ABSTRACTS OF ORAL PRESENTATIONS

ECOHAB – GULF OF MAINE SESSION

ECOHAB-GOM: THE ECOLOGY AND OCEANOGRAPHY OF TOXIC *ALEXANDRIUM* BLOOMS IN THE GULF OF MAINE

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A five-year program called ECOHAB-GOM was initiated to address several fundamental issues regarding *Alexandrium* blooms in the Gulf of Maine: 1) the source of the *Alexandrium* cells that appear in the fresh water plumes in the western Maine coastal current (WMCC); 2) *Alexandrium* cell distribution and dynamics in the eastern Maine coastal current (EMCC); and 3) linkages among blooms in the WMCC and the EMCC. Utilizing a combination of numerical modeling, hydrographic, chemical, and biological measurements, moored and drifting current measurements, and satellite imagery, the project will characterize the structure, variability and autecology of the major *Alexandrium* habitats in the Gulf of Maine.

In the western Gulf, *Alexandrium* blooms and patterns of PSP have been linked to a coastal current or plume of low salinity river outflow (the WMCC). One major project goal is to investigate an area near Casco Bay implicated as the major "source region" for the toxic cells that populate that coastal current. Field surveys will elucidate the biological, chemical, and physical processes that control bloom initiation and development, the delivery of cells from that source region into the WMCC, and the manner in which late-season, localized blooms are retained there to re-seed future blooms with cysts. The second major set of objectives is to characterize the linkage between toxic blooms and the EMCC, to investigate the role of tidal mixing, frontal systems, and upwelling / downwelling in *Alexandrium* dynamics, and to define the linkage between EMCC *Alexandrium* populations and those in both the WMCC (downstream) and the Bay of Fundy (upstream). A series of "process" studies will focus on discrete blooms or patches of cells and quantify such parameters as in situ growth rates and grazing losses of *Alexandrium*, the nutritional physiology, vertical migration behavior and transport of this species, and the partitioning of toxins within the food web. A hierarchy of coupled physical-biological models are being used together with ECOHAB-GOM data for investigation of: 1) detailed structure within each habitat; 2) interconnections among habitats; and 3) the role of the larger Gulf-scale circulation in the long-term maintenance of *Alexandrium* populations in the region. ECOHAB-GOM is thus a combined modeling/observational program, utilizing the most current and innovative technologies in an approach commensurate with the multiple scales and oceanographic complexity of PSP phenomena in the Gulf of Maine. More details on this project, including an update of cruise activities and results can be found at the project web page at: http://crusty.er.usgs.gov/ecohab/

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ALEXANDRIUM CYST DYNAMICS IN THE GULF OF MAINE

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Alexandrium fundyense, the dinoflagellate responsible for Paralytic Shellfish Poisoning (PSP) in the Gulf of Maine (GOM), has a dormant cyst stage in its life history that plays a critical role in bloom dynamics. Given the highly seasonal nature of PSP outbreaks in the region, the cold winters, and the temperature requirements for *A. fundyense* growth (5-21 ˚C), blooms of this species presumably begin from germinated cysts. Surveys of cyst abundance in GOM sediments document high cyst concentrations in deeper, offshore basins, grading to much lower concentrations in shallow waters. It was hypothesized that the most significant input of *A. fundyense* cells would be from cysts in shallow waters that would be exposed to high light levels and more rapid temperature increases than the cysts in the darker and colder offshore waters, even though cyst concentrations are 1-2 orders of magnitude higher offshore. During the ECOHAB-GOM project, several parallel efforts were made to estimate the magnitude and timing of cell input via cyst germination. These efforts, and their present status, are summarized as follows:

1) Cyst germination traps: Several benthic chambers designed to capture germinated cells were collected. Thus far, none of these have provided reliable data.

2) Cyst fluorescence: Surface sediments were collected at a number of stations and *A. fundyense* cysts examined for signs of chlorophyll fluorescence, thought to be a sign of impending germination. Data thus far show no meaningful patterns or trends.

3) Modeled cyst germination: Laboratory experiments were conducted to characterize *A. fundyense* cyst germination rates as functions of temperature, light and time of year (the latter reflecting control of germination by an internal clock). In conjunction with a large-scale cyst map of the region, these data are being used in model simulations driven by environmental and hydrographic conditions observed and modeled in 1993. Results thus far provide a cell inoculum to the water column that varies temporally and spatially. In particular, it appears that the deep water cyst seedbed dominates the inoculum process, but that seedbed is not in the proper location to account for observed blooms in the Casco Bay area. An eastward extension of this seedbed, lying outside the modeled domain could be the source of *A. fundyense* cells to the Casco Bay region. Model simulations have suggested a mechanism whereby upwelling-favorable winds move nearshore waters over the deep-water cyst seedbeds, capturing germinated cells that are then carried to shore when winds shift to downwelling-favorable. Details of this modeling effort will be provided by McGillicuddy et al. in a separate presentation. Armstrong et al. and Thompson et al. present posters with details of similar cyst germination rate experiments for eastern Gulf of Maine populations which will allow an extension of the modeling effort.

These observations on cyst distribution and dynamics will be discussed in the context of observed *Alexandrium* motile cell distribution in 1998 and 2000.

*This work began in collaboration with the late Dr. Maureen D. Keller.

ALEXANDRIUM SP. BLOOMS IN THE WESTERN GULF OF MAINE

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The first outbreaks of PSP in the Gulf of Maine are reported nearly every year along the Western Maine coastline, especially in the Casco Bay region. Previous research has shown that this region is strongly influenced by the Kennebec River, one of the major freshwater inputs into the Western Gulf of Maine, and that surface populations of *Alexandrium* are generally associated with the river plume. Throughout the spring of 1998 and 2000, intensive sampling efforts were mounted in Casco Bay and the adjacent Kennebec River plume area to observe current and water properties during the onset of the *Alexandrium* bloom and subsequent shellfish toxicity. Moorings were deployed at several sites and weekly hydrographic surveys were conducted from April through June. The hydrographic survey data included vertical profiles of temperature, salinity, PAR, fluorescence and water velocity. Further information on the current field was obtained from satellite-tracked drifters, deployed during most surveys. Water samples were collected at the surface and at various depths down to 20m to determine *Alexandrium* abundance in the water column. In addition, mussels were deployed at the mooring sites and analyzed weekly to complement data from the ME shellfish monitoring program.

Early in the field season (i.e. late April) prior to shellfish toxicity, *Alexandrium* cells were observed in inshore waters within Casco Bay as well as in offshore waters beyond the influence of the Kennebec River plume. During both years, shellfish toxicity was recorded at the inshore monitoring stations and at the mussel moorings during mid-May following downwelling favorable conditions. At those times, the surface concentration of toxic cells was quite patchy and ranged from <100 to > 1000 cells $liter^{-1}$ with no evidence that the higher concentrations were associated with any particular water mass. Populations were observed within the less saline (<30psu) waters of Casco Bay inshore and the less-saline waters of the Kennebec plume offshore. In the "upstream" waters, higher populations were consistently observed in intermediate salinity waters associated with the Penobscot River plume as well as in higher salinity waters (>32psu) further offshore. A 24 hour study within the offshore Kennebec plume waters suggested that *Alexandrium* cells did not vertically migrate since the cells predominantly occupied the stratified, surface waters even during the night. When a subsurface population was observed below the pycnocline, it was linked to advection from the upstream coastal areas. Occasionally, high abundances of cells were found near a frontal boundary where the offshore waters converge with the Kennebec River Plume (a feature also found near the Penobscot plume). This convergence is most apparent during downwelling favorable conditions where a coastal "jet" drives the cells rapidly alongshore with the Western Maine Coastal Current. Populations within Casco Bay are protected and generally retained in a slower flow and are less likely to be transported down the coast with the coastal plume leading to the higher toxicities observed there. These results indicate a rather wide distribution of *Alexandrium* in this highly dynamic region where growth and accumulation contribute to higher concentrations sufficient to cause high shellfish toxicity. The study is ongoing with another field year remaining to eludiate the dominant processes responsible for initiation and development of *Alexandrium* blooms in the region.

MODELING *ALEXANDRIUM* SPP. BLOOMS IN THE GULF OF MAINE

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Convolution of coastal circulation with the population dynamics of *Alexandrium* spp. creates enormously complex patterns in the abundance of this organism. Herein we attempt to diagnose the physical--biological interactions that control *Alexandrium* blooms using coupled models together with observations from ECOHAB-GOM and prior studies. One of the main gaps in knowledge identified in earlier work was the lack of information about the source function for the input of new cells into the water column. Two new data sets relevant to this issue have emerged from early ECOHAB-GOM results: (a) surveys of the cyst distribution, and (b) laboratory work which has documented the functional dependence of germination on environmental parameters such as light and temperature. Use of this information has led to an improvement in our ability to model the observed distributions of *Alexandrium* spp. observed in 1993, as compared with earlier simulations based on a riverine input (Franks and Signell, 1997). In particular, cell concentrations in the vicinity of Casco Bay are much more realistic. Further downstream, the differences are less pronounced. From these simulations, it appears that the non-linear response of the river plume to wind forcing provides a mechanism for cross-isobath transport of *Alexandrium* cells. Under upwelling conditions, the plume thins and extends far offshore where it is inoculated by upward-swimming cells that germinated from the offshore cyst bed. When the winds shift to favor downwelling, the plume thickens and moves onshore, thereby exposing the coast to high concentrations of *Alexandrium*.

Aspects of the large scale ECOHAB-GOM surveys have been examined using finite-element particle tracking simulations based on the climatological circulation fields for the region. The synopticity of the 1998 surveys was evaluated by adjusting the station Positions for advection by the mean flow and then comparing distribution maps with those created using the original station locations. Although some differences are visible, the main features of the observed *Alexandrium* distribution appear to be robust with respect to the mean advective transport. Additional particle tracking simulations examine the potential source regions for the peaks in distribution observed in the interior of the Gulf of Maine. These suggest that Penobscot Bay and its estuaries to its west made little contribution to the observed peaks. Areas surrounding Mt. Desert Island and Grand Manan Island were possible sources of cells, while the Bay of Fundy appears to provide a source to the western Gulf. Inflow from the Scotian Shelf is another potentially important pathway.

Work is underway to test and expand upon the pathways identified by the climatological modeling using time dependent simulations for the 1998 and 2000 field years over the entire Gulf of Maine. Of particular interest is the linkage between *Alexandrium* populations in the eastern and western gulf. Initial simulations show clear transport events that bridge the two regions, and future simulations will test the conditions under which the eastern population might seed the western gulf blooms.

References:

Franks, P.J.S and Signell, R.P, 1997, Coupled Physical-Biological Models for Study of Harmful Algal Blooms, Open-File Report 97-498, U.S. Geological Survey. 21 p.

Thompson, A., 1999. Analysis of 1998 cruise data through finite element particle tracking simulations. WHOI summer student fellow report.

Thompson, A., 2000. Analysis of *Alexandrium* distributions and hydrographic fronts in the Gulf of Maine during the summer of 1998. Honors Thesis, Dartmouth College, Hanover, New Hampshire. 88 pp.

PHYSIOLOGICAL DIAGOSTICS AND BEHAVIOR OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM FUNDYENSE,* IN CASCO BAY, MAINE – EVIDENCE OF NITROGEN LIMITATION

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One challenge in phytoplankton ecology is to measure species-specific physiological responses to changes in environmental conditions. Of particular importance are harmful algal bloom (HAB) species such as the toxic dinoflagellate *Alexandrium fundyense* which typically inhabit coastal regions where they are usually not dominant. Within the Gulf of Maine, environmental factors, specifically nitrogen, are likely to be a controlling factor for *A. fundyense* blooms. Therefore, the ability to ascertain the nutritional status of this species in field assemblages in critical to understanding its bloom dynamics.

Since *A. fundyense* usually inhabits coastal areas that are frequently limited by nitrogen, behavioral adaptations and intracellular responses to nitrogen availability are a primary consideration. It was therefore desirable to identify diagnostic indicators and behavioral adaptations of *A. fundyense* to nitrogen stress. Using laboratory water columns, nitrogen (N)-starved batch cultures, and N-limited, semi-continuous cultures, indicators of different N-nutritional states were identified. It was determined that low N concentrations in the surface of a mesocosm did not induce a Casco Bay *A. fundyense* isolate to vertically migrate to deep nutrient pools. Prolonged N-stress caused dramatic changes intracellular biochemistry, specifically chlorophyll a, carbohydrate, and protein content, as well as C:N, toxin content and composition. Ratios of different toxin derivatives were identified that increased with increasing N-stress and appear to be sensitive and robust indicators of N-status.

Once indicators were developed for N-stress, variability in toxin content and composition were examined in the coastal waters of Casco Bay, Maine during an *A. fundyense* bloom in the spring of 1998. Over the course of the field season, toxin compositional changes occurred that were generally consistent with increasing levels of N-stress as the bloom progressed and N levels decreased. As shown in N-limited culture, large increases in some toxin ratios (e.g., GTX1,4:STX and NEO:STX) were observed during the latter portion of the field season, coinciding with low N:P ratios and undetectable levels of dissolved inorganic nitrogen in ambient waters. Overall, the toxin compositional trends were quite remarkable and suggest that this approach may provide valuable species-specific physiological information without the need for elaborate cell separation schemes such as flow cytometry or immunomagnetic bead sorting. Further laboratory studies are underway to better characterize the toxigenic response of *A. fundyense* isolates to environmental stresses before this suite of toxin indicators can be considered robust.

AN OVERVIEW OF INTERACTIONS BETWEEN ZOOPLANKTON GRAZERS AND *ALEXANDRIUM* SP., AND EFFECTS OF GRAZING ON BLOOM DYNAMICS IN THE NEAR-SHORE ENVIRONMENT OF THE GULF OF MAINE

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Laboratory and field studies were conducted during an ECOHAB targeted individual project, to investigate the mechanisms and biology of zooplankton feeding and potential impacts of feeding during blooms of *Alexandrium fundyense* in the near-shore Gulf of Maine. Laboratory experiments established that copepods are capable of discriminating toxic cells from non-toxic cells, and that non-toxic *Alexandrium* sp. cells are preferred to many similar algal types while toxic cells are frequently avoided. Selective feeding was not however universal, and also appeared to depend on the concentration of toxic *Alexandrium* sp. cells as well as the species composition of the prey field experienced by zooplankton. Selective feeding by zooplankton in *Alexandrium* sp. blooms is possible or likely, but not easily predicted, since dominant grazers, prey species composition, and *Alexandrium* sp. concentration must be known to predict response with any confidence. When zooplankton consume toxic *Alexandrium* sp. cells, some toxins are accumulated, but laboratory experiments demonstrate that retention of toxins in body tissues is very inefficient (<5% of ingested toxin). Zooplankton may act as vectors of toxin to higher trophic levels, but are not efficient vectors (see poster by Teegarden et al.).

The impact of zooplankton grazing on *Alexandrium* sp. was investigated in field studies during the spring of 1998 and 1999 in coastal waters of the Gulf of Maine. Samples were collected at weekly intervals from several stations for zooplankton and phytoplankton abundance, biomass, species composition, and toxin content. Grazing rates of the dominant zooplankton species were determined using natural water samples. Dominant zooplankton included copepods (primarily *Acartia hudsonica* and *Calanus finmarchicus*) and barnacle nauplii (*Semibalanus* sp.). The feeding behavior of the zooplankton on *Alexandrium* sp. was species specific: *Acartia* was non-selective, *Calanus* was somewhat selective, and *Semibalanus* avoided ingesting *Alexandrium* sp. During 1998, there was a moderate bloom of *Alexandrium* with peak concentrations reaching 3000 cells/L in late May at one of the inshore stations. In contrast, in 1999 concentrations were very low throughout the study period. During both years zooplankton biomass was low during the early spring but increased exponentially over the study period. In 1998, grazing impacts increased from 0 to 80% day^{-1} in concert with the increase in zooplankton biomass and appeared to contribute to the bloom's demise. Our findings suggest that grazing can be an important source of mortality and will depend on zooplankton filtration rates, degree of selective feeding, and the biomass and species composition of both the phytoplankton and zooplankton communities (see poster by Campbell et al.).

OFFSHORE BLOOMS OF THE RED TIDE DINOFLAGELLATE, *ALEXANDRIUM* SP., IN THE GULF OF MAINE

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We conducted three large-scale surveys of the coastal and offshore waters of Gulf of Maine during the summer of 1998, and two similar surveys in spring and summer of 2000. Hydrographic data were collected and concentrations of phytoplankton chlorophyll, inorganic nutrients and densities of *Alexandrium* cells were measured in discrete water samples. Moorings, measuring Doppler current profiles and temperature and conductivity at several depths, were deployed within the Eastern Maine Coastal Current (EMCC) system during each of the field seasons. During the 1998 the moored program focused on the alongshelf coherence within the EMCC, while in 2000 the empahsis was on flow structures in the frontal region near Penobscot Bay and the linkages to the Western Maine Coastal Current (WMCC). Daily NOAA AVHRR and NASA SeaWiFS satellite data were downloaded and processed to provide coincident spatial patterns of sea surface temperature and chlorophyll over each cruise period.

Our specific objectives were to:

(1) Investigate the physical oceanography, nutrient chemistry, and abundances and distributions of *Alexandrium* in the coastal and offshore waters of the northern Gulf of Maine and to identify the factors that regulate *Alexandrium* population dynamics.

(2) Determine the linkages between the major Gulf-wide current systems, in particular the Eastern Maine Coastal Current and western Maine coastal waters, and between the Gulf of Maine and the Bay of Fundy with respect to freshwater, nutrients, and *Alexandrium* cells.

(3) Identify environmental factors that result in the initiation of *Alexandrium* blooms in Gulf of Maine waters, and how those blooms are controlled with respect to their spatial and temporal distributions.

Our most complete results, as of this writing, are for the 1998 field season. Both surface and subsurface concentrations of *Alexandrium* displayed maximum cell densities in the offshore waters of the Gulf on all cruises. Highest cell densities in surface waters (ca. 5.5×10^3 cells L^{-1}) were observed in two broad patches: one in the Bay of Fundy and another in shelf and offshore waters of the central and eastern Gulf of Maine in association with the Eastern Maine Coastal Current. Highest subsurface densities of cells appeared to be associated with the frontal edges beyond the cold surface waters associated with the Eastern Maine Coastal Current. As the summer progressed, the highest surface densities of *Alexandrium* receded toward the eastern portions of the Gulf and the Bay of Fundy. Locations of high cell densities were described and interpreted using a non-dimensional light-nutrient parameter, computed as the ratio of the depth of the 10% surface irradiance to the depth of 4 _M NO_3 concentration.

We suggest that the offshore distributions of relatively high densities of *Alexandrium* are naturally-occurring and can be related to seasonal and vertical patterns of inorganic nutrient concentrations/ratios and the ambient light field. Possible mechanisms responsible for periodic development of PSP outbreaks in nearshore shellfish beds are discussed in light of our recent findings, and placed in the context of an historical overview of past observations in the Gulf of Maine region.

ACCUMULATION OF PSP TOXINS IN ZOOPLANKTON ASSEMBLAGES IN THE GULF OF MAINE

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The transfer of algal toxins through marine food webs can affect the health of both humans and wildlife, as well as negatively impact the trophic structure of ecosystems. In the case of PSP toxins produced by dinoflagellates, the most common route of entry into the food web is via direct consumption of toxic algae by filter-feeding bivalves as a component of their natural diet. Another potentially important, although poorly understood, vector for the transfer of these toxins into higher trophic levels is the zooplankton grazer community, which represents the prey of certain marine mammals as well as various planktivorous fish species. Such fish can, in turn, experience reductions in fecundity and recruitment with exposure to PSP toxins, and may also transfer toxins to their predators. PSP toxins contained in mackerel were, in fact, implicated in an unusual mortality event several years ago involving humpback whales. Moreover, a recent study by our group in Massachusetts Bay demonstrated not only the accumulation of PSP toxins in zooplankton, but also the preferential movement of toxin into larger size fractions although these animals did not numerically dominate the grazer assemblage. Some larger zooplankton species comprise a majority of the diet for the North Atlantic right whale and thus pose a potential health threat to these endangered marine mammals.

Given the paucity of detailed information on the role of zooplankton grazers as vectors for PSP toxins, we initiated a study of zooplankton mediated trophic transfer of these toxins as a component of the ECOHAB Gulf of Maine regional program. We will be reporting the results of detailed survey cruises in the Casco Bay region of the Gulf of Maine conducted in 1998, which describe the PSP toxin distribution among several plankton size fractions (20-64, 64-100, 100-200, 200-500, >500 µm) as well as the taxonomic composition of these size classes. As expected, the predominant PSP toxin signal was detected in the 20-64 µm size class containing toxic *Alexandrium* spp., and changed according to fluctuations in the cell concentrations of these dinoflagellates. Nonetheless, toxicity was also associated with all other size classes examined at some point during the study, with the distribution pattern varying markedly among stations within a cruise and also between cruises. Unlike our previous work in Massachusetts Bay, toxin signals were occasionally detected in the 64-100 µm size class. Preliminary analyses indicated that these samples did not contain *Alexandrium* cells, but rather were dominated by tintinnid ciliates as well as larger, non-toxic dinoflagellates. The overall pattern of toxin accumulation in the remaining three size fractions was similar to that observed previously, with toxicity occurring consistently in the 200-500 and/or >500 µm size fractions, which were generally dominated by copepods of the genera *Calanus*, *Centropages*, and *Pseudocalanus* - all potential grazers of *Alexandrium* cells. Although toxicity was not associated with the 100-200 µm fraction as frequently, there were several cases in which this fraction, comprised mostly of copepod nauplii, adults of small copepods (e.g., *Oithona similis*), and rotifers, accumulated as much or more toxin than the larger size classes. More detailed analyses of zooplankton taxonomic composition, *Alexandrium* cellular toxicity, and implications for PSP toxin transfer to higher trophic levels will be discussed.

ABSTRACTS OF POSTER PRESENTATIONS

ECOHAB – GULF OF MAINE SESSION

TEMPERATURE-CONTROLLED GERMINATION OF CYSTS OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM TAMARENSE*, FROM BAY OF FUNDY, CANADA

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The physiology of the eastern Gulf of Maine *Alexandrium* populations is unknown and laboratory work is required to develop an understanding of *Alexandrium* dynamics in this area. To this effect, an experiment was conducted to determine the rates of cyst germination as a function of temperature (2, 4, 6, 8 and 15°C) and light (light vs. dark), following the experimental protocol used previously by Bronzino et al. for cysts of the Casco Bay region. Preliminary observations of the percentage of cyst germination in individual sediment samples kept at 8°C over time show that the germination rate increased exponentially after 12 days. On the other hand, excystment at this temperature under dark conditions shows the same pattern as under light conditions but with lower abundance. Results from the various incubation temperatures will be compared. Germination functions will be developed and compared with those derived for the Casco Bay *Alexandrium* isolates.

This work was started under the leadership of the late Dr. Maureen D. Keller.

VERTICAL DISTRIBUTION OF *ALEXANDRIUM* SP. IN THE WATER COLUMN

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Much research has been done on the species of *Alexandrium* that are the cause of PSP in the Gulf of Maine, trying to establish under what conditions dinoflagellate blooms are likely to occur. Past surveys of *Alexandrium* in the GOM have focussed on the phytoplankton populations in the surface layers. With evidence published by McIntyre et al in 1997 that *Alexandrium* were capable of diel vertical migration under laboratory conditions of nutrient stress, the question of whether this occurs in natural populations arises. My research is an investigation into the vertical distribution of *Alexandrium* in the Gulf of Maine, and how that distribution changes over time.

In June of 2000, two sites in the Gulf of Maine were chosen for 24-hour sampling of the vertical profile. Both sites were well offshore and chosen because of the presence of *Alexandrium* in the net tow and the absence of the dense diatom population that we saw near shore. Water samples were taken at 5-meter intervals between the surface and 55 meters, a total of 12 depths collected each hour for 25 hours. All seawater was collected in 5 L Niskin bottles attached to the CTD rosette. The ship followed a 25 m drogue during each experiment. Hydrographic data was collected and water was drawn from each sample for measurements of chlorophyll, inorganic and organic nutrients and *Alexandrium* counts. Cell counts were done using a stain comprised of an antibody, specific to the genus *Alexandrium*, attached to a fluorescent tag. All counts were done using an epifluorescence microscope.

Results will be presented, showing the depth distribution of *Alexandrium* populations in relation to total chlorophyll, light and nutrients.

THE ROLE OF ZOOPLANKTON GRAZERS IN *ALEXANDRIUM* SP. BLOOM DYNAMICS IN THE NEAR-SHORE ENVIRONMENT OF THE GULF OF MAINE

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The role of zooplankton grazers in *Alexandrium* sp. bloom dynamics was investigated in coastal waters of the Gulf of Maine during the spring of 1998 and 1999. We collected samples at weekly intervals from several stations for zooplankton and phytoplankton abundance, biomass, species composition, and toxin content. In addition, grazing rates of zooplankton were determined using natural water samples. Dominant zooplankton included copepods (primarily *Acartia hudsonica* and *Calanus finmarchicus*) and barnacle nauplii (*Semibalanus* sp.). The feeding behavior of the zooplankton on *Alexandrium* sp. in moderate bloom conditions (≤3000 cells/L) was species specific; *Acartia* was non-selective, *Calanus* was somewhat selective, and *Semibalanus* avoided them. Toxin was measured in zooplankton tissues collected from the field stations and was present for several weeks after the decline of the bloom. Despite our laboratory findings that toxin retention was inefficient (see abstract by Teegarden), it appears that retention is sufficient to pose risks to higher trophic levels. *Acartia* was by far the most abundant zooplankton species at the near-shore stations and its filtration rates on *Alexandrium sp.* was positively related to temperature but independent of ambient chlorophyll concentrations. The filtration rates did not saturate over the range of food concentrations encountered during the study, and consequently, measured grazing impacts were in good agreement with estimates from temperature and biomass measurements alone. During 1998, there was a moderate bloom of *Alexandrium*; peak concentrations reached 3000 cells/L in late May at one of the inshore stations. In contrast, in 1999 concentrations were very low throughout the study period. During both years zooplankton biomass was low during the early spring but increased exponentially over the study period. In 1998, grazing impacts during the peak and subsequent decline of the bloom increased from 25 to 80% day^{-1} in concert with the increase in zooplankton biomass and appeared to contribute to the bloom's demise. Our findings suggest that grazing can be an important source of mortality and will depend on zooplankton filtration rates, degree of selective feeding, and the biomass and species composition of both the phytoplankton and zooplankton communities.

DIFFERENTIAL EFFECTS OF TOXIC *ALEXANDRIUM* SP. ON NORTHERN VERSUS SOUTHERN POPULATIONS OF THE COPEPOD *ACARTIA HUDSONICA*

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The frequency and duration of harmful algal blooms have been increasing worldwide with detrimental effects including fish kills and human illness. Locally, along the east coast of North America, the toxic dinoflagellate *Alexandrium* sp. appears to have spread in its geographical distribution. The consequences of this spreading to grazers are not understood. Our goal is to examine the effects of *Alexandrium* on the copepod *Acartia hudsonica* (=*clausi*) from five different regions along the coast of North America (New Brunswick, Maine, Cape Cod, Connecticut, and New Jersey) which have differences in the frequency and toxicity of *Alexandrium* blooms. We hypothesize that, in the presence of local adaptation, copepod populations from regions which experience regular and highly toxic *Alexandrium* blooms will exhibit enhanced fitness when feeding on toxic *Alexandrium* compared to copepod populations from regions where the blooms rarely occur and are less toxic. To test this hypothesis, we performed laboratory experiments examining the ingestion, egg production and hatching rates of *A. hudsonica* feeding on a toxic strain of *Alexandrium*. For all of the copepod populations, ingestion and egg production rates increased with concentration of *Alexandrium* (ranging from 25-500 µgC/l). However, consistent with our hypothesis, the northern populations (Cape Cod, Maine and New Brunswick) exhibited significantly higher ingestion and egg production rates than the southern populations. In another experiment, the five copepod populations were given toxic and non-toxic *Alexandrium*, and non-toxic *Tetraselmis* sp. (a green flagellate) at a high (500 _gC/l) and low concentration (100 _gC/l). Again, we found that the populations from Connecticut and New Jersey exhibited significantly lower ingestion and egg production rates when fed the toxic *Alexandrium* strain than the other two diets. Thus is appears that local adaptation has occurred in northern *A. hudsonica* populations enabling them to resist the toxic effects of *Alexandrium* better than the southern populations. Possible causes for the observed decrease in rate processes of the southern copepod populations will be discussed in light of experiments examining the possibility of physiological incapacitation.

OCCURRENCE OF *ALEXANDRIUM OSTENFELDII* IN THE GULF OF MAINE

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Phytoplankton samples were collected in the Gulf of Maine during April-May and June of 2000 as part of the broadscale survey cruises of the GOM-ECOHAB Program. Samples were examined for the presence of *Alexandrium* sp. based on an immunofluorescent technique that involves a genus-specific antibody-antigen assay. In this procedure, a secondary antibody is conjugated to a fluorescent compound, fluorescein isothiocyanate (FITC), which allows visual detection using an epifluorescence microscope. Enumeration of the samples revealed two distinct categories of size ranges, those cells of diameter 30-35_m, indicative of *A. tamarense* and/or *A. fundyense*, and larger cells, greater than 40_m diameter, with some reaching a maximum of 55_m. These larger cells exhibited unique intracellular characteristics in the form of golden-colored inclusions when viewed under epifluorescence. Inclusions found in these large cells numbered from one to three per cell and usually appeared round to elongate in shape and lacked internal structure. The presence of these inclusions may indicate mixotrophy. We suspect that these larger cells are *Alexandrium ostenfeldii* (Jacobson and Anderson, 1996; Balech and Tangen, 1985).

Highest surface (2m depth) densities of *A. ostenfeldii* were observed in the June 2000 samples, although maximum densities were <100 cells L^{-1}; cell densities were <50 cells L^{-1} in April-May. The distributions of *A. ostenfeldii* in April-May showed no single center of abundance but were generally wide-spread. Concentrations on the order of 50-100 cells L^{-1} occurred offshore of Penobscot Bay, in the Casco Bay region and at the mouth of the Bay of Fundy. In June, the greatest concentration of cells occurred at the mouth of the Bay of Fundy and along the axis of the Eastern Maine Coastal Current. Distributions of *A. ostenfeldii* were similar to those of total *Alexandrium* sp., except that *A. ostenfeldii* was absent from Jordan Basin in June, while other *Alexandrium* spp. were abundant. Possible correlations with nutrients and hydrography will be presented.

RELATIONSHIPS BETWEEN SEDIMENT TYPE AND *ALEXANDRIUM TAMARENSE* CYST MEASUREMENTS IN THE WESTERN GULF OF MAINE AND CASCO BAY REGIONS

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To date, the source of the harmful algae *Alexandrium tamarense* in the Western Gulf of Maine and Casco Bay has not been well quantified and is a topic of debate. One possible source of cells lies in the sediment in the form of dormant cysts. Sediment samples analysed in the Anderson Lab at the Woods Hole Oceanographic Institution (WHOI) show a patchy distribution, with neighboring stations have very different values. As a result, the cyst map is very sensitive to interpolation techniques. In an effort to improve the map, we analysed the sediment samples for sediment type, with the goal being to use well established sediment type (Poppe *et al*) and sediment environment maps (Kelley *et al*) to improve the cyst map. These sediment maps express the patchy nature of sediment in this energetic and bathymetrically complex region. The cyst map is an important input into a physical biological model of *alexandrium tamarense*, which will yield insights toward predicting blooms.

If *Alexandrium* cells are forming cysts due to environmental stresses (*e.g*. seasonal changes in water temperature), these cysts might settle in a fashion similar to some sediment type, and even be transported along with the sediment. Cyst counts were mapped onto a ternary diagram to show that most of the high cyst counts were found in silty/clayey regions, but not all such regions contained cysts. Then the sediment types were compared with those predicted by the sediment type map. A spatially higher resolved map of sedimentary environment (accumulation, erosion, or reworking) was also used for comparison.

The analyses did not yield a great improvement of the original cyst map due to the relatively larger spatial scales of the current model grid, potential aliasing of the cyst data due to selectively coring in 'soft' areas (where coring is possible), and a lack of agreement with the sediment type map itself. However, the future possibility of a higher resolution model grid and good agreement with the highly resolved sedimentary environment map keep the analysis viably useful.

Reference:

Poppe *et al*. Map Showing Distribution of Surficial Sediment, Gulf of Maine and Georges Bank. USGS MAPI-1986-A. Published 1989.

Kelley *et al*. Surficial Geology of the Maine Inner Continental Shelf. Maine Geological Survey Geologic Map No. 96-9. 1996.

COMPARISON OF MOLECULAR PROBES FOR THE IDENTIFICATION AND ENUMERATION OF *ALEXANDRIUM* SP. FROM THE GULF OF MAINE

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Over the last several years, molecular probes for the identification and enumeration of HAB species have become more readily available, but a comparative evaluation of natural samples has not been well documented. Three different methods that target *Alexandrium* sp. were tested on samples collected during the ECOHAB-Gulf of Maine (GOM) cruises in the spring of 1998 and 2000. Two of these, an oligonucleotide probe (NA-1) that binds to large-subunit ribosomal RNA (LSU rRNA) of the North American *Alexandrium* ribotype and a monoclonal antibody probe (M8751-1) that recognizes the outer cell surface antigens of several *Alexandrium* species were used in fluorescent whole cell microscopic assays. In addition, analyses were performed using the NA-1 probe on sample lysates empolying a semi-quantitative sandwich hybridization (SH) assay where the resulting color intensity is proportional to the *Alexandrium* cell density.

During 1998, about 70 samples were analyzed using the 3 methods. Comparison of the two whole cell assays indicated that estimates of *Alexandrium* sp. were consistently higher with the immunofluorescent method vs. the oligonucleotide probe method. Re-examination of the samples revealed that the NA-1 probe recognized only the smaller *Alexandrium* cells that did not contain autofluorescent inclusion bodies, while the M8751-1 probe labeled both larger and smaller *Alexandrium* sp., some of which contained inclusion bodies. The SH method detected cells when densities were high enough to cause toxicity (~200 cells liter^{-1}), but did not detect very low abundances of *Alexandrium* prior to the bloom.

Based on the observed differences in the 1998 counts, the 2000 immunofluorescent counts were improved by discriminating the counts into 4 categories: <50 µm cells with inclusion bodies, <50 µm cells without inclusion bodies, > 50 µm cells with inclusion bodies and >50 m cells without inclusion bodies. The cells <50µm without inclusion bodies were identified as the *Alexandrium tamarensis* complex (i.e., *fundyense*, *tamarense*), while the cells >50um with inclusion bodies were identified as *A. ostenfeldii*. Sensitivity of the SH assay was improved by collecting more volume per sample and by enhancing the detection of the target LSU rRNA. As a result of these modifications, better agreement of the 3 methods was achieved. These data suggest that different probes recognize *Alexandrium* species differently, and that at least two *Alexandrium* species co-occur in GOM waters during the spring months. Thus, the choice of probe is not always clear. A researcher must consider these issues along with other factors such as ease of preservation and long-term sample integrity when choosing an enumeration tool. Ultimately, a suite of probes must be developed to accurately distinguish all the potential toxic species of *Alexandrium* that are present in the sample.

ALEXANDRIUM SPP. HYPNOZYGOTE CYSTS IN THE WATER COLUMN IN THE GULF OF MAINE

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Alexandrium spp. in the Gulf of Maine are known to produce hypnozygote resting cysts as part of their life cycle. These cysts are thought to form near the end of the growing season and fall to the bottom where they undergo mandatory quiescence on the order of a few months. Excystment of these benthic cysts in response to some environmental cue is thought to initiate the annual spring and summer blooms of *Alexandrium*. Light and temperature have been determined to affect excystment in the laboratory, but photoperiod, physical, or chemical cues have not been eliminated as possible mechanisms. After excystment, the newly germinated planomeiocite cells undergo division, establishing the spring *Alexandrium* vegetative cell population.

Assuming that the excystment cue is light or temperature related, this conceptual model is adequate for shallow water environments where light and vernal warming reach the benthos where the cysts lie. In deeper waters such as near coastal Gulf of Maine and moderately deep waters with extensive tidal mixing and subsequent turbidity such as in the mouth of the Bay of Fundy, the conceptual model falls short. Evidence exists, however, for offshore bloom initiation (see Townsend et al abstract, this volume), which suggests some other source of cysts.

One possible source is the water column, and especially the bottom nepheloid layer. Physical processes (winter convection, tidal mixing) may resuspend cysts or slow the descent of newly formed cysts such that the entire dormancy period passes while the cysts remain in the water column. Either scenario would result in cysts near enough to the surface to receive seasonal environmental cues to trigger excystment.

To begin researching this possibility, samples were collected during a cruise in the Gulf of Maine in February of 2000. Samples were taken in 30 L Niskin bottles from three depths: 5 m above the bottom, the top of the nepheloid layer as located by transmissometer, and 2 m below the surface. In the lab, samples were sonified and stained with the fluorochrome stain primulin, then examined for cysts using an epiflourescence microscope.

Cysts are indeed present in the water in the Gulf of Maine in February. Fourteen of fifteen samples taken from 5 sample sites around Grand Manan Island and the mouth of the Bay of Fundy contained cysts. Concentrations in those fourteen samples ranged from 90 to >2500 cysts per cubic meter. The near bottom sample from southern Jordan Basin had the highest concentration of cysts recorded, at around 9000 cysts per cubic meter.

The presence of cysts in the water column makes offshore bloom initiation a possibility. Evaluating the efficacy of cysts in the water column initiating blooms hinges on knowing how many cysts are needed to inoculate a bloom, which in turn depends on vegetative growth rate which is at present not known with any certainty. These early results also raise questions regarding previous assumptions concerning bloom dynamics, such as the importance of benthic cyst beds and the physical origin of bloom initiation.

***ALEXANDRIUM* POPULATION AND NUTRIENT DYANMICS IN CASCO BAY, MAINE**

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Since 1972, blooms of the toxic dinoflagellate, *Alexandrium tamarense*, have occurred almost every spring in the coastal region of Casco Bay, Maine. Seven cruises from April to June, 1998 were undertaken in this region to elucidate the physical, chemical, and biological environment of waters containing these harmful blooms. Samples were combined ("pooled") from depths of 1, 3.5, and 7 m in an attempt to obtain a homogenous water sample representative of the mixed surface layer. Consistent with previous work, blooms of *Alexandrium* in this region were concurrent with low inorganic nutrient conditions. Because these blooms typically occur soon after the spring diatom bloom, as the surface waters warm, dissolved inorganic nutrient concentrations are generally low. The highest *Alexandrium* cell densities (~1000 cells/L) were observed during mid May near shore (within the Kennebec River plume) and in an area approximately 25miles offshore. Near shore aggregations of *Alexandrium* cells were likely the result of downwelling favorable winds (from the northeast), which commenced in early May. Areas marked by high cell densities (>400 cells/L) were associated with dissolved inorganic nitrogen concentrations of less than 2.5 µM and N:P ratios lower than 6:1. In areas where *Alexandrium* cell counts exceeded 200 cells/L, 82-99% of the total dissolved nitrogen (TDN) was in the organic form. Average DON concentrations in areas where blooms exceeded 200 cells/L were 8.5 µM, while in bloom areas of 100 cells/L or less DON concentrations averaged approximately 6.5 µM. It is hypothesized that *Alexandrium* may be able to obtain some of its nutrient requirements from the dissolved organic nitrogen pool, but further work is needed to determine this.

RELATIONSHIPS BETWEEN OCEANOGRAPHIC SATELLITE DATA AND *ALEXANDRIUM* DISTRIBUTIONS IN THE GULF OF MAINE

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Satellite image time-series of sea surface temperature and ocean color coincident with comprehensive field measurements of both *Alexandrium* and associated oceanographic parameters from 1998 and 2000 were collected and processed. Here we present the preliminary results of relationships between satellite measured patterns and field data from the three 1998 cruise periods. Sea surface temperature images from NOAA AVHRR were pre-filtered with a custom cloudmasking routine that was applied individually for each cruise period and for each satellite (NOAA 12, 14, and 15). In addition, within each cruise, sea surface temperatures were normalized to reduce the effects of anomalous atmospheric attenuation and diurnal variability. SeaWiFS data were processed using the latest (2000) NASA global coefficients. Both individual and composite scenes formed over each cruise period were examined. Qualitative relationships between the patterns seen in the satellite data and the *in situ* distributions of *Alexandrium* are shown using contours overlaid on the composite images. Initial results show that in June and July the distributions of *Alexandrium* generally followed the cold plume of the eastern Maine coastal current. In August, this association was weaker. In each month, the contours show that a recirculation feature, evident as a warm temperature pool at the mouth of the Bay of Fundy, is also coincident with a large population of *Alexandrium*. Distributions of *Alexandrium* suggest that, in the June, elevated concentrations are most closely associated with advective patterns evident in the SeaWiFS data, but in July and August concentrations are highest in association with boundaries between elevated coastal chlorophyll and more oligotrophic offshore waters. Preliminary results from regression analyses between satellite and ship-measured parameters will be shown to demonstrate quantitative relationships.

PROROCENTRUM LIMA IN THE GULF OF MAINE: SHOULD WE CARE?

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The dinoflagellate *Prorocentrum lima* has been found at several sites along the coast of Maine during the summer in 1998 and 1999, some in areas where shellfish are harvested commercially. Identity was confirmed by scanning electron microscopy (SEM). Although *P. lima* is known to produce toxins (okadaic acid and derivative compounds), no incidence of diarrhetic shellfish poisoning (DSP) has been conclusively documented so far in the Gulf of Maine, despite toxicity events in the early 1990's in Nova Scotia, Canada. *Prorocentrum lima* was first observed near Georges Bank in 1994 in an offshore plankton tow. In coastal waters, samples containing the dinoflagellate came from wild mussel populations collected at low tide, while others originated from aquaculture sites. Many of the cells were isolated from water samples and net tows, and on a few occasions were found in association with epiphytic algae.

Our survey suggests that *P. lima* is relatively rare at most stations. However the widespread distribution of this toxin producer, its recurrence two years in a row and over several months, and presence close to mussels and in the plankton warrant increased monitoring to address public health concerns and foster a better understanding of its ecology, especially in light of anticipated shifts in shellfish cultivation methods.

DOES *ALEXANDRIUM FUNDYENSE* IN THE BAY OF FUNDY MIGRATE VERTICALLY?

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A number of dinoflagellates have demonstrated a nocturnal downward migration in order to utilize nutrients and growth-promoting substances. The organism, *Alexandrium fundyense*, is responsible for producing paralytic shellfish (PS) toxins in the Bay of Fundy resulting in many shellfish areas being closed to harvesting for a period of time each year.

A study of *A. fundyense* in Bay of Fundy was conducted east of Grand Manan Island over a 54 hr period (1000AM, July 27-1600PM, July 29) where samples were collected at 3 h intervals from the surface and depths of 5 m, 10 m, 20 m, 30 m, 50 m, and 90 m. Analyses included dominant phytoplankton species with particular emphasis on total *A. fundyense* concentrations which included its various life cycle stages – fusing cells, duplets, planozygotes and newly formed resting cysts. Highest concentrations of total *A. fundyense* cells were detected in 15 of the 19 surface samplings with the highest numbers detected at 1000PM. The remaining four samplings had highest concentrations detected at 5 m and all were between the hours of 1000 AM and 1600 PM. Surface concentrations ranged from 8.88 x 10^4 to 1.65 x 10^5 cells l^{-1}. Although cells were observed throughout the water column, *A. fundyense* numbers decreased with depth throughout the study and numbers at the 90 m depth ranged from 100-1900 cells l^{-1}. Similarly, planozygotes were observed throughout the water column with concentrations greatest in surface samples with the highest number 2.6 x 10^5 cells l^{-1} observed at 1300 PM. Duplets were observed to a depth of 20 m but the majority were detected in the surface waters with no duplets detected at depths of 50 and 90 m. Highest numbers of duplets were 9.46 x 10^5 cells l^{-1} at 700 AM. Numbers of fusing cells were significantly lower that those for duplets although the maximum density 5.11 x 10^3 cells l^{-1} was also detected at the surface. Newly formed resting cysts were also observed during the study, but only at depths of 50 and 90 m. Concentrations ranged from 20-300 cells l^{-1}.

Mean surface and 90 m temperatures were 12.1°C and 7.9 °C, respectively. Salinities ranged from 31.0-32.9 psu. Other phytoplankton species observed during the study included *Scrippsiella trochoidea*, *Mesodinium rubrum*, *Ptychocylis* sp., *Helicostomella* sp., *Favella* sp., and various other tintinnid species.

Further research is required to determine why the Bay of Fundy *A. fundyense* strain does not migrate vertically. Attempts to reconstruct the study and do further analyses have been unsuccessful as a result of the extremely low concentrations in recent years.

INHIBITION OF *ALEXANDRIUM FUNDYENSE* STR. CB301-A BY MARINE BACTERIA

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Bacterial influences on toxic algal blooms have been studied extensively in several regions of the world but remain poorly understood in the Gulf of Maine, where *Alexandrium* spp. blooms have become a chronic problem. In the current study, we describe a bacterial assemblage and two bacterial isolates that either inhibited or killed *Alexandrium fundyense*. A bacterial assemblage from a non-axenic, late-exponential phase *A. fundyense* culture was inoculated into axenic *A. fundyense* str. CB301-A, which has been maintained as an axenic culture for about one year. This bacterial assemblage was found to cause the decline of *A. fundyense* CB301-A cells within 6 days of inoculation. Three members of this assemblage were isolated on agar plates containing f/2 medium amended with 0.5% peptone, 0.5% glucose, and 0.5% yeast extract. Of the three bacteria isolated, two strains (B1 and B2) inhibited CB301-A growth, while the third (B3) had no effect. 16S rRNA phylogenetic analysis revealed that isolate B1 was most closely related to strain S34, an uncharacterized member of the alpha-*Proteobacteria* that was isolated from the Sargasso Sea. Strain B2 was extremely closely related to *Marinobacter* sp. PCOB-2, a member of the family *Alteromonodaceae* isolated from a marine algal culture. We are currently continuing our characterization of these bacterial isolates and plan to determine their effects on other *Alexandrium* strains as well as non-toxic marine algae found in the Gulf of Maine.

PSP TOXIN RETENTION BY ZOOPLANKTON FEEDING ON *ALEXANDRIUM FUNDYENSE* – VECTOR OR SINK?

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Zooplankton can consume toxic *Alexandrium* spp. dinoflagellates in the Gulf of Maine and retain PSP toxins, potentially acting as vectors of toxin. Experiments were designed to determine toxin budgets for common species of coastal copepods feeding on toxic *Alexandrium fundyense*, offered as monocultures or in mixtures of algal prey, by comparing calculated toxin ingestion rates and toxin content of copepod body tissue and fecal pellets. Both copepod tissue and fecal pellet fractions accounted for $\leq$5% each of the calculated ingested toxin, and thus $\geq$90% was lost as dissolved fraction toxin. The presence of alternate food did not significantly alter the efficiency of toxin retention. Experiments using varying concentrations of *A. fundyense* and alternate non-toxic species show no effect of cell concentration on toxin retention efficiency. Sloppy feeding is the probable mechanism for release of dissolved toxin.

Selective feeding was evident at high, but not low, concentrations of *A. fundyense*, suggesting that PSP toxins deter grazers more effectively when higher *Alexandrium* spp. cell concentrations are achieved. Total toxin retained and efficiency of retention varied among copepod species. Toxin profiles (% molar composition) of dinoflagellates and copepod tissues differed, indicating some metabolic transformation, while fecal pellets had toxin profiles that were intermediate in composition between copepods and dinoflagellates. Because of their low toxin retention efficiency, copepod grazers are probably a net sink for PSP toxins produced by *Alexandrium* spp., especially when cell concentrations are low ($<10^4$ cells L^{-1}) and relatively high zooplankton clearance rates are attained. Nevertheless, zooplankton can attain toxin body burdens sufficient to contribute to propagation of PSP toxins to other trophic levels.

ANNUAL EXCYSTMENT OF *ALEXANDRIUM* HYPNOZYGOTES FROM EASTERN GULF OF MAINE POPULATIONS

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A germination experiment was begun last year in order to explore the possibility that an annual endogenous clock may help determine excystment of the Alexandrium hypnozygote from eastern Gulf of Maine populations, as has been shown for populations from Casco Bay, in the western side of the Gulf. Sediment samples were collected from three stations in the eastern Gulf of Maine (off Monhegan and Matinicus Islands and in the Bay of Fundy); subsamples have been kept refrigerated, under nitrogen, in the dark. Subsamples are incubated in f/2 growth medium biweekly, under constant temperature (15°C) and light conditions, and excystment determined over a 6-week period. Preliminary results show that germination started in February, continued through May-June, and decreased to a minimum by the end of July in all 3 stations, except for off Matinicus Is. Where it ended in mid-September. This information is significant in bloom prediction models.

This work was started under the leadership of the late Dr. Maureen D. Keller.