LONG-TERM PATTERNS OF NARRAGANSETT BAY PHYTOPLANKTON DRIVEN BY DECADAL SHIFTS IN PHYTOPLANKTON HABITAT

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A 38-year (1959 to 1996) time series of weekly observations of Narragansett Bay phytoplankton was analyzed to evaluate the interactions of climate, physical, chemical and biological variables on the selection and succession of HAB and benign red tide phytoplankton. The phytoplankton community was diatom dominated from 1959 until 1978 and from 1990 until 1996. During the intervening period of the 1980s (1979 to 1989) diatom abundance declined and flagellate abundance increased such that flagellate abundance rivaled that of diatoms. Abundance of most of the 17 HAB and nuisance species recorded in the time series occurred in the 1980s, implying flagellate-favorable modification of the phytoplanktonic habitat during that period. Peak abundance of HAB and nuisance species including *Dinophysis acuminata, Heterocapsa triquetra*, *Heterocapsa rotundata*, *Prorocentrum minimum*, *Prorocentrum micans* as well as summer blooms of the brown tide pelagophyte *Aureococcus anophagefferens* occurred during the 1980s.

De-trended and de-seasonalized timeseries of phytoplankton habitat variables show several potential mechanisms driving the 1980s flagellate increase. Estimates of flushing time (t) derived from salinity and riverflow observations indicate that the early 1980s were characterized by elevated flushing time, and a relative increase in the tidal component of estuarine circulation. Coincident with the elevated flushing time was an increase in nitrate concentration, which peaked at a level two-fold the long-term mean level in the 1980s. Combined with a long-term decline in phosphorus, the 1980s peak in dinoflagellate abundance coincided with a peak in DIN:DIP ratio.

While flagellate abundance was on the increase in the 1980s, diatom abundance, driven by a ca. 40% post-1980 decline in *Skeletonema costatum* abundance, was declining. Fluctuations in *Skeletonema* abundance and bloom pattern can be partially explained by variation in a large-scale proxy indicator of winter weather patterns, the North Atlantic Oscillation Index (NAOI). In Narragansett Bay, and other temperate estuaries, competition for the summer phytoplankton niche is usually between a diatom (typically *Skeletonema*) and one or more small flagellates (*Prorocentrum minimum, Heterosigma akashiwo*). The 1980s increase in flagellate abundance occurred during a decline in diatom abundance suggesting a release of competitive exclusion for the 'open' summer phytoplankton niche that favored flagellates over diatoms in the 1980s.

CAN NATURAL SELECTION FOR RESISTANCE TO PARALYTIC SHELLFISH POISONING TOXINS IN SOFTSHELL CLAM, *Mya arenaria*, POPULATIONS OCCUR DURING EARLY LIFE HISTORY STAGES?

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We have previously documented significant differences in the prevalence of individuals resistant to paralytic shellfish poisoning (PSP) toxins, measured by burrowing incapacitation and *in vitro* nerve resistance to saxitoxin (STX), among populations of juvenile (~30-40 mm in shell length) softshell clams, *Mya arenaria*, on both Atlantic and Pacific coasts of North America. Site-specific differences in the percentage of resistant clams generally correlate with the history of exposure to PSP, with highly resistant populations occurring in regions recurrently affected by red tides.

The main goal of this study is to test the hypothesis that natural selection for resistance to PSP toxins could potentially occur during early life history stages of *M. arenaria*. Progeny from a naïve, sensitive population from the Lawrencetown River estuary, southeastern Nova Scotia (NS) was used as a laboratory test population. Clam veliger larvae fed a non-toxic algal suspension spiked with a high-toxicity *Alexandrium tamarense* strain (PR18b, 29 to 80 µg STX equivalents $cell^{-1}$, mean diameter = 35 µm) for 1 wk showed no difference in survival or growth rate relative to non-toxified controls. This indicates that ingestion of toxic cells is required for adverse effects of PSP toxins to occur, as *Alexandrium* cells are too large for ingestion by bivalve larvae. In contrast, paralysis and significant mortalities of small juveniles occurred within 4 h of exposure to toxic *Alexandrium* cells, resulting in 95% cumulative mortalities of 4 mm juveniles (spat) following only one week of exposure. Mortalities are attributed to anoxia of the pallial cavity resulting from toxin-induced muscular paralysis and reduced irrigation of the pallial cavity. This rapid mortality of early post-settlement stages contrasts with previous findings for large (35-42 mm) juveniles from the same source population, in which mortalities varied considerably between experiments and were only initiated after 8-10 days of toxin exposure. Summer blooms of *A. tamarense* in the Bay of Fundy coincide with the main period of spawning and larval development of *M. arenaria* and also with the occurrence of small, year-2 spat. This study demonstrates that in post-settlement stages lethal effects of PSP toxins and thus strong selection of more resistant individuals could potentially occur within a few days of exposure to a highly toxic *Alexandrium* bloom. We are currently testing progeny (larvae and spat) from a resistant population in Lepreau Basin, Bay of Fundy for comparison.

THE ROLE OF ZOOPLANKTON GRAZERS IN THE BLOOM DYNAMICS OF THE TOXIGENIC DINOFLAGELLATES *Alexandrium* SPP. IN THE GULF OF MAINE

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Increasing incidence of harmful algal blooms threatens public health and economic activities (fisheries and aquaculture) in coastal environments. In the Gulf of Maine region of the northeastern United States, toxigenic dinoflagellates of the genus *Alexandrium* may form moderately dense aggregations in surface waters during spring, summer and fall of each year. Blooms of *Alexandrium* spp. are the source of the potent neurotoxins responsible for paralytic shellfish poisoning (PSP) in New England. We investigated the role of zooplankton grazers in the bloom dynamics of *Alexandrium* spp. in different regions of the Gulf of Maine. In the near-shore environment of the western Gulf of Maine, moderate blooms (<3000 cells/l) of *Alexandrium* spp. occurred and it was a minor component of the phytoplankton assemblage. Here, the copepod *Acartia hudsonica*, the most important grazer, fed non-selectively and had a significant grazing impact on the *Alexandrium* population. In contrast, in the Grand Manan Basin in the Bay of Fundy, *Alexandrium* spp. concentrations were much greater (>50,000/l) and it was a dominant component of the bloom assemblage. In this region, the copepod *Calanus finmarchicus*, the dominant grazer, had reduced grazing rates and did not appear to have a significant impact on the *Alexandrium* population. In both instances, despite low toxin retention efficiencies, toxin accumulation in zooplankton was significant and posed risks to higher trophic levels.

TOXICITY OF *Prorocentrum lima* AND THE POTENTIAL FOR DIARRHETIC SHELLFISH POISONING ALONG THE NEW ENGLAND COAST ON THE UNITED STATES

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Following the occurrence of several unexplained incidents of shellfish-related gastroenteritis, field studies were conducted to determine if diarrhetic shellfish poisoning (DSP) toxins were present in the coastal waters of New England states. Previous studies have found the toxic dinoflagellate, *Prorocentrum lima*, is widespread in New England coastal waters. The abundance and seasonality of this toxin producer was followed within the planktonic and epibiotic community. Samples were collected bimonthly at eight sites from Rhode Island, New Hampshire, and Maine**.** In an effort to evaluate the potential for diarrhetic toxins to contaminate shellfish resources, the digestive glands of wild and cultured shellfish were collected.

The epiphytic samples and digestive glands were analyzed for potential okadaic-acid activity using the fluorometric protein phosphatase inhibition assay. Samples positive for protein phosphatase activity were analyzed for okadaic acid and related congeners using the ADAM-HPLC method. Epiphytic samples showed a seasonal trend in both the population of *P. lima* and total toxicity, with increase cell number and toxin content during summer months. Analysis of these samples showed the production of both dinophysis toxin-1 and dinophysis toxin-2. Like previous studies, no okadaic acid was detected. Cultures of *P. lima* isolated from the sample area showed a similar toxin profile with a predominate production of dinophysis toxin-1 and little okadaic acid production. The digestive glands did display very low protein phosphatase activity. Although the presence of DSP-type toxins in shellfish digestive glands indicate uptake, the levels are well below the maximum accepted concentration.

CROSS-FRONTAL ENTRAINMENT OF PLANKTON INTO A BOUYANT PLUME: THE FROG TONGUE MECHANISM

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A mechanism for the cross-frontal entrainment of plankton by a buoyant plume influenced by wind stress is described and tested using an idealized numerical model. Under the right circumstances, plankton may enter a buoyant plume during an upwelling wind stress, then be transported shoreward during a subsequent downwelling wind stress. In order for the plankton to enter the plume, they must swim upward at a velocity (w_p) bounded by

$$H_{plume} / T < w_p < k / H_{mix}$$

where H_{plume} is the thickness of the buoyant plume, H_{mix} is the thickness of the upper oceanic mixed layer ($H_{mix} > H_{plume}$), k is the magnitude of vertical mixing within the mixed layer, and T is the time between upwelling and downwelling events. In words, this equation states that the plankton must swim slow enough so that they are evenly distributed through the mixed layer, so that the buoyant plume may override the plankton patch during upwelling. Once the plume has overridden the patch, in order to enter the plume, the plankton must swim fast enough to be able enter the plume in the time while it is over them. These two bounds on the swimming rate suggest that, given various physical parameters, there may be a range of swimming speed that will maximize entrainment into a plume. Numerical experiments corroborate the feasibility of the proposed mechanisms and associated scaling.

PHYLOGENETIC DIVERSITY OF BACTERIA ASSOCIATED WITH *Alexandrium* spp. AND OTHER PHYTOPLANKTON FROM THE GULF OF MAINE

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Previous work relying on the measurement of bulk community parameters has indicated a close link between bacteria and phytoplankton dynamics in marine environments. However, little is known about how these communities interact at a species-composition level or whether there are specific associations between bacteria and phytoplankton. Studies of the interactions between several harmful algal bloom (HAB) species and associated bacteria have suggested that these interactions can be specific and may be important controlling factors for HAB events. The objective of the current study was to determine whether there are specific interactions between diverse phytoplankton and the bacteria that are closely associated with them. Our analysis included representatives of the major taxonomic groups of phytoplankton in the Gulf of Maine (GOM), as well as several strains of the toxic dinoflagellates, *Alexandrium* spp., from the GOM and elsewhere. We determined the molecular phylogenetic diversity of bacterial assemblages associated with xenic, uni-algal phytoplankton isolates that were chosen to (1) represent a broad taxonomic range; (2) represent a broad geographic range for *Alexandrium* spp. isolates; (3) grow under similar cultivation conditions; (4) have a minimal length of time since the original isolation; and (5) have been isolated from a vegetative cell (not from a cyst). DNA was extracted from xenic cultures using a FastDNA Spin Kit and used as template in PCR amplification of 16S rDNA fragments with a primer set that targets most *Bacteria*. The PCR products were analyzed using denaturing gradient gel electrophoresis (DGGE), and resolved DGGE bands were recovered from the gel and either sequenced directly or cloned into *E. coli* and then sequenced. DGGE analysis revealed little similarity among bacterial assemblages from different phytoplankton cultures, with only a few bacteria associated with more than one phytoplankton culture. However, sequence analysis indicated that similar bacterial phylogenetic groups were dominant across distantly related phytoplankton taxa. In particular, the *Rhodobacter* and *Cytophaga-Flavobacterium-Bacteroides* groups were important members of assemblages in most phytoplankton cultures. These groups are known to include important colonizers of marine particulates and bacteria that have both positive and negative interactions with phytoplankton. Taken together, these results indicate that specific bacteria-phytoplankton interactions may exist, but that they are not the result of coevolutionary relationships that would be expected to occur in tight symbioses.

A MESOCOSM STUDY EXAMINING THE INFLUENCE OF NUTRIENTS ON *Alexandrium tamarense/fundyense* TOXIN CONCENTRATION AND COMPOSITION

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Laboratory and field studies exploring the influence of varying nutrient regimes on *Alexandrium tamarense/fundyense* have revealed that they can have dramatic effects on toxin content and composition. Therefore, we hypothesize that certain "generic" toxin composition profiles, or ratios of the various saxitoxin derivatives, may be indicators of a cell's nutrient status.

As part of a larger study to investigate the impact of a number of different environmental parameters on toxin content and composition, a field-deployed mescosm study was conducted along the shoreline of Salt Pond, Eastham, MA in the spring of 2003. The primary objective of this study was to determine if nutrient variability would result in discernable patterns in toxin composition in both natural and cultured *Alexandrium* sp. populations.

Two, eight-foot diameter, fiberglass pools, each containing six, 230-liter, polyethylene tanks were used to house the experiment. Salt Pond water was pumped into the 2 pools at a rate of 170 liters per minute to regulate the tanks to ambient water temperature. At the beginning of the *Alexandrium* bloom, nine of the 230-liter tanks were filled with 64 µm-filtered pond water (to remove grazers) while the other 3 tanks were filled with 20 µm-filtered water (to remove *Alexandrium* and grazers). Three of the 64 µm-filtered tanks were enriched with f/20 levels of nutrients (nutrient replete), while 3 were nitrogen limited and 3 were phosphorus limited. The 20 µm-filtered tanks were inoculated with the *A. tamarense* culture ATSPG5-1 (a clonal Salt Pond isolate) at an initial density of 1000 cells per liter. One of these tanks was nutrient replete, one nitrogen deplete and one phosphorus deplete. During the course of the month-long study, samples were collected from each tank at least twice daily for chlorophyll content, cell density, cell volume and life cycle status, cellular nutrient status, saxitoxin content, dissolved and particulate nutrients. A pond sample taken through the pool cooling water discharge line was also collected and was assessed for the same parameters as the tanks.

Results of HPLC analysis of saxitoxin, as well as analysis of the other ecological parameters, will be presented and evaluated to identify generic trends in cellular toxin content and composition in relation to nutrient availability.

A MOLECULAR APPROACH SPECIFIC FOR THE DETECTION OF *Prorocentrum lima* IN NEW ENGLAND COASTAL WATERS

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In the eastern US and Canada, the source of DSP toxins in shellfish is closely linked to the presence of the epibenthic/epiphytic dinoflagellate *Prorocentrum lima*. Although *P. lima* is generally easy to recognize under light microscopy, its presence within the epibiota of filamentous seaweeds complicates sampling and renders quantification difficult and very time-consuming. In an attempt to accelerate sample processing, a molecular approach using two *P. lima*-specific oligonucleotide probes is currently under investigation with New England coastal populations.

The two fluorescent probes, originally developed at AWI, are based on target sequences of the small subunit 18S and the large subunit 28S rRNA. Probe reactivity and specificity, first determined by DNA dot blot hybridization (digoxygenin system), have been verified in a whole-cell hybridization format with fluorescence microscopy using *P. lima* strains from Rhode Island and Maine coastal waters and dinoflagellates likely to co-occur with *P. lima*. Each probe satisfactorily reacts with the target organisms from U.S. northeast coastal waters. However non-specific probe-binding to cell surfaces is particularly problematic with one strain of *P. mexicanum*, an occasionally co-occurring species. Enumeration of *P. lima* in natural as well as *P. lima*- enriched field samples yields comparable cell concentrations, whether a traditional method based on microscopy or the developed molecular approach is used. The two methods of processing field samples will be compared using data on cell concentration within the epibiota, time expended to process the samples, difficulty of application and expenses.

LOSS OF TOXICITY AND CHARACTERIZATION OF ASSOCIATED BACTERIA IN A SINGLE CLONE OF *Alexandrium lusitanicum*

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Here we examine a specific case whereby a culture of *A. lusitanicum* from Obidos Lagoon, Portugal appears to have lost the ability to produce saxitoxins. Two subcultures of this isolate were established and maintained as separate isolates. Three independent toxin analysis methods (mouse bioassay, HPLC and mouse neuroblastoma receptor binding assay) show that while one of the variants maintains the same levels of toxicity and the toxin profile as the initial culture, the other no longer produces detectable toxin levels. Through morphological analysis and sequencing of three domains of the ribosomal gene the two cultures proved to be identical.

Several explanations can be suggested for the loss of the ability to produce saxitoxins, including mutations in one or more genes, or the change in associated bacterial flora due to continued antibiotic treatment of one of the subcultures. The hypothesis that bacteria living in close association with the dinoflagellate cells are directly or indirectly involved with toxin production is currently under investigation. The total bacterial assemblage (culturable and non-culturable) of both toxic and non-toxic subculture was identified using two different molecular methods. Denaturing gel electrophoresis and a clone library were carried out using 16s rDNA amplified with universal primers from total dinoflagellate DNA. Results of both methods show that the associated bacteria differed significantly between the toxic and the non-toxic dinoflagellate culture. The identity of associated bacteria was obtained through sequence comparison with entries in GeneBank. These results and new data from ongoing studies of bacterial involvement in toxin production will be discussed.

MECHANISMS REGULATING THE LARGE-SCALE SEASONAL DEVELOPMENT OF *Alexandrium fundyense* BLOOMS IN THE GULF OF MAINE (U.S.A)

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Observations of *Alexandrium fundyense* in the Gulf of Maine indicate three salient characteristics of the vegetative cell distributions: (1) patterns of abundance are gulf-wide in geographic scope, (2) their main features occur in association with the Maine Coastal Current, and (3) the center of mass of the distribution shifts upstream from west to east during the growing season from April to August. The mechanisms underlying these aspects are investigated using coupled physical-biological simulations that represent the population dynamics of *A. fundyense* within the seasonal mean climatological flow. A model that includes germination, growth, mortality, and nutrient limitation is qualitatively consistent with the observations. Germination from resting cysts appears to be a key aspect of the population dynamics that confines the cell distribution near the coastal margin, as simulations based on a uniform initial inoculum of vegetative cells across the Gulf of Maine produces blooms that are broader in geographic extent than is observed. In general, cells germinated from the major cyst beds (in the Bay of Fundy and offshore of Penobscot and Casco Bays) are advected in the alongshore direction from east to west in the ambient coastal current. Growth of the vegetative cells is limited primarily by temperature from April through June throughout the gulf, whereas nutrient limitation occurs in July and August in the western gulf. Thus the seasonal shift in the center of mass of cells from west to east can be explained by changing growth conditions: growth is more rapid in the western gulf early in the season due to warmer temperatures, whereas growth is more rapid in the eastern gulf later in the season due to severe nutrient limitation in the western gulf during that time period. A simple model of encystment based on nutrient limitation predicts deposition of new cysts in the vicinity of the observed cyst bed offshore of Casco and Penobscot Bays, suggesting a pathway of re-seeding the bed from cells advected downstream in the coastal current. Seasonal spinup of a retentive gyre at the mouth of the Bay of Fundy would tend to favor re-seeding that cyst bed from local populations.

TEMPORAL AND SPATIAL VARIABILITY IN THE CHARACTERISTICS OF *Alexandrium fundyense* BLOOMS IN THE COASTAL ZONE OF THE BAY OF FUNDY

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The abundance of *Alexandrium fundyense* has been monitored at four locations in the Bay of Fundy (eastern Canada) at seasonally varying weekly to monthly intervals since 1987. The date at which Alexandrium first appears in samples varied from day of year 105 to 179. This corresponds with a range of 74 days and a standard deviation of 16 days. The mean and median date of the first appearance of *Alexandrium fundyense* varied by only a few days between stations. The mean (median) days of first appearance estimated were 135 (132), 128 (130), 128 (129) and 128 (133) for sampling stations 3, 15, 16 and 17 respectively. The overall mean (median) date of first appearance was day 136 (134). The null hypothesis that the date of first appearance varied randomly (alpha 0.05) could not be rejected (alpha=0.05) by a two-sided runs test. The dates of the maximum concentration of *Alexandrium* cells vary by about 30 days between stations and years. The maximum cell counts occur earliest at the inshore estuarine station (day 172-175) and latest at the offshore station (day 197-203). The day of maximum cell counts at the other two stations is 188 and 194. The standard deviation in the date of maximum cell count ranges from 15 to 28 days. The time series of dates visually suggest the possibility of a low frequency trend suggesting an earlier date of maximum cell count in the more recent years. However, a non-parametric runs test is unable to reject the null hypothesis that the variation in the date of maximum concentration is random. The annual maximum concentration of *Alexandrium fundyense* varies by about three orders of magnitude and there is a range of about one order of magnitude in the station mean concentrations. The mean concentrations form a gradient from inshore to offshore with the mean and median cell concentrations being least in the inshore estuarine station and greatest in the offshore stations. Although the time series may appear to have a low frequency trend suggesting a lower maximum concentration in the more recent years, a non-parametric runs test is unable to reject the null hypothesis that each series is a random series of values. The duration of *Alexandrium* blooms ranged from 42 to 205 days. The mean (median) duration of the bloom was 114 (112) days. The mean bloom duration is 20-30 days shorter at inshore estuarine stations than at the other stations. The temporal character of the *Alexandrium* bloom also varies interannually with some blooms consisting of 1 to 3 pulses per year.

BIOLOGICAL AND PHYLOGENETIC CHARACTERIZATION OF *Amoebophrya* sp. ex *Alexandrium tamarense*

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Amoebophrya ceratii is a parasitic dinoflagellate that infects several dinoflagellate genera. The first report of infections in *Alexandrium tamarense* was made by Jacobson (1987), although cultures were not established at the time. In May 2003, infected *Alexandrium tamarense* were again observed during a bloom in Salt Pond, Eastham, MA. Water samples were collected and filtered through a 20-mm sieve, enriched with f/2-Si medium, and kept at 15ºC. Five to ten infected *Alexandrium* cells were isolated by microcapillary technique under a fluorescence microscope. The cells were then placed in each well of a 96-well plate containing f/2-Si medium as well as uninfected *Alexandrium tamarense* isolated from the same location. The plates were maintained at 15ºC. Free-living zoospores were observed after 1 to 2 days, but no vermiforms were found. Late-stage infections were seen after 4 to 5 days in several wells. These were then transferred to new wells with uninfected hosts. Basic features of the parasite and its life cycle were documented using video fluorescence microscopy (*in vivo*). Phylogenetic analysis and other biological characterizations are underway and will be presented at the symposium.

Culture experiments revealed that this parasite can infect several *Alexandrium* isolates from Salt Pond, as well as isolates from the Gulf of Maine (GTCA28, GTCA29), Alaska (PW06), Northern Europe (BAHME184), South Africa (SA2), Hong Kong (HK, HK1), Japan (OFO41) and Australia (ACPPO9). Surprisingly, this parasite from *Alexandrium tamarense* was also capable of infecting other dinoflagellate species including *Prorocentrum minimum* (CCMP1329), *Prorocentrum micans* (CCMP21), *Scrippsiella trochoidea* (SA2 Scripp) and *Heterocapsa triquetra* (Het), all of which are known previously to harbor *Amoebophrya* infections themselves. However, the infection was quickly lost in *P. micans*. The parasite did not infect the athecate dinoflagellates, *Akashiwo sanguinea* (GSBL) and *Gymnodinium instriatum* (GIAL177). Recent work suggests that *Amoebophrya ceratii* consists of a species complex with a large degree of host specificity (Coats et al. 1996; Coats and Park, 2002). However, these findings suggest that certain isolates of *Amoebophrya* may have a wider host range than previously found. Further studies planned with this isolate may provide new insights into this issue.

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GERMINATION OF DINOFLAGELLATE RESTING CYSTS FOLLOWING DEPOSIT-FEEDER GUT PASSAGE AND PELLET ENCAPSULATION

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As dinoflagellate cysts may remain viable in marine sediments for months to years, they may pass through the guts of deposit feeders many times before conditions become favorable for germination. Little is known, however, about how dinoflagellate cysts are affected by deposit-feeder digestion, fecal pellet formation, and translocation within the sediment column by bioturbation. To answer the question whether gut passage leads to cyst mortality, we fed cysts of the dinoflagellate *Scrippsiella lachrymosa* to three species of deposit feeders, *Capitella* sp., *Streblospio benedicti*, and *Polydora cornuta*. These species differ in the extent to which they form fecal pellets. To examine the effects of longer gut-passage times, we incubated cysts in *Arenicola marina* digestive fluid for up to 24 h. We then monitored the cysts to determine rates of germination. We found that cysts were remarkably resistant to digestion by deposit feeders and that they were capable of germinating even within the robust fecal pellets of *Capitella*. Although burial due to bioturbation by deposit feeders might reduce cyst germination in the field, we expect that gut passage and pelletization does not result in substantial mortality of dinoflagellate resting stages.

LONG-TERM BLOOM BEHAVIOR OF *Prorocentrum* SPECIES IN NARRAGANSETT BAY

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The annual bloom dynamics of *Prorocentrum micans, P. minimum, P. scutellum* and *P. triestinum* have been followed over a 38-year period in Narragansett Bay based on weekly sampling. Arrayed along their mean annual abundance: *P. triestinum* > *P. minimum* > *P. scutellum*> *P. micans*. Significant interannual and seasonal bloom patterns were observed. Annual mean abundance of *P. minimum* increased between 1959 to 1982; there has been a long-term decline in *P. triestinum* since 1966; *P. scutellum* has become rare since 1983, while mean abundance of *P. micans* has been more or less invariant over the four decades of sampling. While the bloom duration varied, the maximum mean weekly abundance occurred between weeks 23 to 25 for *P. micans, P. minimum* and *P. triestinum,* and week 30 for *P. scutellum*. Within these patterns, there is considerable interannual variability in whether *Prorocentrum* will bloom and, if selected for, which of the four species will bloom. There appears to be a repetitive sequence of high annual *Prorocentrum* abundance followed by a year of lower abundance. This variability in *Prorocentrum* bloom behavior is partly linked to whether there is a *Heterosigma akashiwo* bloom, an event influenced by a variety of factors, including grazer structure and growth of the important diatom *Skeletonema costatum.* Some aspects of this "open niche" and the influence of the long-term regime shift that has occurred in Narragansett Bay from a diatom to flagellate dominated system on *Prorocentrum* bloom behavior are discussed.

The long-term relationships between *Heterosigma akashiwo* and *Prorocentrum* blooms, and between the latter and *Skeletonema costatum* are discussed. The associations between long-term changes in climate, proxied as the North Atlantic Oscillation Index, the Groundwater Index and Palmer Drought Index, and habitat conditions of nutrients, their ratios and flushing intensity on *Prorocentrum* bloom behavior are also considered.

EVALUATING HYPOTHESES FOR THE INITIATION AND DEVELOPMENT OF *Alexandrium fundyense* BLOOMS IN THE WESTERN GULF OF MAINE USING A COUPLED PHYSICAL-BIOLOGICAL MODEL

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A coupled physical/biological model and observations are used to investigate the factors governing the initiation and development of an *Alexandrium fundyense* bloom in the western Gulf of Maine during the spring of 1993. The physical circulation is modeled with a 3D primitive equation model forced by climatological elevation fields and observed winds, irradiance, and river outflow. This is coupled with a biological model constructed from laboratory and field data that estimates the germination and growth rates of *A. fundyense* as a function of environmental conditions. Four biological model structures of increasing complexity are considered, with each structure representing a hypothesis for factors controlling bloom initiation and development. The model/data fit is optimized over the uncertainty in the parameters to which the model is most sensitive. The significance of changes in the model/data fit between structures is quantified using a maximum likelihood ratio test. Biological models incorporating a strong dependence of growth on dissolved inorganic nitrogen (DIN) produce results that are significantly better (>90% confidence) at matching observations than those that do not. The optimal simulation generally reproduces mean regional cell levels in time and space, but considerable misfits remain at individual points. Diagnosis of the best-fit model solution suggests that cysts germinating from sediments at greater than 50 meters depth account for the majority of cells within the study area. However, cells germinating from cysts in shallow waters (< 50m), and the inflow of cells from the eastern Gulf of Maine can make significant contributions to the cell budget, particularly late in the spring and inshore. The growth of *A. fundyense* is strongly limited by low water temperatures until mid-May and by low levels of DIN afterward. These alternating limitations prevent growth from dramatically enhancing the initial cyst-driven source. This is consistent with previous work (Franks and Anderson, 1992) stating that patterns in cell abundance and toxicity in the region are consistent with the along-shore advection of established populations of *A. fundyense*.

References:

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EFFECTS OF *Alexandrium fundyense* CELL CONCENTRATION AND CELLULAR TOXICITY ON COPEPOD FEEDING BEHAVIOR

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Mesozooplankton such as copepods are major grazers of phytoplankton, and their feeding activity can potentially control harmful algal blooms (Campbell et al., 2000; Teegarden et al., 2001), as well as provide vectors for food web transfers of toxin (White, 1981; Teegarden and Cembella, 1996; Turner et al., 2001; Teegarden et al., 2003). Laboratory experiments suggest that copepods can and will avoid toxic *Alexandrium* sp. cells in mixtures of prey types, and that cellular toxin content is a principal cue for selective feeding (Teegarden, 1999). Field studies of zooplankton grazing on *Alexandrium* sp. at lower concentrations indicate that non-selective feeding is common when toxic cells are only a minor portion of available food (Teegarden et al., 2001). From these studies we hypothesized that selective feeding on *Alexandrium* spp. is dependent on cell concentration, or cellular toxicity, or both. To address and prioritize these hypotheses, we performed a series of experiments challenging three species of copepod grazers (*Acartia hudsonica*, *Centropages hamatus*, *Eurytemora herdmani*) with mixtures of natural water samples containing non-toxic algae (diatoms and flagellates) and three clones of *Alexandrium* spp. – GTCN16 (toxin content below detection), GTCA28 (moderate toxicity, ~25 pgSTXeq cell^{-1}), and BC1 (high toxicity, ~75 pgSTXeq cell^{-1}), each at high (10^5 cells L^{-1}) and low (10^4 cells L^{-1}) concentrations.

Ingestion of *Alexandrium* sp. by all copepod species depended more on concentration than cellular toxicity. Within any one copepod species and *Alexandrium* sp. clone treatment, clear differences existed in clearance rate, selection indicated by electivity index, *Alexandrium* cells ingested, and total food ingested. In low *Alexandrium* sp. concentration treatments, copepod clearance rates on *Alexandrium* were usually higher and electivity indices less negative, indicating less avoidance compared to high concentration treatments. Effects of cellular toxicity were not however consistent and predictable. For example, there was little difference between *E. herdmani* rates of consumption or selectivity of *Alexandrium* between the BC1 high toxin clone treatment and the GTCN16 low toxin clone treatment, while the moderate toxicity GTCA28 clone was shunned. In high toxicity (BC1) treatments, one result consistent among copepod species was that total food consumption was lower at high *Alexandrium* sp. concentrations, suggesting that high cellular toxicity and high cell concentration suppresses overall feeding, but this is not solely a result of selective *Alexandrium* sp. avoidance, as all food species were consumed at lower rates. The low toxicity GTCN16 clone was also consistently consumed by all copepod species at the highest rates and greatest positive electivity compared to more toxic clones fed to the same grazer species, but differences were not dramatic. To summarize, toxicity of individual *Alexandrium* sp. cells appeared to have only minor influence on feeding selectivity, unless combined with the factor of high concentration. Concentration however had an effect on selectivity and total food consumption at any level of cellular toxicity.

References:

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IMPACT OF ZOOPLANKTON GRAZING ON *Alexandrium* spp. BLOOMS IN THE OFFSHORE GULF OF MAINE

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Zooplankton grazing was investigated in 22 shipboard experiments during natural blooms of *Alexandrium* spp. (*A. fundyense* and *A. ostenfeldii*) in the offshore Gulf of Maine in spring and/or summer of 1998, 2000, and 2001. Grazing studies were done in conjunction with studies of accumulation of *Alexandrium* toxins in the zooplankton, as part of the ECOHAB-Gulf of Maine regional program. Several species of copepods, marine cladocerans, and appendicularians were allowed to graze upon natural phytoplankton assemblages at natural abundances, and ambient temperatures (14-17^{o}C). Grazing was measured by quantitative microscopic analyses of disappearance of cells in experimental, compared to initial and control suspensions, preserved in Utermöhl's solution. Thus, we were able to examine grazing upon *Alexandrium* in comparison to grazing on all other co-occurring phytoplankton taxa. Even during *Alexandrium* "blooms," this dinoflagellate was a minor component of the overall phytoplankton assemblage, present at stations where grazing experiments were conducted at low levels of 0.12-5.11 cells ml^{-1}, or 0.03-3.9% of total phytoplankton cells present. Phytoplankton assemblages were dominated by athecate microflagellates (3-6 μm diameter), and secondarily by diatoms and non-toxic dinoflagellates. Microflagellates were present at abundances of 159.62-793.93 cells ml^{-1}, accounting for 60.6-95.6% of total cells. Ingestion of *Alexandrium* spp. and microflagellates accounted for up to 3.2% and 35.6-98.2% of total grazing, respectively. Grazing was significantly non-selective, with *Alexandrium* spp. and microflagellates being ingested in similar proportions to their availability in food assemblages. There were no apparent adverse effects on grazers during incubations of 18-24 hours, and grazer survival was 100%. Multiplication of daily experimental rates of ingestion of *Alexandrium* spp. per grazer by *in situ* abundances of grazers used in experiments permitted estimations of the proportions of the *in situ* populations of *Alexandrium* removed daily by experimental grazers. Experimental grazers removed a mean of 5.79% of *Alexandrium* spp. cells (range = 0-117%), but 18 of 22 experiments (81.8%) exhibited experimental grazer impacts of < 1% of *in situ* populations of *Alexandrium* spp.. The few instances where grazing impact was high were more due to high abundances of experimental grazers than to high ingestion rates of individual grazers, or unusually low abundances of *Alexandrium* spp. These estimates are undoubtedly conservative, in that they do not account for additional potential grazing by abundant zooplankters such as copepod nauplii and the copepod *Oithona similis* that were not used as experimental grazers. However, concurrent studies of toxin accumulation in smaller zooplankton size fractions that were dominated by such small zooplankters revealed low levels of toxin accumulation, suggesting that they do not appreciably ingest *Alexandrium* spp.. We conclude that due to its low *in situ* abundance in the offshore Gulf of Maine, *Alexandrium* appears to be an unimportant component of the diets of its grazers. Thus, the antipredation effects of high concentrations of *Alexandrium* on some grazers that have been reported from laboratory studies probably occur in nature only rarely, due to low *in situ* abundances of *Alexandrium* spp., and dilution of adverse effects of *Alexandrium* toxins on their grazers by ingestion of other co-occurring food sources. Further, the impact of zooplankton grazing on *Alexandrium* spp. populations in the Gulf of Maine appears to be minimal, except in rare cases where abundances of grazers are unusually high.