendations formulated on the information needs and policy actions to overcome them. A similar transitioning process is underway for ECOHAB-GoM, so GOMTOX can benefit from the existing network of stakeholders.

4. EXPECTED RESULTS, BENEFITS, OUTPUTS AND OUTCOMES

At its completion, this program and its predecessor will have produced a comprehensive understanding of the dynamics and forcing mechanisms underlying *A. fundyense* blooms and the associated toxicity of nearshore and offshore shellfish across a vast and highly complex region. Important hydrographic pathways and branch points will have been identified, and key features and processes characterized. Conceptual models will have been formulated to explain blooms and toxicity throughout the region, and sophisticated numerical models developed and tested that simulate physical, chemical, and biological processes at a detailed level over the entire region. Combined with state of the art efforts to utilize data assimilation methodology, we will have made major advances towards the goal of operational bloom forecasting in the region. Yet another outcome will be the development and transfer of knowledge that can be used by the shellfish industry and federal and state officials to create multi-million dollar offshore surfclam, ocean quahog and roe-on and whole-scallop fisheries where none exist at present.

5. GENERAL PROJECT INFORMATION

Interaction with other programs. Considerable cost savings accrue to GOMTOX through its cooperation with other programs, agencies and industry. The project will share two major cruises with projects funded through the Woods Hole Center for Oceans and Human Health, a savings of ~$400,000. Four scallop and two surfclam survey cruises conducted by NMFS vessels will provide critical regional offshore samples, with significant cost savings. Likewise, our fishing industry partners will collect shellfish on twice-yearly trips to Georges Bank and Nantucket Shoals, at either no cost or a reduced-rate charter. Cooperation with the SBMS will further reduce project costs through shared use of their vessel and diving assistance. More cost savings accrue from federal scientists from the US and Canada who will participate actively in GOMTOX but will not require salary support (three PI's from the FDA, one from the NMFS, one from SBMS, and one from the Canadian DFO). Additional savings will be from the thousand or more toxin analyses to be run by

FDA scientists for only the cost of supplies and an intern. It is difficult to estimate the total value of all of these savings, but it clearly amounts to several million dollars over the life of the project. Without this in-kind support, GOMTOX would be neither practical nor economically viable.

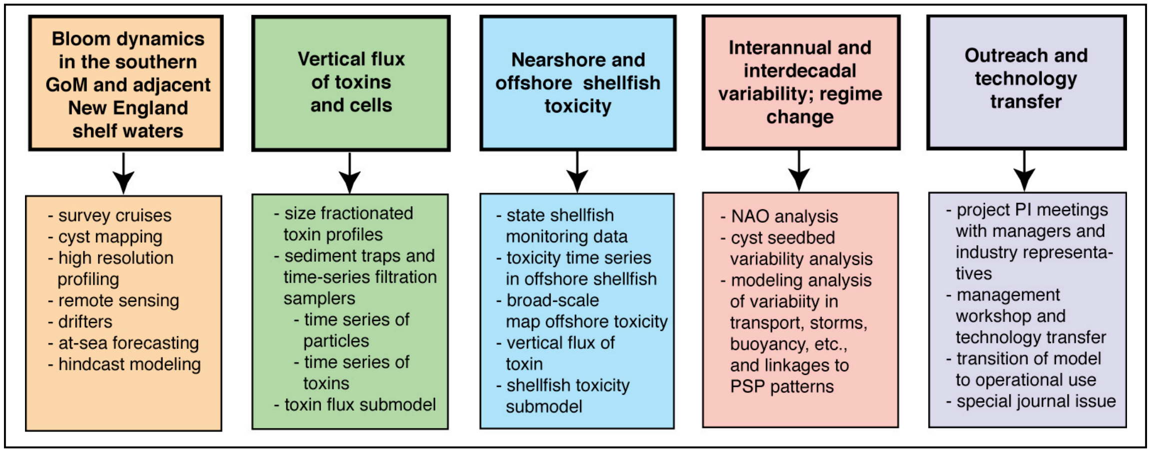
GOMTOX will utilize the PSP data of the Maine Department of Marine Resources, the New Hampshire Department of Environmental Services, and the Massachusetts Division of Marine Fisheries. These agencies in turn will benefit significantly from our survey and shellfish toxicity data, which complements their measurements on shore. A similar relationship exists with the Canadian Department of Fisheries and Oceans through one of our PIs, J. Martin (support letter). We will interact with a project on ECOHAB remote sensing and shellfish toxicity time series study (PIs Thomas and Xue), and a project documenting cyst deposition and resuspension fluxes in the northern GoM (PIs Pilskaln and Anderson). GoMOOS moorings and data products will be utilized throughout, facilitated by co-PI Pettigrew, who directs the observation aspects of that program. Although 2006-2007 funding for GoMOOS fell short of the level required to maintain the full system, revenue from other sources is helping to fund the gap. Funds are secured to maintain buoys A, B, C, E, I, and N through summer 2007. We believe that all shelf and basin buoys will be funded prior to the GOMTOX field operations, and anticipate that these buoys will be maintained throughout the program.

Facilities. All of the facilities and expertise needed to accomplish the stated objectives are available to the GOMTOX team, or will be purchased with grant funds. This includes sophisticated microscope and analytical chemistry facilities for toxin, particulate, and plankton analyses, mooring hardware, shipboard instruments, sampling bottles, CTDs, ADCPs, access to data streams of various types, molecular probes and laboratory equipment for WC and SHA cell counts, nutrient analysis, autonomous underwater vehicles, drifters, numerical models, and so forth.

Project management and responsibilities. Overall project coordination will be by D.M. Anderson, with assistance from a steering committee to be selected from GOMTOX PIs. As with the ECOHAB-GoM program, small teams will coordinate activities and communication in particular subject areas (e.g., an "Offshore Shellfish" team, and a "Modeling Team"). A restricted listserver will be established, and a WWW homepage set up as a site where investigators can place and access data. This will function as a central node for GOMTOX communication. State-of-the-art visualization techniques are a hallmark of several of our PIs, and these approaches will be used for all project elements.

The following are the primary program activities of each PI: **Don Anderson:** General program management, budgeting, and oversight, *A. fundyense* bloom dynamics, direct involvement in numerous subtasks and project elements; **Monica Bricelj:** shellfish biology, toxin dynamics in shellfish tissues, toxin submodel parameterization; **Jonathan Deeds:** toxin analysis; **Stacey Etheridge:** offshore shellfish collection and toxin analysis**; Sherwood Hall:** regulatory aspects of offshore shellfish toxicity**; Ben Haskell:** coordinate SBMS activities; **Ruoying He:** ROMS model formulation and development**; Bruce Keafer:** cruise logistics, *A. fundyense* bloom dynamics, cyst surveys, WTS traps; **Jim Manning:** drifter activities, and will assist with remote sensing effort; real-time processing/serving of data streams for modeling activities; **Jennifer Martin:** *A. fundyense* and PSP toxicity data from the BoF; **Dennis McGillicuddy:** supervision of all modeling activities, data assimilation, and toxin submodel development; **Neal Pettigrew:** large-scale hydrographic CTD surveys and deployment of Slocum gliders, geostrophic velocity fields, integration of time-series data from GoMOOS buoys with survey data and modeling activities, assist with high resolution vertical profiling program; **Cindy Pilskaln:** responsible for all mooring activities and oversight of sediment traps, analysis of all sediment trap samples and data production, and mooring-based current meter and CTD/transmissometer data delivery; **Andy Thomas:** remote sensing data collection and analysis**; Dave Townsend:** high resolution profiling , nutrient analysis, NAO analysis; **Jeff Turner:** size-fractionated plankton and toxin profiles, fecal pellet analysis, general grazing issues.

Figure 5. Research framework and timeline for the GOMTOX program. In the box diagram below, the general categories of research are broken out under broad themes that incorporate all GOMTOX objectives.



Bloom dynamics in the southern GoM and adjacent New England shelf waters
- survey cruises
- cyst mapping
- high resolution profiling
- remote sensing
- drifters
- at-sea forecasting
- hindcast modeling
Vertical flux of toxins and cells
- size fractionated toxin profiles
- sediment traps and time-series filtration samplers
- time series of particles
- time series of toxins
- toxin flux submodel
Nearshore and offshore shellfish toxicity
- state shellfish monitoring data
- toxicity time series in offshore shellfish
- broad-scale map offshore toxicity
- vertical flux of toxin
- shellfish toxicity submodel
Interannual and interdecadal variability; regime change
- NAO analysis
- cyst seedbed variability analysis
- modeling analysis of variabiity in transport, storms, buoyancy, etc., and linkages to PSP patterns
Outreach and technology transfer
- project PI meetings with managers and industry representa-tives
- management workshop and technology transfer
- transition of model to operational use
- special journal issue

Project Timeline / Project Year:	Year 1											
Activity / Calendar Year:	2006				2007							
Month:	S	O	N	D	J	F	M	A	M	J	J	A
Bloom dynamics in the southern GoM												
Large-scale survey cruises									X	X		
High resolution profiles									X	X	X	X
Drifter release and tracking									X	X	X	X
Remote sensing observations	X		X		X		X		X		X	
Vertical flux of toxins and cells												
Sediment and filtration trap deployment								X	X	X	X	X
Vertical profiles of size-fractionated toxin									X	X	X	X
Toxin time series in shellfish	X	X	X	X	X	X	X	X	X	X	X	X
Broad-scale toxin mapping in offshore shellfish		X		X		X		X		X		X
Interannual/interdecadal variability												
Cyst mapping survey												
Analysis of blooms, cysts & toxicity					X	X	X					
NAO analysis						X	X					
Modeling analysis												
At-sea forecasting								X	X	X	X	
Seasonal hydrodynamic hindcasts											X	X
Seasonal *A. fundyense* hindcasts												
Toxicity submodel												
Outreach and Communication												
PI meetings		X						X				
Management workshop & tech. transfer												
Transition of model to operational use												
Special journal issue												

Project Timeline / Project Year:	Year 2											
Activity / Calendar Year:	2007				2008							
Month:	S	O	N	D	J	F	M	A	M	J	J	A
Bloom dynamics in the southern GoM												
Large-scale survey cruises									X	X		
High resolution profiles									X	X	X	X
Drifter release and tracking									X	X	X	X
Remote sensing observations	X		X		X		X		X		X	
Vertical flux of toxins and cells												
Sediment and filtration trap deployment	X	X	X	X	X	X	X	X	X	X	X	X
Vertical profiles of size-fractionated toxin									X	X	X	X
Toxin time series in shellfish	X	X	X	X	X	X	X	X	X	X	X	X
Broad-scale toxin mapping in offshore shellfish		X		X		X		X		X		X
Interannual/interdecadal variability												
Cyst mapping survey												
Analysis of blooms, cysts & toxicity					X	X	X					
NAO analysis						X	X					
Modeling analysis												
At-sea forecasting								X	X	X	X	
Seasonal hydrodynamic hindcasts	X	X	X	X							X	X
Seasonal *A. fundyense* hindcasts	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity submodel			X	X	X	X	X	X	X	X	X	X
Outreach and Communication												
PI meetings		X						X				
Management workshop & tech. transfer												
Transition of model to operational use												
Special journal issue												

Project Timeline / Project Year:	Year 3											
Activity / Calendar Year:	2008				2009							
Month:	S	O	N	D	J	F	M	A	M	J	J	A
Bloom dynamics in the southern GoM												
Large-scale survey cruises												
High resolution profiles									X	X	X	X
Drifter release and tracking												
Remote sensing observations	X		X		X		X		X		X	
Vertical flux of toxins and cells												
Sediment and filtration trap deployment	X	X	X	X	X	X	X	X	X	X	X	X
Vertical profiles of size-fractionated toxin									X	X	X	X
Toxin time series in shellfish	X	X	X	X	X	X	X	X	X	X	X	X
Broad-scale toxin mapping in offshore shellfish		X		X		X		X		X		X
Interannual/interdecadal variability												
Cyst mapping survey		X										
Analysis of blooms, cysts & toxicity					X	X	X					
NAO analysis						X	X					
Modeling analysis												
At-sea forecasting												
Seasonal hydrodynamic hindcasts	X	X	X	X								
Seasonal *A. fundyense* hindcasts	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity submodel	X	X	X	X	X	X	X	X	X	X	X	X
Outreach and Communication												
PI meetings		X						X				
Management workshop & tech. transfer												
Transition of model to operational use												
Special journal issue												

Project Timeline / Project Year:	Year 4											
Activity / Calendar Year:	2009				2010							
Month:	S	O	N	D	J	F	M	A	M	J	J	A
Bloom dynamics in the southern GoM												
Large-scale survey cruises									X	X		
High resolution profiles									X	X	X	X
Drifter release and tracking									X	X	X	X
Remote sensing observations	X		X		X		X		X		X	
Vertical flux of toxins and cells												
Sediment and filtration trap deployment	X	X	X	X	X	X	X	X	X	X	X	X
Vertical profiles of size-fractionated toxin									X	X	X	X
Toxin time series in shellfish	X	X	X	X	X	X	X	X	X	X	X	X
Broad-scale toxin mapping in offshore shellfish		X		X		X		X		X		X
Interannual/interdecadal variability												
Cyst mapping survey												
Analysis of blooms, cysts & toxicity					X	X	X					
NAO analysis						X	X					
Modeling analysis												
At-sea forecasting								X	X	X	X	
Seasonal hydrodynamic hindcasts											X	X
Seasonal *A. fundyense* hindcasts	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity submodel	X	X	X	X	X	X	X	X	X	X	X	X
Outreach and Communication												
PI meetings		X						X				
Management workshop & tech. transfer												
Transition of model to operational use												
Special journal issue	X		X		X		X		X		X	

Project Timeline / Project Year:	Year 5											
Activity / Calendar Year:	2010				2011							
Month:	S	O	N	D	J	F	M	A	M	J	J	A
Bloom dynamics in the southern GoM												
Large-scale survey cruises												
High resolution profiles												
Drifter release and tracking												
Remote sensing observations												
Vertical flux of toxins and cells												
Sediment and filtration trap deployment	X	X										
Vertical profiles of size-fractionated toxin												
Toxin time series in shellfish												
Broad-scale toxin mapping in offshore shellfish												
Interannual/interdecadal variability												
Cyst mapping survey												
Analysis of blooms, cysts & toxicity					X	X	X					
NAO analysis						X	X					
Modeling analysis												
At-sea forecasting												
Seasonal hydrodynamic hindcasts	X	X	X	X								
Seasonal *A. fundyense* hindcasts	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity submodel	X	X	X	X	X	X	X	X	X	X	X	X
Outreach and Communication												
PI meetings		X						X				
Management workshop & tech. transfer			X							X	X	X
Transition of model to operational use										X	X	X
Special journal issue	X	X	X	X	X	X	X	X	X	X	X	X

Note: Alternating X's indicate a sustained, though intermittent, activity.

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