

Alexandrium fundyense cyst dynamics in the Gulf of Maine

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Abstract

The flux of cells from germinated cysts is critical in the population dynamics of many dinoflagellates. Here, data from a large-scale cyst survey are combined with surveys in other years to yield an *Alexandrium fundyense* cyst distribution map for the Gulf of Maine that is massive in geographic extent and cyst abundance. The benthic cyst population extends nearly 500 km alongshore. Embedded within it are several distinct accumulation zones or “seedbeds,” each 3000–5000 km² in area. Maximal cyst abundances range from 2–20 × 10⁶ cysts m⁻². Cysts are equally or more abundant in deeper sediment layers; nearshore, cysts are fewer by a factor of 10 or more. This cyst distribution reflects sedimentary dynamics and the location of blooms in overlying surface waters. The flux of germinated cells from sediments was estimated using a combination of laboratory measurements of cyst germination and autofluorescence and observations of cyst autofluorescence in the field. These measurements constrained a germination function that, when applied to the cyst distribution map, provided an estimate of the germination inoculum for a physical/biological numerical model. In the laboratory studies, virtually all cysts incubated at different temperatures and light regimes became autofluorescent, but the rate of development was slower at lower temperatures, with no difference between light and dark incubations. Germination rates were highest at elevated temperatures, and were 2-fold greater in the light than in the dark. Laboratory and field fluorescence measurements suggest that > 70% of the cysts in the top cm of sediment would germinate over a 60–90 day period in offshore waters. The combination of laboratory germination experiments and numerical modeling predicts nearly 100% germination of cysts in the top cm of sediment and resulting early season cell concentrations that are comparable in magnitude to observed cell distributions. It cannot account for late-season peaks in cell abundance that are heavily influenced by vegetative growth. Cyst germination flux from deep-water (> 50 m) cyst seedbeds is 14X the flux in shallow waters.

A conceptual model is proposed that is consistent with observed and modeled *A. fundyense* cyst and motile cell distributions and dynamics in the Gulf of Maine. Cysts germinate within the Bay of Fundy seedbed, causing localized, recurrent blooms that are self-seeding and “propagatory” in nature, supplying cells that populate the eastern segment of the Maine Coastal Current (MCC) and eventually deposit cysts offshore of Penobscot and Casco Bays. These cysts serve as a seed population for western Maine blooms that are transported to the west by the western segment of the MCC, where cells are removed either by mortality or advection from the region. Without the localized, “incubator” characteristic of the

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Bay of Fundy bloom zone, *A. fundyense* populations in the Gulf of Maine should diminish through time. Their persistence over many decades highlights the effectiveness of the mechanisms described here.

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1. Introduction

A number of key harmful algal bloom (HAB) species have dormant, cyst stages in their life histories, including *Alexandrium* spp., *Pyrodinium bahamense*, *Gymnodinium catenatum*, *Cocchlo dinium polykrikoides*, *Pfiesteria piscicida*, *Chattonella* spp., and *Heterosigma akashiwo* (reviewed in Matsuoka and Fukuyo, 2003). Accordingly, an important aspect of many HABs is their reliance on life history transformations for bloom initiation and decline. For the dinoflagellates, resting cysts remain in bottom sediments or near-bottom nepheloid layers when conditions in the overlying waters are unsuitable for growth (Wall, 1971; Dale, 1983). Several factors regulate the length of that resting state. Some are internal, including a mandatory maturation period after encystment lasting days to months depending on the species (e.g., Anderson, 1980; Bravo and Anderson, 1994) and an endogenous annual clock as observed in *Alexandrium fundyense* (Anderson and Keafer, 1987; Perez et al., 1998; Matrai et al., 2005). Germination is also controlled by external, environmental factors, such that the resting state of a mature cyst will continue (termed quiescence) if external conditions are unfavorable for growth. Temperatures above and below a “window” or range that supports germination can maintain quiescence (Dale, 1983; Anderson, 1998). Likewise, cysts of some species require light for germination, while others germinate more slowly in darkness than in light (Anderson et al., 1987). A primary stimulus for excystment is a shift in temperature to favorable levels, as occurs in seasonal warming or cooling in temperate waters (Anderson and Morel, 1979).

Oxygen (or the lack thereof) can have a dramatic effect on cyst germination. Most dinoflagellate species examined to date have an absolute requirement for oxygen during germination (Anderson et al., 1987; Rengefors and Anderson, 1998; Kremp and Anderson, 2000). Cysts buried deep in the sediment can thus remain quiescent for years, their fate being either eventual death if anoxia persists, or

germination should they be transported to the sediment surface or overlying water. The longevity of buried cysts is difficult to determine, but quantitative cyst profiles, radioisotope measurements, and a simple model suggest that the half-life of *A. tamarensense* cysts in anoxic sediments is approximately 5 years (Keafer et al., 1992).

Once germination occurs, the water column is inoculated with a population of cells that begins to divide asexually via binary fission to produce a bloom. At the end of the bloom, vegetative growth ceases and the cells undergo sexual reproduction, often in association with nutrient limitation (Pfiester and Anderson, 1987; Anderson and Lindquist, 1985). Gametes are formed that fuse to form the swimming zygotes that ultimately become dormant cysts. Clearly, the location of cyst accumulations in bottom sediments (termed “seedbeds”) can be an important determinant of the location of resulting blooms, and the size of the cyst accumulations and the timing and extent of excystment and encystment can directly affect the magnitude of the blooms (Wall, 1971).

Among the cyst-forming HAB dinoflagellates, *Alexandrium* species such as *A. tamarensense*, *A. fundyense*, *A. catenella*, and *A. minutum* are perhaps the best studied with respect to population dynamics because of their links to paralytic shellfish poisoning (PSP). Relatively little is known, however, about the quantitative role of cysts in these toxic *Alexandrium* blooms. This reflects the dynamic nature of marine coastal waters and the logistical difficulties encountered in attempting to directly measure germination fluxes or the timing and extent of cyst formation and deposition.

In temperate waters such as the Gulf of Maine, blooms of *A. fundyense*¹ are highly seasonal (Anderson, 1997), consistent with the view that excystment

¹Both *A. tamarensense* and *A. fundyense* occur in the Gulf of Maine (Anderson et al., 1994). We consider these to be varieties of the same species (Anderson et al., 1994; Scholin et al., 1995). Thus, for the purpose of this study, the name *A. fundyense* is used to refer to both forms, as this is the dominant variety observed in the study area (Anderson et al., 1994)

and encystment are major regulatory factors. During the Ecology and Oceanography of Harmful Algal Blooms (ECOHAB)-Gulf of Maine (GOM) project, several attempts were made to directly measure the flux of germinated cells that emerged from the surface layer of this massive seedbed through time. These were unsuccessful, however, so an indirect method was attempted using a combination of laboratory measurements of cyst germination rates, laboratory and field measurements of cyst autofluorescence, a map of benthic cyst abundance, and a numerical model. A function estimating the germination rate under different environmental conditions was constructed using laboratory germination data. This function was incorporated into a hydrodynamic model of the Gulf of Maine and applied to the cyst distribution map to provide a time varying estimate of the germination source. This estimate was further refined and constrained using the measurements of cyst autofluorescence, since *Alexandrium* cysts synthesize chlorophyll as they prepare to germinate and emit red autofluorescence (Anderson and Keafer, 1985). Such observations can be used as an indicator of the fraction of the sampled cyst population that is in the process of germinating. When combined with analogous autofluorescence measurements in the laboratory, these measurements provide guidance for the application of the laboratory-derived germination function to the field. In this study, field autofluorescence measurements are mainly used to constrain the depth of sediment over which cysts can germinate. However, they have the potential to provide more detailed estimates of the influence of sediment dynamics on the germination source and may thus be integral to further model improvement.

Here, we present the fluorescence and germination time-course experiments (the latter used to specify germination rates in the model), as well as the conclusions drawn from estimates of excystment fluxes. More details on the model formulation, its extension to include vegetative growth, and other aspects of *A. fundyense*'s population dynamics are given in Stock et al. (2005), and McGillicuddy et al. (2005).

2. Methods

2.1. Cyst mapping

In October 1997 during a cruise on the *R.V. Endeavor*, sediment samples were collected from 79 stations in offshore and nearshore waters

between Portsmouth, NH, and the Canadian border (Fig. 1). Station locations were determined in part from historical toxicity patterns as well as from sediment deposition maps. The timing of the collection was scheduled to be well after new cysts have formed and settled in the spring, but before any potential germination of mature cysts occurs the following year when light and temperature are optimal. Additional samples were taken in 2001 along a single transect south of Penobscot and Casco Bays to provide data in a region where more resolution was needed to define the spatial extent of the large cyst seedbeds located there. Another special survey was mounted in 2003 to examine cyst abundance along several transects north of Cape Ann, MA and at the western boundary of the 1997 survey.

Samples for these surveys were collected using a hydraulically damped corer (Craib, 1965), which provides undisturbed sediment samples. The top three cm of sediment were collected, stored, and processed according to Anderson et al. (1982). Core samples were processed into the top 0–1 cm and 1–3 cm layers, sonicated with a Branson Sonifier 250 at a constant 40-W output for 1 min, and sieved to yield a clean, 20–75 μm size fraction (Anderson et al., 2003). *A. fundyense* cysts were counted in 1-ml Sedgewick Rafter slides according to standard methods for cyst identification and enumeration (Anderson et al., 2003) using primulin to stain the cysts (Yamaguchi et al., 1995). Briefly, 9 ml of processed sediment were preserved by the addition of 1 ml of 10% paraformaldehyde and returned to 2–4°C for at least 30 min. This sample was then centrifuged for 10 min, overlying water decanted, brought up to 10 ml with methanol, and stored in the same refrigerator for at least 48 h. The sample was centrifuged and decanted as before, brought up to 9 ml with deionized water plus 1 ml of primuline stain (2 mg ml⁻¹). After staining for 30 min, the sample was centrifuged and decanted for the final time and brought up to 5 ml with deionized water. Two 1 ml counts of each final, stained subsample were made on a Zeiss Axioskop microscope under blue light epifluorescence at 160X with a chlorophyll filter set (band pass 450–490 nm, long pass 520 nm). Stained cysts were easily and rapidly counted at low magnification. Note that some stained cysts with no contents may be counted with this method. These are few in number, however, as empty *A. fundyense* cysts are easily deformed during sonication and sieving, and most empty cysts

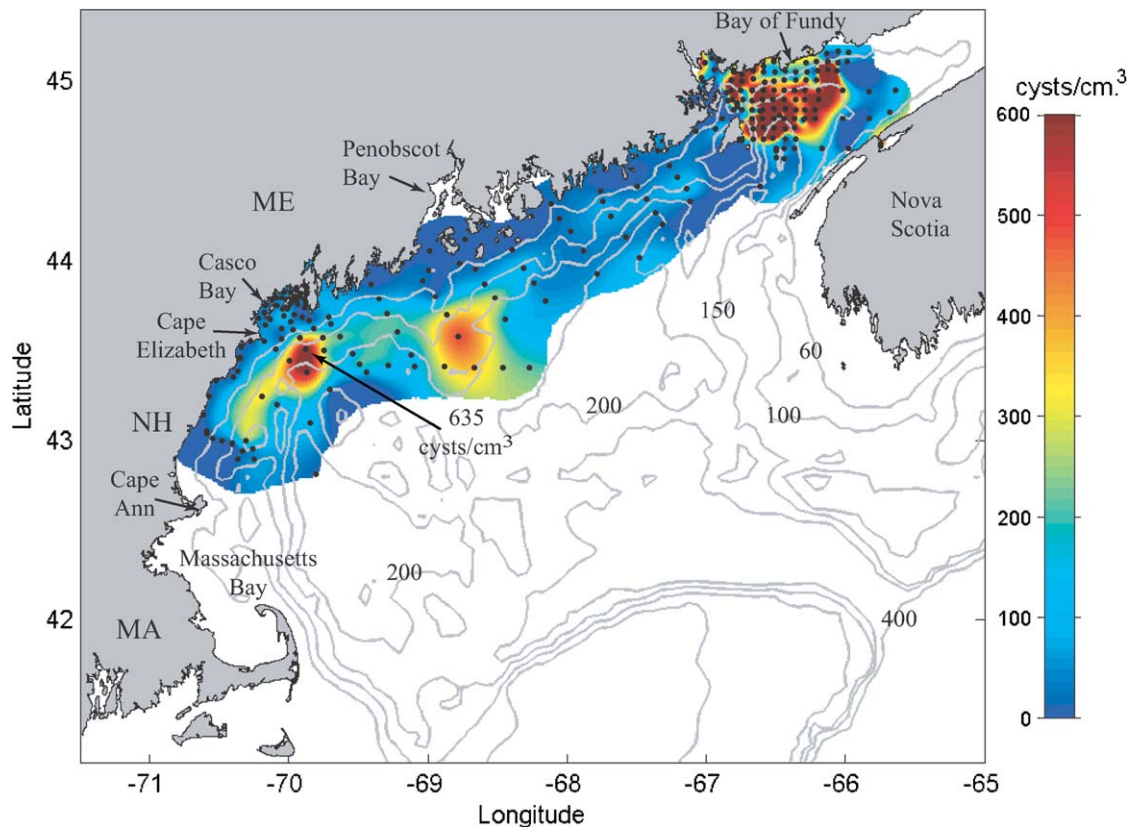


Fig. 1. Distribution and abundance of *A. fundyense* cysts in surface sediments (top cm) of the Gulf of Maine. Station locations are shown as filled circles. Data are combined from multiple surveys. Samples in the Bay of Fundy are predominately from White and Lewis (1982), Martin and Wildish, (1994), and J. Martin (unpublished data), while most other samples between the Bay of Fundy and the western edge of the sampling domain are from the 1997 ECOHAB-GOM cyst survey. Seven samples were taken in 2001 along a single horizontal transect south of Penobscot and Casco Bays near the offshore edge of the sampling domain. Data are also included from a 2003 survey north of Cape Ann, MA at the western boundary of the 1997 survey (nine samples).

therefore do not have the intact, elongate morphology used as a diagnostic feature for counting.

To provide a more complete cyst distribution map, *A. fundyense* cyst abundance data from surveys in the BOF in 1982, 1983, and 1984 (White and Lewis, 1982; Martin and Wildish, 1994; J. Martin, unpub. data) were incorporated into the Gulf of Maine dataset. These BOF samples were collected with a Hunter and Simpson Grab (0.1 m²), and processed as follows. A subsample of sediment 4.5 cm in diameter and 2.0 cm in depth was taken from the initial sample, and a 1.0 cm³ subsample was removed and resuspended with cold filtered seawater in a 15 ml centrifuge tube. Samples were sonicated for approximately 1 min in a Millipore 40-W, 55 kHz sonifier, then washed with cold filtered seawater onto a mesh sieve. Material on the sieve was backwashed into the centrifuge tube and centrifuged at 750g for 5 min. Following

centrifugation the volume of the sample was reduced to 3.0 ml, the sample was shaken and *A. fundyense* cysts were counted in 0.1 ml Palmer Maloney counting chambers using brightfield illumination. All samples were counted in triplicate. To make all cyst data consistent for the composite map, multiple vertical profiles of *A. fundyense* cyst abundance were examined to determine the abundance of cysts in the top 1 cm relative to those in the top 2 cm. We found approximately equal numbers of cysts in these two layers, so BOF concentrations for the 0–2 cm layer could be used to approximate concentrations for the top cm alone.

2.2. Cyst fluorescence — field samples

Cores were taken at two locations along an offshore transect near Casco Bay in the western Gulf of Maine region (Stations B1 and B9–43°25.50'N,

69°55.22' W and 43°41.43' N, 70°02.17' W). These stations were revisited 11 times from April through July 1998. Data from the two stations were averaged to give a single measurement of the percentage of *A. fundyense* cysts that were fluorescing at each sampling time. Fluorescence data also were obtained from a core from a nearby station in February 1998. The top cm of freshly collected cores was carefully extruded, its outer edges discarded to avoid contamination from lower layers (Anderson et al., 1982), and the resulting sample stored in the dark at 4 °C until analysis, typically within 24 h. Samples were processed and sieved as above, and the resulting size-fractionated material reconstituted to 5 ml with filtered seawater. Of this live sample 1 ml was observed in a Sedgewick–Rafter counting chamber using an epifluorescence microscope. The slide was scanned at 200× magnification with tungsten light to locate at least 30 cysts. Each of the cysts was observed under blue light with a chlorophyll filter set (band pass 450–490 nm, long pass 520 nm) to determine the percentage of cysts exhibiting visible red chlorophyll autofluorescence.

2.3. Cyst germination and fluorescence in the laboratory

Experiments were conducted utilizing a range of temperatures appropriate for the Gulf of Maine, each with and without light, to demonstrate the effects of temperature and light on the chlorophyll autofluorescence and germination of *A. fundyense* cysts in field samples. Germination data derive from several different experiments using sediments and cysts from different regions of the Gulf of Maine. The sediment used for laboratory germination and fluorescence studies of western Gulf of Maine (WGOM) cysts was collected from offshore station No. 38 in Casco Bay, Maine (43°30.29'N, 69°52.72'W). This station was chosen because of the high concentration of cysts (540 cysts per cc of sediment in the 2–3 cm layer). For the eastern Gulf of Maine (EGOM) experiments, sediment was collected at station No 4 in the Bay of Fundy (44° 42' N, 66° 30' W).

The WGOM and EGOM sediment samples were treated in similar ways, though container types, volumes of added medium, and other details are slightly different. All sediment manipulations were conducted under red light in near darkness. Most cysts also were shielded from light by the mass of sediment surrounding them. For the WGOM

sediments, the 2–6 cm layer of sediment was stored under anoxic conditions at 2 °C in the dark until a subsample of the cysts was shown to have a 90–93% germination potential at 15 °C in a 14:10 h light:dark cycle. Then 50 ml of f/2 medium (Guillard and Ryther, 1962) were distributed into 145 flasks and chilled to 4 °C. A slurry was made up of one part sediment to seven parts f/2 medium. While keeping the sediment slurry well mixed, 10-cc aliquots were placed in each flask, with all manipulations in a 4 °C darkroom.

For the EGOM sediments, mud was stored in filled sampling cups (120 cc) without any headspace. Caps were sealed with Parafilm, and placed in a light blocking gas-sampling bag. The bag was then nitrogen flushed and samples stored under anoxic conditions at 2 °C in the dark. Germination experiments commenced in June 2, 2000 when data from the endogenous clock experiment (Matrai et al., 2005) showed that cysts from station 4 were germinating at rates higher than 90%. Equal quantities of mud from each of the BOF stations 4-A, B, and C were mixed together in a darkroom dimly lit by a red bulb and maintained at a temperature between 2–4 °C. A mud slurry was then made in f/2 medium, and 10-ml aliquots of this slurry delivered to 250-ml polycarbonate flasks containing 50 ml of the same medium. The 60-ml content of each flask was gently swirled, thoroughly mixing the added slurry with the medium.

For both WGOM and EGOM sediments, flasks were randomly separated into seven or eight different incubation conditions with 18–26 flasks per treatment. Flasks were placed into incubators at 2, 4, 6, and 8 °C, with EGOM sediments exposed to 15 °C as well. Half of the experimental flasks were exposed to light at 5–15 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ (14 h:10 h L:D cycle), while the others were kept in the dark using multiple layers of tin foil. Six remaining flasks were processed to determine the average initial cyst count per flask and the initial chlorophyll autofluorescence. All flasks were thoroughly swirled once a week throughout the experiment in order to ensure a consistent exposure to light and/or oxygenated media. The flasks were harvested at specific intervals, with the non-harvested flasks remaining in the incubators. Most of the flasks were harvested in duplicate (two from the light and two from the dark), but individual flasks were occasionally harvested in order to conserve samples. For germination determinations, the harvested flask was thoroughly rinsed and the

contents sonicated, sieved, stained with primulin, and counted as described above. The difference between the initial cyst number and the cyst number after incubation was used to calculate the percentage of cysts that had germinated over 3 weeks. For autofluorescence determinations, randomly selected flasks from each temperature were sonicated, sieved, and cyst autofluorescence determined as described above.

2.4. Germination model

The modeled rate of germination (G , % of initial cysts day^{-1}) is a function of bottom water temperature (T , °C), irradiance (E , W m^{-2}), and the endogenous clock ($EC(t)$, where t is the year-day)

$$G(T, E, t) = EC(t) \times G(T, E).$$

Temperature and light dependence were constructed using data from the germination time series described above. The slope of a linear fit was used to associate a germination rate with each experimental condition. The fitting procedure was complicated by the asymptotic behavior of the data points as the germination potential was approached. In order to capture the slope during the period over which the majority of cysts germinate, only points prior to the attainment of 90% germination and those taken less than 75 days after the beginning of the time series are included in the fit. The fitted lines were not forced through the origin to minimize the influence of any necessary acclimation to experimental conditions after transfer from cold, dark storage. Laboratory fluorescence rates were similarly estimated and are presented herein, although they are not used within the germination model.

These discrete germination rates were interpolated and extrapolated to other temperatures by fitting to the function

$$G(T, E_{\text{igt}}) = G_{\text{min}}(E_{\text{igt}}) + \frac{(G_{\text{max}}(E_{\text{igt}}) - G_{\text{min}}(E_{\text{igt}}))}{2} \times \{ \tanh(\alpha(E_{\text{igt}})T - \beta(E_{\text{igt}})) + 1 \},$$

$$G(T, E_{\text{drk}}) = G_{\text{min}}(E_{\text{drk}}) + \frac{(G_{\text{max}}(E_{\text{drk}}) - G_{\text{min}}(E_{\text{drk}}))}{2} \times \{ \tanh(\alpha(E_{\text{drk}})T - \beta(E_{\text{drk}})) + 1 \},$$

where E_{igt} and E_{drk} refer to the irradiance under the “light” and “dark” experimental conditions. The hyperbolic tangent function above allows for a linear relationship over moderate temperatures (slope = α) that is consistent with many previous investigations of seed germination behavior (Bewley and Black, 1982). It also allows for maximum germination rates (G_{max}) and minimum germination

rates (G_{min}) to occur over broad ranges, which is suggested by previous observations of *A. fundyense* germination behavior (Anderson, 1998).

The light response is interpolated and extrapolated to other values using the following:

$$G(T, E) = G(T, E_{\text{igt}}) \quad E \geq E_{\text{igt}},$$

$$G(T, E) = G(T, E_{\text{drk}}) \quad E \leq E_{\text{drk}},$$

$$G(T, E) = G(T, E_{\text{drk}}) + (G(T, E_{\text{igt}}) - G(T, E_{\text{drk}})) \times \frac{E - E_{\text{drk}}}{E_{\text{igt}} - E_{\text{drk}}} \quad E_{\text{igt}} > E > E_{\text{drk}}.$$

For the purpose of this calculation, E_{drk} is taken to be 1% of E_{igt} . Sensitivity analyses (see Stock et al., 2005) show that model results in the Gulf of Maine are insensitive to the choice of this parameter when it is varied over several orders of magnitude. To approximate the light reaching the cysts within the sediment, observed surface shortwave irradiance is exponentially attenuated in the water column ($k_w = 0.2 \text{ m}^{-1}$) and within the sediment ($k_s = 3.5 \text{ mm}^{-1}$). The former value was chosen to be representative of the relatively clear coastal waters of the Gulf of Maine, while the latter is based on the findings of Kuhl and Jorgensen (1994).

The endogenous clock function ($EC(t)$) was formulated using data from Anderson and Keafer (1987), Matrai et al. (2005), and Anderson et al. (unpublished data). Germination potential data from seven different isolates was used. In each data set, germination of cysts was measured over 4–6 week periods throughout the year. The total number of cysts germinated relative to the total cysts in the experiment defines the germination potential. In order to minimize the influence of any shift in the period of the endogenous cycle expected after removal from the natural environment (see

Discussion in Matrai et al., 2005), only the first year of data after cyst isolation was used. Points were binned by month based on the mid-point of the experimental period. The median germination potential of each monthly bin was calculated, and the points connected using a piecewise linear function to form a representative endogenous clock. The median was used to prevent the creation

of a representative cycle that, because of averaging in variable areas, has properties that are dissimilar from any of the data sets contributing to it. Most notably, use of the mean would lead to a greatly damped oscillation. The peak germination potential would only be $\sim 85\%$, while the minimum would be $\sim 30\%$. The median focuses the fit on characteristics common to the majority of the cycles: a sharp late fall/winter low in germination potential followed by an abrupt spring rise to just under 100%, then maintenance of the high potential throughout the spring and majority of the summer until a more gradual late summer, early fall decline.

The germination function is applied to the cyst map described above to provide an estimate of the cyst flux (F_g)

$$F_g = \int_0^{1 \text{ cm}} G(T, E(z), t) \times [\text{Cysts cm}^{-3}]_0 dz,$$

where $[\text{Cysts cm}^{-3}]_0$ is the initial number of cysts in the top centimeter of the sediment.

2.5. Hydrodynamic model

In order to assess the transport pathways of newly germinated cells, the predicted germinated cyst flux is specified as an input to a hydrodynamic model of the region. Because our objective is to investigate large-scale transport by the mean currents, we utilize a model of the seasonal climatological flow of the Gulf of Maine/Georges Bank region (Lynch et al., 1996). This three-dimensional finite element model is hydrostatic, nonlinear, and incorporates advanced turbulence closure. Published solutions for the climatological mean circulation, broken down into six bi-monthly periods, are demonstrably consistent with available observations (Naimie, 1996; Naimie et al., 2001). Lynch et al. (1997) described simulations of springtime circulation in the Maine Coastal Current that are particularly relevant in the present context. Archived model results are stored in a form that is available for use in an off-line transport code that solves a depth-averaged form of the advection-diffusion-reaction equation on the same mesh. This code constitutes the basis for *A. fundyense* transport simulations in which the ex-cystment flux described above is specified as the “reaction” term.

3. Results

3.1. Field data

Cyst distributions. A large-scale mapping survey of cyst abundance was mounted in 1997, spanning the region from Cape Elizabeth, ME to the Bay of Fundy (BOF). *Alexandrium fundyense* cyst abundance data from surveys in the BOF in 1982, 1983, and 1984 (White and Lewis, 1982; Martin and Wildish, 1994; J. Martin, unpub. data) also were incorporated into the Gulf of Maine dataset, as were data from a small cruise in October 2003 near Cape Ann (see below).

Fig. 1 shows the composite map of *A. fundyense* cyst abundance in top 1 cm of bottom sediments throughout the Gulf of Maine. In addition to a broad, low-density distribution that spans nearly 500 km, several accumulation zones with high cyst concentrations are apparent that can be considered potential “seedbeds”. Cysts were most abundant in the Bay of Fundy where maximum abundance approached 2000 cysts cm^{-3} in the top cm. Elsewhere, the maximum cyst concentration was 635 cysts cm^{-3} offshore of Casco/Penobscot Bays, within a fairly large region with values from 400–600. Cysts were equally or more abundant in the 1–3 cm layer. For the 1997 survey, for example, the mean cyst concentration in the top cm was 87.7 cysts cm^{-3} , whereas the 1–3 cm layer mean was 111.4 ($n = 79$).

Within the Gulf of Maine, the offshore seedbeds lie beyond the 75 m isobath, with dense cyst accumulations at 150 m and deeper in the basins. The accumulation zones offshore of Penobscot and Casco Bays appear to represent two distinct but interconnected maxima. The total area of this combined western seedbed is 5100 km^2 , with an average cyst concentration of 275 cysts cm^{-3} , equivalent to 1.4×10^{16} total cysts in the surface sediments of the seedbed. The BOF seedbed covers 3500 km^2 and contains an average cyst concentration of 524 cysts cm^{-3} , equivalent to 1.8×10^{16} total cysts. Nearshore, cyst concentrations ranged from 0 to 100 cysts cm^{-3} , typically at least an order of magnitude lower than in offshore waters. Cysts were predominantly associated with the clay-silt fraction, but abundance was highly variable within that fraction (Hyatt et al., 2000). Indeed, the major cyst deposits in the BOF and offshore of Casco and Penobscot Bays occur where clay and silt are the major sedimentary constituents (Fig. 2).

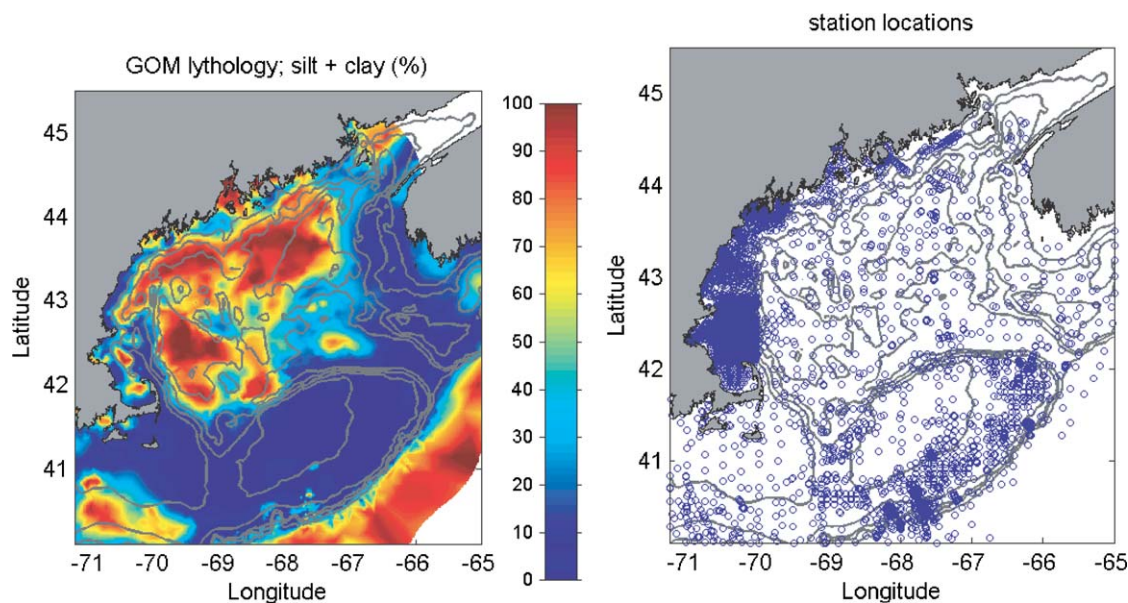


Fig. 2. Left: silt and clay fraction of sediments in the Gulf of Maine region. Right: station positions. Data source: Poppe et al. (2003).

In assembling the large-scale cyst map, an effort was made to include results from prior cyst surveys to the west, near and within Massachusetts Bay in 1983 and 1984 (Anderson and Keafer, 1985). However, unlike the BOF situation where several recent surveys confirmed the continued existence of a large cyst seedbed to the north and east of Grand Manan Island (Martin and Wildish, 1994; J. Martin, unpublished data), comparison of cyst abundances in the westernmost samples from the 1997 large-scale ECOHAB-GOM cyst survey with those from similar stations sampled in 1983 and 1984 by Anderson and Keafer (1985) showed large discrepancies, with the latter being substantially higher than the former. Accordingly, a special cruise was conducted in October 2003 to resample sediments to the immediate north and east of Massachusetts Bay. Counts of *A. fundyense* cysts in those samples were consistently lower than the 1983 and 1984 counts, generally by an order of magnitude or more. For example, two stations where more than 1000 cysts cm^{-3} were counted in 1983 had 1/10th that number in 2003, whereas another with 200 cysts cm^{-3} in 1983 had only 20 cysts cm^{-3} in 2003. Therefore, the 2003 data were consistent with the 1997 large-scale survey results for that specific region (and are thus included in the composite figure), suggesting that the westernmost edge of the cyst bed offshore of Casco Bay is reduced compared to the earlier 1980s report. Anderson and Keafer's

(1985) cyst data for 1983 and 1984 near Massachusetts Bay are not included in the regional, composite cyst map produced for this study.

3.1.1. Chlorophyll autofluorescence in cysts

No cysts in surface sediments collected in February 1998 had visible red autofluorescence (Fig. 3A). At the two stations near Casco Bay that were sampled repeatedly during subsequent months, the percentage of cysts in the top cm of sediment that were fluorescing increased, reaching 74% in May and June, suggesting that most of the cysts within this sediment depth will germinate. The cysts within the top centimeter were therefore used for the modeling efforts described below. A general trend of increasing fluorescence through time followed by a decrease in June and July is suggested in the field fluorescence data, but there is considerable variability. Bottom temperatures increased from 4.7 to 6–8 °C over that time interval. In the overlying water column, average *A. fundyense* motile cell abundance increased gradually in April and May, peaking near 205 cells l^{-1} in mid-May and decreasing to undetectable levels by late June (Fig. 3B).

3.2. Laboratory studies

3.2.1. Cyst fluorescence

Laboratory studies of the development of chlorophyll autofluorescence in cysts incubated under

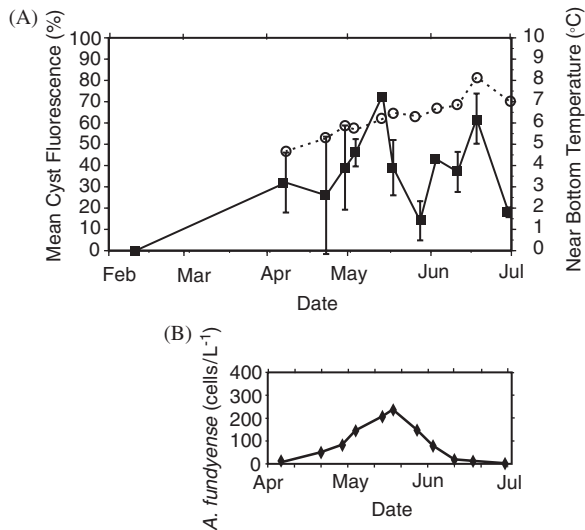


Fig. 3. Time course of *A. fundyense* cyst fluorescence (% of total cysts which had distinct red chlorophyll autofluorescence), *A. fundyense* motile cell abundance, and bottom water temperature near Casco Bay. (A) Average percentage of cysts with red autofluorescence at two stations (B1 and B9) near Casco Bay (■). Error bars denote one standard deviation) and average near bottom temperature (○) and (B) Average *A. fundyense* motile cell concentrations (cells/L⁻¹) in surface waters along transects near stations B1 and B9 during the 1998 bloom season (2–4 transects sampled each time period (see Keafer et al., 2005a).

different temperature and light conditions were conducted for both WGOM and EGOM cysts (Fig. 4). Under all incubation conditions, most of the cysts eventually became fluorescent, whereas those not removed from long-term 2 °C dark storage did not fluoresce. Differences were observed in the timing of fluorescence development at different temperatures, with a slower rate of development at lower temperatures in both the light and the dark. WGOM cysts appeared to fluoresce slightly more quickly than those from the EGOM. Fluorescence rates were calculated from the slopes of these time series in a manner analogous to that outlined for germination (Table 1). The rates at which the cyst populations became fluorescent were similar in the light and the dark for each specific temperature (e.g., Figs. 4A and B).

Calculated uncertainties in the rates are based only on the linear regression to points within the increasing portion of the curve (see the fitting criteria in Section 2). This results in large uncertainties in rates based on time series with few observational points in the specified interval (e.g.,

fluorescence time series at moderate to high temperatures). The statistical uncertainty in such cases is listed for completeness, but is conservative given that constraints imposed by the points within the asymptotic region of the curve are not included in the calculation. For example, consider the WGOM isolate incubated at 8 °C under light conditions, for which the inferred rate is $13.92 \pm 13.14\% \text{ day}^{-1}$ (Table 1). Clearly, the lower bound of this estimate is not realistic, given there are eight points near 100% fluorescence in this time series (Fig. 4). Cases where the number of points in the increasing portion is small are noted in Table 1 and Table 2, and their uncertainty should be interpreted cautiously.

3.2.2. Cyst germination

Laboratory studies of germination success at different temperature and light conditions for both WGOM and EGOM *A. fundyense* cysts are summarized in Figs. 5 and 6 and Table 1. In excess of 90% germination was achieved in most treatments. The observed time courses and calculated germination rates were generally similar for EGOM and WGOM cysts (e.g., Figs. 5A and B vs. Figs. 5B and C; Table 1), although rates in the dark were somewhat faster for EGOM isolates, particularly at 8 °C in the dark. At low temperatures, germination was slow in the light and in the dark, with the rate increasing as temperatures increased. For example, at 2 °C, 50% germination required 40–60 days of incubation, with germination rates of 1.74 and 1.21% day⁻¹ in the light and dark, respectively, for EGOM cysts (Table 1). At 8 °C, 50% germination was observed after approximately 10–15 days in the light and in the dark, with the associated germination rates being 8.72 and 4.24% day⁻¹ for EGOM cysts. Light germination rates were often significantly greater than those in the dark. This was particularly true of WGOM strains, where significant ($p < 0.1$) increases in the germination rate were observed at 4, 6, and 8 °C. For the EGOM strain, differences were most significant at 15 °C ($p < 0.1$). Fig. 6 shows a composite of all germination data for cysts from both regions.

Comparison of fluorescence and germination time courses revealed that maximal germination took approximately twice as long to achieve as maximal fluorescence in the light, regardless of temperature. This difference was approximately 3–4-fold in the dark. For example, at 2 °C in the light, fluorescence

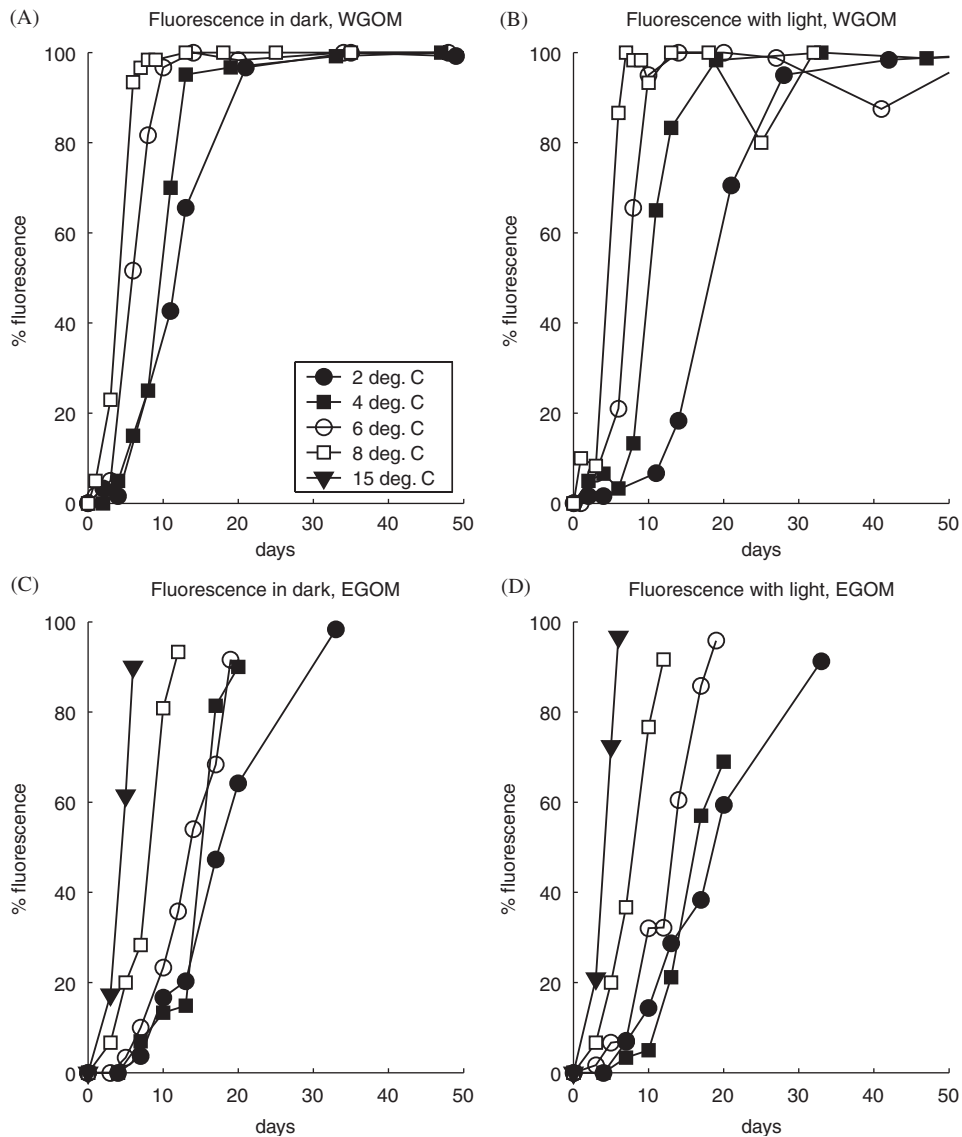


Fig. 4. Time course of chlorophyll autofluorescence development in *A. fundyense* cyst populations incubated at different temperatures. WGOM cysts incubated in darkness (A) and light (B) at 2 (●), 4 (■), 6 (○), and 8 °C (□), and EGOM cysts incubated in darkness (C) and light (D) at 2 (●), 4 (■), 6 (○), 8 (□), and 15 °C (▼). There are no 15 °C rates for the WGOM cysts.

peaked after 30–35 days for WGOM and EGOM cysts, whereas germination was maximal after 60–80 days (Figs. 4 and 5). At 6 °C, fluorescence was maximal after 15 days, and germination after 30 days, again in the light. At 15 °C in the light, fluorescence peaked after approximately 5 days, with germination being maximal after 10 days. In the dark at 6 °C, fluorescence was maximal after 20 days, while germination peaked after 60–90 days.

3.3. Model simulation results

The temperature responses of the germination model derived from the time series experiments above (Fig. 6) exhibit a broad region of minimum germination rates (<4 °C) and maximum rates (>10 °C) intersected by a limited region of linear increase, consistent with the findings of Anderson (1998) and Bewley and Black (1982). Values of the fitted parameters are reported in Table 3. The light

Table 1

Fluorescence rate estimates (% day⁻¹) and the width of the 90% uncertainty interval for *A. fundyense* cysts from the western and eastern Gulf of Maine (WGOM, EGOM) incubated at different temperatures in the light and dark

Temperature (°C)	Dark rate (WGOM)	Dark rate (EGOM)	Light rate (WGOM)	Light rate (EGOM)
2	5.04 (1.94)	3.31 (1.09)	2.95 (1.76)	3.00 (0.81)
4	6.01 (3.02)	4.05 (3.18)	6.38 (3.06)	3.70 (1.55)
6	10.53 (4.21)	4.32 (1.05)	7.41 (4.81)	5.04 (1.52)
8	15.76 (8.77) ^{a,b}	7.71 (4.51)	13.92 (13.14) ^b	7.64 (3.38)
15	NA	11.78 (28.17) ^a	NA	13.88 (32.46) ^b

NA = not available.

Uncertainty intervals were estimated using only the linear portion of the curves shown in Fig. 4.

^aThe first point above 90% was included for this rate estimate due to the lack of a data point between 25% fluorescence and 90% fluorescence in this time series.

^bThese rates were estimated with <5 data points in the region of linear increase and the uncertainties should be treated with caution (see details in text).

Table 2

Germination rate estimates (% day⁻¹) and the width of the 90% uncertainty intervals for *A. fundyense* cysts from the western and eastern Gulf of Maine (WGOM, EGOM) incubated at different temperatures in the light and dark

Temperature (°C)	Dark rate (WGOM)	Dark rate (EGOM)	Light rate (WGOM)	Light rate (EGOM)
2	0.91 (0.25)	1.21 (0.34)	0.89 (0.27)	1.74 (0.62)
4	0.82 (0.24)	1.43 (0.30)	1.69 (0.22)	1.74 (0.77)
6	1.19 (0.17)	1.70 (0.37)	2.03 (0.52)	1.58 (0.34)
8	1.40 (0.32)	3.32 (0.60)	6.55 (1.76)	4.03 (0.60)
15	NA	4.24 (0.48)	NA	8.72 (3.51)

NA = not available.

All conditions contained at least eight points in the linear region except the light rate at 15 °C, which contains only five. Uncertainty intervals were estimated using only the linear portion of the curves shown in Fig. 5.

response (Fig. 7) shows that, under modeled light attenuation conditions, cysts resting at the surface of the sediment are germinating under “dark” conditions at depths greater than ~50 m. This is compounded by light attenuation within the sediment, which, with $k_s = 3.5 \text{ mm}^{-1}$, is capable of reducing typical irradiance levels (e.g., 1200–1500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) at the sea-surface below the E_{drk} threshold by ~3 mm.

The endogenous clock function (Fig. 8) exhibits high germination potential from March through June. The increasing portion of the function is very sharp, while the decline is more gradual. The data show a considerable amount of scatter (see Anderson, 1998; and Matrai et al., 2005 for discussion of underlying mechanisms). However, the pattern of rapid ascent in the germination potential in the late winter/early spring, maintained high potential through the spring into early summer, followed by a slower decline and lows throughout the fall and early winter is apparent in the majority of the isolates tested.

Incorporation of *A. fundyense* germination into the circulation model provides predictions of vegetative cell distributions resulting from the combined effects of hydrodynamic transport and spatial and temporal variations in excystment (Fig. 9). Early season peaks in vegetative cell concentrations occur in association with the major cyst seedbeds located at the mouth of the Bay of Fundy and offshore of Casco and Penobscot Bays. Under the influence of the Maine Coastal Current system (Lynch et al., 1997), newly germinated cells are advected downstream toward the south and west, such that by the end of the simulation a broad population peak exists in the western Gulf of Maine. Traces of the cell population extend far to the south and east, where cells have been entrained into the clockwise circulation around Georges Bank. Low cell concentrations are evident all the way to the outflow boundary south of Cape Cod. Clearly, a great deal of spatiotemporal structure results simply from the input of cells from the cyst

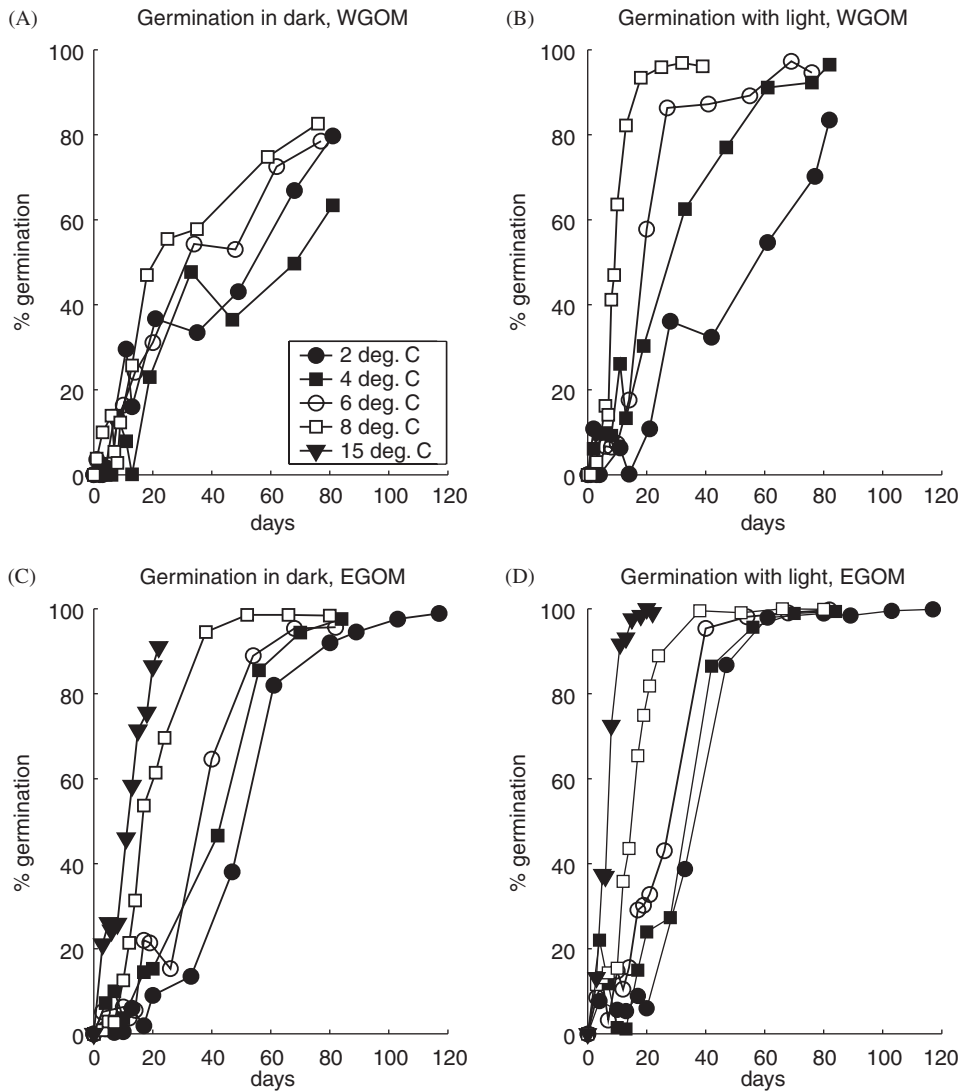


Fig. 5. Time course of *A. fundyense* cyst germination at different temperatures. WGOM cysts incubated in darkness (A) and light (B) at 2 (●), 4 (■), 6 (○), and 8 °C (□), and EGOM cysts incubated in darkness (C) and light (D) at 2 (●), 4 (■), 6 (○), 8 (□), and 15 °C (▼).

seedbeds into the complex circulation of this region. Also, note that the model predicts nearly complete germination of the mapped cyst population specified in the initial condition (not shown).

4. Discussion

The flux of cells from germinated resting cysts in bottom sediments is exceedingly difficult to quantify, particularly in deeper coastal waters and over large study domains. This is, however, a critical parameter in the population dynamics of cyst-forming HAB species such as *A. fundyense*. As the first step in a study of cyst germination dynamics of

this species within the Gulf of Maine, a large-scale cyst mapping survey was conducted. Those data, combined with results from other cyst surveys in the region, yielded a map of *A. fundyense* cyst distribution and abundance that extends from the BOF to Cape Elizabeth, ME (Fig. 1). This regional cyst population is massive in geographic extent and cyst abundance. An indirect method was used to estimate the flux of germinated cells that emerged from the surface layer of this massive seedbed through time using laboratory measurements of cyst germination and autofluorescence, and observations of cyst autofluorescence in the field. These measurements were used to constrain a germination

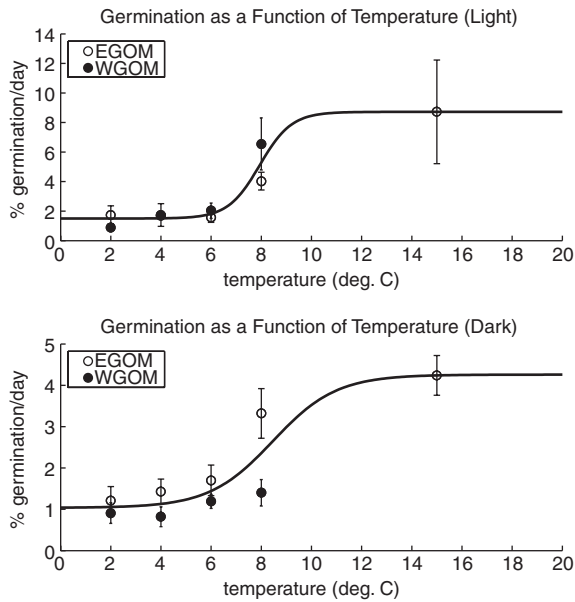


Fig. 6. Germination as a function of temperature at $E = E_{\text{lgt}}$ (top panel) and $E = E_{\text{drk}}$ (bottom panel). The symbols are experimental time series rate estimates with 90% uncertainty bounds (Table 1). The dark line is the functional fit which drives the germination model.

Table 3
Fitted parameters of the germination model

Parameter	Units	$E = E_{\text{lgt}}$	$E = E_{\text{drk}}$
α	($^{\circ}\text{C}$) $^{-1}$	0.790	0.394
β	Dimensionless	6.27	3.33
G_{max}	% day $^{-1}$	8.72	4.26
G_{min}	% day $^{-1}$	1.50	1.04

function that, when applied to the cyst distribution map, provided an estimation of the germination-driven source of *A. fundyense* cells for a coupled physical/biological numerical model. The combination of laboratory data, field data, and numerical modeling yielded realistic and detailed flux estimates and provided useful insights into *A. fundyense* cyst and motile cell dynamics. A conceptual model now can be proposed that is consistent with observed and modeled *A. fundyense* cyst and motile cell distributions and dynamics in the Gulf of Maine.

4.1. Cyst distribution and abundance

4.1.1. General considerations

All estimates of the *A. fundyense* germination flux from Gulf of Maine sediments must be based on knowledge of the regional distribution and abun-

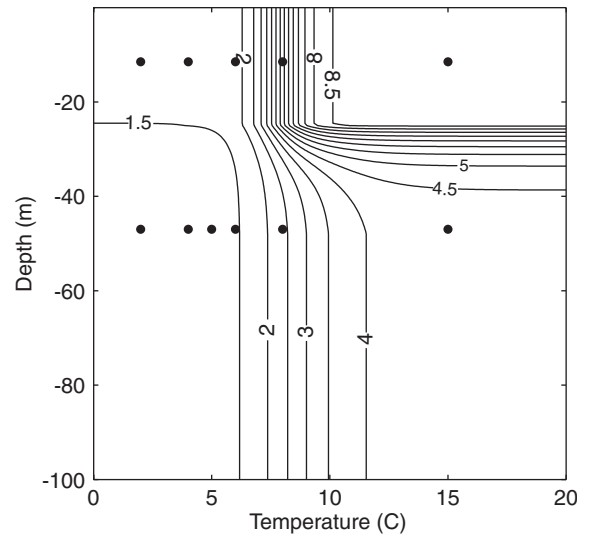


Fig. 7. Contours (interval = 0.5% day $^{-1}$) of the model estimated germination rate (% day $^{-1}$) at the sediment surface at various water depths as a function of light and temperature ($G(T,E)$). Light has been transformed to an equivalent depth using an exponential attenuation coefficient $k_w = 0.2 \text{ m}^{-1}$ and a typical day-averaged irradiance of 345 W m^{-2} (8 AM–6 PM). Filled circles show temperatures where experiments were carried out for light conditions (upper row) and dark conditions (bottom row). Note that these estimates are at the sediment surface, and there is additional light attenuation within the sediment ($k_s = 3.5 \text{ mm}^{-1}$) which is not shown.

dance of viable cysts. Our original intention was to use only data from the large-scale survey in 1997, which spanned from Cape Elizabeth to the mouth of the BOF. It soon became clear, however, that the western and eastern boundaries of this survey area omitted significant *A. fundyense* cyst accumulations that could have a major bearing on the population dynamics of this species. Accordingly, Fig. 1 represents a composite of the 1997 large-scale survey data plus that from surveys conducted in previous and subsequent years.

There is strong rationale for compiling a single cyst map that covers as much of the Gulf of Maine and the BOF as possible. A composite map can be used as a realistic approximation of the cyst distribution in the region in any given year, and forms the basis of numerical modeling exercises and other efforts to assess regional germination fluxes. We recognize the uncertainties inherent in this approach, but believe that within the precision of our modeling efforts, the key cyst seedbeds within the region were either adequately sampled by the large scale 1997 survey or are sufficiently persistent over long time intervals (i.e., the BOF — see below)

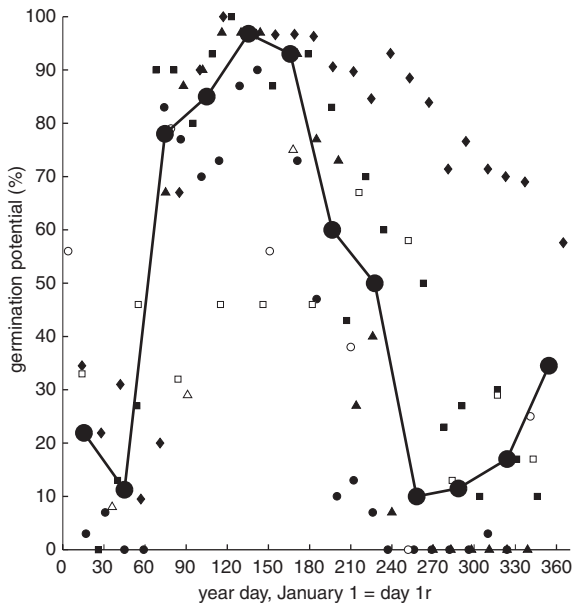


Fig. 8. The representative endogenous clock fit to the median (—●—), of various germination studies. Data are: Matrai et al. (2005): station 61 (●), station 94 (■), station 5 (▲); Anderson (unpublished, ◆); and Anderson and Keafer (1985); 1984 isolation (○), 1985 isolation (□), and 1987 isolation (△). Data are from the first year after isolation and are plotted against the mid-point of the experimental duration.

to be representative of the typical conditions during the period over which we are trying to simulate blooms.

4.1.2. Interannual variability

In support of our contention that the BOF seedbed is persistent between decades, three repeated surveys in the BOF for 1981–1983 showed no significant difference in *A. fundyense* cyst abundance year to year ($p < 0.05$). Over a larger time interval, Martin and Wildish (1994) document that the *A. fundyense* cyst abundance and distribution at the mouth of the BOF in the fall 1992 were essentially the same as observed during that same season 10 years earlier. Furthermore, more recent cyst counts in that region have again revealed cyst concentrations in the same general range as those depicted in Fig. 1 (J. Martin, unpublished data).

One area where interannual variability was observed in the cyst concentrations was in the western Gulf of Maine near Cape Ann, MA, where the high cyst accumulations observed 20 years ago (Anderson and Keafer, 1985) were no longer present, based on both our 1997 large-scale survey,

and a special survey mounted in 2003 to specifically reexamine those waters. In that area, which lies at the westernmost boundary of the large cyst seedbed located offshore of Penobscot and Casco Bays (Fig. 1), *A. fundyense* cyst concentrations decreased at least an order of magnitude between the 1984 (Anderson and Keafer, 1985) and the 1997 and 2003 surveys. The apparent disappearance of cysts in this region is noteworthy, as it may relate to the decreasing trend in PSP incidence in Massachusetts Bay and southern waters over the last decade. The large cyst accumulations observed in that region in 1983 and 1984 may have originated from the massive *A. fundyense* red tides that occurred in the Gulf of Maine in 1972–1974, some of which extended down the coast to Massachusetts Bay (Hartwell, 1975; Mulligan, 1975). Thereafter, PSP events were frequent within Massachusetts Bay in the 1980s and early 1990s. There has been, however, virtually no toxicity within the Bay since 1993 (D. Whittaker, pers. comm.) It is possible that the gradual loss of cysts from the western edge of the Penobscot/Casco Bay seedbed through germination and other loss processes, and the lack of new cysts resupplied to that boundary region may be an underlying cause for the decrease in PSP incidence in southern Gulf of Maine waters. This is clearly a speculative inference, but it is consistent with toxicity trends, and thus worthy of future study.

What might be the cause of the decrease in cyst abundance observed at the southwestern edge of the cyst deposit offshore of Casco and Penobscot Bays? Temporal variations in the abundance of cysts presumably reflect a change in balance between supply (encystment) and removal (excystment) processes. Given that interannual variations in temperature and light are relatively modest, the specific rate of excystment probably does not vary significantly from year to year. Therefore, spatial variations in deposition are the more likely cause of interannual variations in cyst abundance. As described above, the distance between one major seedbed in the Bay of Fundy and the second seedbed offshore of Casco and Penobscot Bays is set by the location within the EMCC at which ambient conditions shift from growth-favorable to encystment-favorable. McGillicuddy et al. (2005) have suggested an encystment trigger associated with the transition to oligotrophy that occurs within the EMCC during its transit westward from the high-nutrient source waters near the mouth of the Bay of Fundy (Townsend et al., 1987). Because this

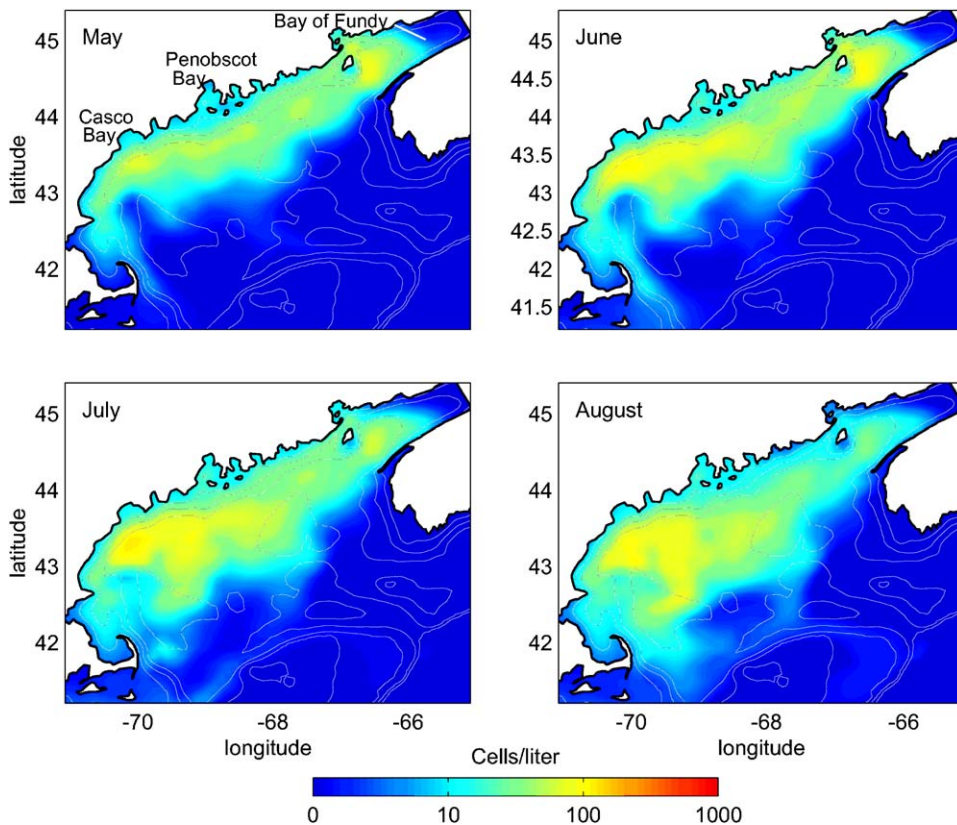


Fig. 9. Simulated *A. fundyense* distribution arising from germination and hydrodynamic transport. Mid-month snapshots of model output from May through August are shown in each row. The following bathymetric contours are overlaid: 60, 100, 150, 200, and 400 m.

transition is subject to interannual fluctuations in a variety of ecosystem properties that determine ambient nutrient concentrations, the precise location of the epicenter of cyst deposition is likely to vary in terms of its downstream position. This could potentially lead to significant fluctuations in the abundance of cysts at the fringes of the “secondary” seedbed, as was observed along its southwestern flank.

4.1.3. Distribution patterns

The regional cyst distribution is massive in geographic extent and contains very high cyst concentrations in certain accumulation zones, particularly in the BOF and offshore of Penobscot and Casco Bays. Abundances are high several cm deep in the sediment, although as described below, these cysts are not expected to contribute substantially to bloom development under normal circumstances (i.e. in the absence of major benthic disturbances). The seedbeds tend to lie offshore of the 75 m isobath, but do not correspond to specific basins or

bathymetric depressions. The observed distribution reflects in part the sediment geology of the Gulf of Maine, since the highest cyst abundances are in areas with predominantly silt and clay deposits (Fig. 2, Martin and Wildish, 1994; Hyatt et al., 2000). Sandy areas, indicative of high energy, erosional environments, had few, if any cysts. The cyst distribution is thus generally consistent with sedimentary dynamics, yet there are areas within the study domain where clays and silts dominate, but in which *A. fundyense* cysts are rare.

There is also a striking relationship between the locations of the two cyst seedbeds and areas of *A. fundyense* motile cell accumulations in the eastern Gulf of Maine and the BOF. Cruises in 1998 documented a persistent bloom to the east of Grand Manan Island in the BOF (Townsend et al., 2001), consistent with past reports of high density, recurrent blooms in that region (e.g., Martin and White, 1988). This is an area where the residence time of the water and blooms is relatively long, so it is not surprising that this is also the site of one of the

A. fundyense cyst seedbeds in Fig. 1. The 1998 cruises also mapped out a large accumulation of *A. fundyense* cells at the distal end of the EMCC, offshore of Penobscot Bay in June, with the center of mass of that population gradually shifting to the east in July and August (Townsend et al., 2001). This decrease in *A. fundyense* abundance is now thought to reflect nutrient limitation in that zone (McGillicuddy et al., 2005), which would induce sexuality and cyst formation in this species (Anderson et al., 1984). Given the underlying circulation, cysts formed in that area would settle to bottom sediments to the south and west, which is where the offshore Penobscot/Casco Bays seedbed is located (Fig. 1). That seedbed presumably reflects repeated bloom and cyst deposition events in that region, consistent with survey cruises in 2000 and 2001, which again documented an offshore population of *A. fundyense* associated with the EMCC as it veered offshore near Penobscot Bay (Townsend et al., 2005b; Keafer et al., 2005b).

4.2. Dynamics of cyst autofluorescence and germination

Under all incubation conditions, 90–100% of the cysts incubated in laboratory experiments eventually became fluorescent, but autofluorescence developed more quickly at higher temperatures (Table 1). Germination rates were often significantly greater in the light than in the dark. This was particularly true of WGOM strains, where significant increases in the germination rate were observed at 4, 6, and 8 °C. For the EGOM strain, differences were strongest at 15 °C.

Maximal germination took approximately twice as long to achieve as maximal fluorescence in the light, regardless of temperature. The difference was approximately 3–4-fold in the dark. A fluorescing cyst will eventually germinate, but the time needed for that to occur will vary with temperature and light exposure.

Field surveys of cyst autofluorescence show an initial increase in fluorescence, peaking at 74% in mid-May, followed by a prolonged period of highly scattered, moderate values (i.e. 15–60%). Incubations in the laboratory also exhibit an initial increase in cyst fluorescence, but generally peak at almost 100% fluorescence, and showed very little scatter. These differences presumably reflect (and therefore provide valuable information about) processes influencing the field data that are not

acting in the laboratory. The lower peak percentage of cysts fluorescing in the field is likely caused by the upward mixing of non-fluorescing cysts from below 1 cm, or by deprivation of oxygen within the top 1 cm of sediment. The high degree of scatter in the field data probably reflects variability in sediment mixing, the deposition of new cysts via encystment, and the spatial patchiness of the cyst distribution relative to the precision of the coring location. Explicit representations of these processes are thus necessary to reproduce the detailed structure of the field autofluorescence measurements.

4.2.1. Sediment depth for germination

For this study, the decision was made to focus all analyses and modeling on the top cm of sediment, for several reasons. First, in repeated sediment profiles during this project and in others (Anderson and Keafer, 1985), autofluorescing cysts were found almost exclusively in the top (0–1) cm. Low levels (~5%) of chlorophyll autofluorescence were occasionally observed in the 1–3 cm layer, but this was infrequent and low compared to the high values observed in surface sediments, which reached 70–80% on occasion (Fig. 3A). Second, sediments in the Gulf of Maine tend to be anoxic a short distance below the sediment surface. Using short-lived isotopes, Keafer et al. (1992) estimated an oxygenated, mixed layer 0.5 cm deep, consistent with reports from Hines et al. (1991) that oxygen is depleted within the upper 0.5 cm. Two or three cm below the sediment surface, oxygen levels are likely to be too low to support germination (Anderson et al., 1987), consistent with the general lack of chlorophyll autofluorescence observed in those layers. Furthermore, even if cysts were supplied with sufficient oxygen to support germination at those sediment depths, it is not clear that the germling cells would be able to reach the sediment-water interface, as they would have to swim the equivalent of several hundred body lengths through the packed sand, silt, and clay particles that surround them before emerging. The top cm of sediment not only contains more oxygen, but it tends to be less dense and more flocculent in nature, and thus more conducive to the escape of germinated cells.

The influence of sediment dynamics has already been identified as a primary source of uncertainty in the germination source (e.g. see Section 4, Stock et al., 2005). The addition of a sediment dynamics model (e.g., Shull, 2001) may provide a means of

investigating this uncertainty and provide a more accurate germination source term. In addition, the combination of field autofluorescence observations and the laboratory studies herein can provide valuable information for the validation and calibration of such a model. However, substantial obstacles to such an effort are imposed by the high variability in the autofluorescence data and the complexity of sediment dynamics (e.g., Wheatcroft and Martin, 1996). In the meantime, the overall high percentage of cysts fluorescing in the top cm in the field further supports the first approximation of the germination source being those cysts within the top cm of sediment.

4.3. Model simulations

Laboratory germination experiments often showed faster germination of *A. fundyense* cysts incubated in the light relative to those incubated in the dark, consistent with a less extensive study by Anderson et al. (1987). Based on the latter study, our initial hypothesis in the ECOHAB-GOM program was that cysts in shallow waters, though less abundant than those accumulating in the offshore seedbeds (Fig. 1), could provide a significant input of germinated cells as a bloom inoculum because of warmer temperatures and higher light conditions. However, bottom water temperatures were often colder in the nearshore waters than the offshore areas because of the water mass characteristics in those areas (Churchill et al., 2005). Furthermore, light is so rapidly attenuated in the sediments that most cysts were essentially germinating in the dark, whether they were located in shallow or deep waters. Accordingly, in the Casco Bay region, where both the inshore and offshore cyst populations are well sampled, the modeled cyst germination flux from deeper waters (> 50 m) was $14 \times$ larger on average than the flux from sediments less than 50 m deep during the spring. This difference is $7 \times$ if the threshold between deep and shallow is set at 75 m. The magnitude of these differences is a direct reflection of the relative abundance of cysts offshore versus onshore.

Hydrodynamic transport simulations fed by excystment fluxes specified from our germination model predict vegetative cell distributions that share salient features with observations in Townsend et al. (2001). Specifically, the simulated cell distributions are gulf-wide in character, and the highest concentrations occur in association with the Maine Coastal Current. However, germination by itself is not

sufficient to explain the seasonal increase in cell abundance in the region, as the simulated concentrations are considerably lower than are observed, particularly during the latter part of the season. Also lacking from this simulation is the observed west-to-east shift in the center of mass of the vegetative cells as the season progresses. Both of these aspects are captured with a more complete model of *A. fundyense* population dynamics (McGillicuddy et al., 2005). In particular, vegetative growth is needed to account for the seasonal increase in cell abundance, and the west-to-east shift in the center of mass appears to result from a transition from temperature to nutrient limitation. Nevertheless, the excystment simulation documented herein illustrates that resting cysts play a key role in *A. fundyense* population dynamics in this region, and provides insights into other processes, such as germination in shallow versus deep waters.

That the excystment simulation produces gulf-wide cell distributions in association with the Maine Coastal Current is not unexpected given that the observed cyst beds lie directly below major elements of the circulation. Once germination begins, hydrodynamic transport swiftly connects population centers emanating from the two major cyst beds, creating a continuous cell distribution along the coast. It is important to note some simplifications of the model that potentially bear on these findings. First, the two-dimensional formulation used herein requires an assumption that newly germinated cells arrive at their target depth immediately. In reality, cells swimming upward from the deep cyst beds at 10 m d^{-1} would take several days to reach the surface as they transit through a sheared current that is weaker near the bottom and stronger near the surface. Instantaneous arrival of the cells in the higher-velocity near-surface strata would therefore tend to overestimate downstream transport. However, these simulations are driven by the vertically averaged velocity field, which is generally weaker than the currents in the near-surface waters where *A. fundyense* resides. This aspect causes the downstream cell transport to be underestimated. Thus, these two aspects tend to compensate for each other.

4.4. A conceptual model

Based on the observed cyst distribution in Fig. 1, as well as large-scale survey cruise data (Townsend et al., 2001, 2005a; Keafer et al., 2005b) and historical patterns of PSP toxicity in shellfish (Bean

et al., 2005), we propose a conceptual model of *A. fundyense* in the Gulf of Maine that highlights the dynamics of cysts. McGillicuddy et al. (2005) present a related conceptual model that represents average conditions (both physical and biological). Our model emphasizes the episodic nature of the interconnections among the various “epicenters” of the *A. fundyense* population — namely the BOF, the EMCC, and the WMCC. Fig. 10 depicts the major features of our model. The focus is on cysts in bottom sediments, but we note that at least in the BOF region where tidal energy is substantial, resuspended cysts also may be important in bloom initiation, as discussed by Kirn et al. (2005).

An important consideration that this model must accommodate is the general east to west flow of the Maine Coastal Current (MCC) system (Lynch et al., 1997) and the mean alongshore flow of the Gulf of Maine. Average conditions therefore are, in effect, a one-way transport system that will move *A. fundyense* cells to the west and south towards Georges Bank, with the mean flow providing limited opportunity for those cells to circulate back into the northeast portion of the domain. Accordingly, if blooms begin with germinating cysts from specific seedbeds, as we hypothesize, the replenishment of those seedbeds with new cysts cannot be from the

cells that have been transported away. The dilemma is that a system in which there is significant alongshore transport must also have features that allow *A. fundyense* motile cell populations to accumulate and deposit cysts at the “upstream” end of the transport pathway.

In this context, the critical component is the population that develops near Grand Manan Island at the mouth of the BOF (Fig. 10). It is well established that there is an eddy system that retains cells to the east of Grand Manan Island, resulting in exceptionally high cell concentrations, sometimes exceeding $60,000 \text{ cells l}^{-1}$ (Martin and White, 1988). In our conceptual model, this area serves as the “incubator” for the region, with many cells remaining within the BOF and completing their life history there, depositing new cysts at the end of the bloom season, and creating the seedbed at the mouth of the BOF seen in Fig. 1. As discussed earlier, this seedbed is a persistent feature that has been mapped on numerous occasions over the last 20 years (White and Lewis, 1982; Martin and Wildish, 1994; J. Martin, unpublished data). There are thus always benthic cysts present in the BOF to initiate blooms in that area.

The BOF blooms are not completely isolated, however, as outflow from the BOF will carry cells

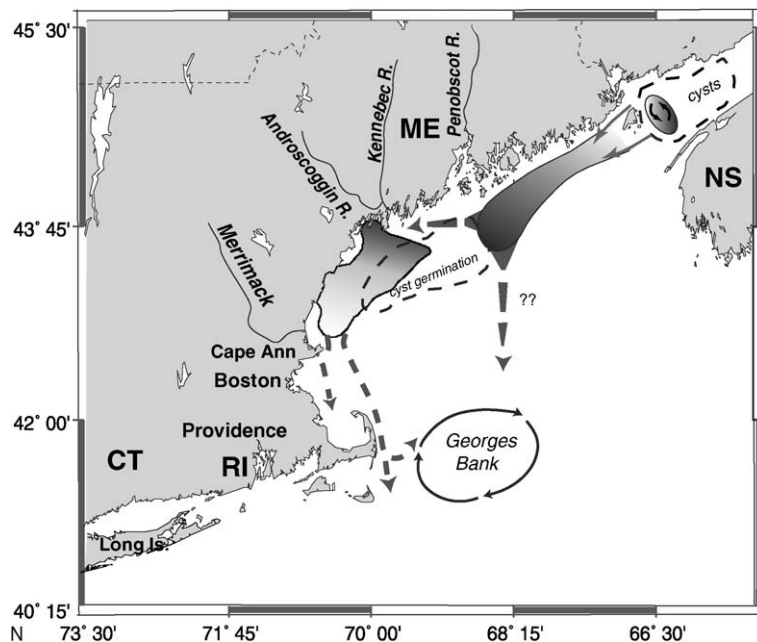


Fig. 10. Conceptual model of *A. fundyense* cyst and motile cell dynamics in the Gulf of Maine. Areas enclosed with dashed lines denote cyst seedbeds that provide inoculum cells. Major current systems are shown with shaded arrows. The shaded areas represent growth and transport of motile cells.

into the Maine Coastal Current system via the EMCC branch. This linkage has been depicted in surveys conducted by Martin and White (1988) and Townsend et al. (2001). As hypothesized by the latter authors, *A. fundyense* cells that enter the EMCC near the BOF do not initially flourish, due to the deep mixing and high turbulence of that water mass. However, as the cells are transported to the west, the water stratifies and allows growth (Townsend et al., 2001). Model simulations of this *A. fundyense* population that include a nutrient dependence (McGillicuddy et al., 2005) show nutrient limitation at the western edge of the EMCC in the mid-summer months. Based on laboratory studies (e.g. Anderson and Lindquist, 1985) nutrient limitation will result in the induction of sexuality in *A. fundyense* populations, leading to the formation of resting cysts that will then fall from the water column. We note that the significant cyst accumulations offshore of Penobscot and Casco Bays are found in the general area where model results suggest nutrient limitation will occur (McGillicuddy et al., 2005), and where Townsend et al. (2001) showed abundant vegetative populations of *A. fundyense* during large-scale surveys.

The *A. fundyense* populations that cause PSP problems in the western Gulf of Maine region have two possible origins. One is from motile cells delivered to the nearshore waters of the WGOM from the EMCC (Townsend et al., 2001; Keafer et al., 2005b; Luerssen et al., 2005; Stock et al., 2005). The other is from the germination of cysts from both the inshore and offshore cyst seedbeds in that region (Stock et al., 2005; Fig. 1). The inshore cysts are not abundant, but may be responsible for the localized blooms and PSP outbreaks that occur within certain estuaries and sounds – as in Lumbo's Hole and Cundy's Harbor in northeastern Casco Bay. These blooms may be self-seeding as well as propagatory, analogous to the BOF blooms described above, although the extent to which cells from these blooms are entrained into the WMCC is not known. The offshore cysts are another potentially important source of inoculum cells for the western Gulf of Maine, as a net shoreward flux of *A. fundyense* cells can arise from the joint effects of upward swimming and the time-dependent response of buoyant plumes to fluctuating wind conditions (McGillicuddy et al., 2003). These are the cysts that presumably originated from the EMCC populations, which in turn originated from the BOF "incubator" blooms. At least a 1-year time lag is

involved in this sequence, since the cysts that are deposited in the offshore Penobscot/Casco Bays seedbed from the EMCC would need to overwinter before they would mature and be able to initiate blooms in the WMCC in subsequent years (Anderson, 1980). The ultimate scenario is thus of cysts that germinate within the BOF seedbed, causing localized, recurrent blooms to the east of Grand Manan Island that are self-seeding as well as propagatory in nature, supplying cells that populate the EMCC. Some EMCC cells are entrained into western Maine waters where they may cause immediate toxicity, while others eventually deposit cysts offshore of Penobscot and Casco Bays. In subsequent years, these cysts serve as a seed population for the western Maine blooms that are transported to the south and west by the WMCC, causing toxicity along the coasts of western Maine, New Hampshire, and Massachusetts before they are either lost due to mortality or advected out of the region. Without the localized, incubator characteristic of the eddy system near Grand Manan Island, one would expect *A. fundyense* populations in the entire Gulf of Maine to diminish through time and the PSP problem to disappear. The fact that PSP has been a persistent problem in the region for a century or more (Prakash, 1975) argues for the effectiveness of the mechanisms described here.

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