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Suspended *Alexandrium* spp. hypnozygote cysts in the Gulf of Maine

Sarah L. Kirn[1], David W. Townsend*, Neal R. Pettigrew

School of Marine Sciences, University of Maine, 5706 Aubert Hall, Orono, ME 04469, USA

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Abstract

The life cycle of dinoflagellates of the genus *Alexandrium* includes sexual reproduction followed by the formation of a dormant hypnozygote cyst, which serves as a resting stage. Negatively buoyant cysts purportedly fall to the benthos where they undergo a mandatory period of quiescence. Previous reports of cysts in the surficial sediments of the Gulf of Maine, where *Alexandrium* blooms are well documented, show a broad distribution of cysts, with highest concentrations generally in sediments below 100 m depth. We report here an exploration of cysts suspended in the water column, where they would be better positioned to inoculate springtime *Alexandrium* populations. During cruises in February, April, and June of 2000, water samples were collected at depths just off the bottom (within 5 m), at the top of the bottom nepheloid layer, and near the surface (1 m) and examined for cyst concentrations. Suspended cysts were found throughout the Gulf of Maine and westernmost Bay of Fundy. Planktonic cyst densities were generally greater in near-bottom and top of the bottom nepheloid layer samples than in near-surface water samples; densities were of the order of 10^2 cysts m^{-3} in surface waters, and 10^2–10^3 cysts m^{-3} at near-bottom depths. Temporally, they were most abundant in February and least abundant in April. Reports by earlier workers of cysts in the underlying sediments were on the order of 10^3 cysts cm^{-3}. We present calculations that demonstrate the likelihood of cyst resuspension from bottom sediments forced by swell and tidal currents, and propose that such resuspended cysts are important in inoculating the seasonal bloom. We estimate that suspended cysts may contribute significantly to the annual vegetative cell population in the Gulf of Maine.

Keywords: *Alexandrium*; *Alexandrium fundyense*; Cysts; Excystment; Gulf of Maine; Harmful algal blooms; HABs

1. Introduction

Three toxic species of the dinoflagellate genus *Alexandrium* have been identified in the Gulf of Maine: *Alexandrium tamarense*, *A. fundyense*, and

*Corresponding author. Tel.: +1 207 581 4367; fax: +1 207 581 4388.
E-mail address: davidt@maine.maine.edu (D.W. Townsend).
[1]Present address: Gulf of Maine Research Institute, 350 Commercial Street, Portland, ME 04101, USA.

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A. ostenfeldii (Anderson et al., 1994; Gribble et al., 2005). Sexual reproduction resulting in a dormant hypnozygote occurs in the life histories of each of these *Alexandrium* species. *A. fundyense* and *A. tamarense* produce an oblong cyst approximately 50 μm × 25 μm (Dale, 1977; Anderson and Wall, 1978), whereas *A. ostenfeldii* cysts have a distinctly different round morphology (MacKenzie et al., 1996). Although both *A. tamarense* and *A. fundyense* occur in the Gulf of Maine, they are thought to be varieties of the same species (Anderson et al., 1994; Scholin et al., 1995). As *A. fundyense* is most abundant, we refer to both forms by the name *A. fundyense* throughout this paper. Here we report a study that investigated the sources of springtime *Alexandrium* populations, the hypnozygote cysts.

The life cycle of *Alexandrium* has been described (Dale, 1977; Anderson and Wall, 1978; Anderson, 1998) and includes several stages. In response to factors not yet fully understood, vegetative *A. fundyense* cells will initiate sexual reproduction (Anderson, 1980). The planozygote cell that results is deeply pigmented and short-lived, transforming into the hypnozygote cyst stage within about a week (Turpin et al., 1978; Anderson, 1980; Anderson et al., 1983; Yentsch et al., 1980; Anderson and Lindquist, 1985). Hypnozygote cysts have a mandatory dormancy period on the order of 2–6 months (Anderson, 1980) that may impart several life history benefits. Apart from ensuring species survival during periods of adverse environmental conditions, this resting stage also might provide a mechanism for dispersal; cysts can almost certainly survive an oceanic passage of several months or longer. Cysts also can establish perennial populations in salt ponds, estuaries, and coastal embayments (Anderson, 1997).

Although cysts can survive extended periods of anoxia, germination will occur only in the presence of oxygen (Anderson et al., 1987; Keafer et al., 1992). Both warmer temperature and increased light enhance germination rates and planomeiocyte success, although the literature reports some variation on the extent to which germination is affected (Anderson and Wall, 1978; Anderson and Morel, 1979; Anderson, 1980; Anderson et al., 1983; Anderson and Keafer, 1987). Field observations in the Bay of Fundy have indicated that cysts begin to show an increase in Brownian motion, taken as a sign of incipient germination, when temperatures rose above 5–6 °C (White and Lewis, 1982; Anderson et al., 1987). Laboratory observations of cysts isolated from a salt pond found chlorophyll within the cysts began to autofluoresce, which is also a sign of germination readiness, when the ambient temperature reached 6–8 °C (Anderson and Morel, 1979). Anderson and Wall (1978) observed that excystment occurred when cultures were incubated at 16 °C.

When temperatures are favorable, light is the next most important environmental factor affecting excystment (Anderson and Keafer, 1985). *A. tamarense* (*G. tamarensis*) cysts isolated from sediments off Cape Ann in the Gulf of Maine germinated 8 times faster in a light treatment at 15 °C (Anderson et al., 1987). More recent work suggests that light enhances germination to a far lesser degree at the lower temperatures found in the Gulf of Maine, on the order of 2.5 times faster than germination in darkness at equivalent temperatures (Anderson et al., 2005). Several experiments suggest that dark germination may occur at a reduced rate (Anderson et al., 1987; Nehring, 1996). Anderson and Keafer (1987) documented the first evidence of an endogenous clock in *Alexandrium* cysts, which Matrai et al. (2005) have shown is capable of preventing germination but incapable of inducing germination. The discovery of this endogenous clock does not alter the effects of light, temperature, and water chemistry on rates of germination nor planomeiocyte survival (Anderson and Wall, 1978; Anderson et al., 1987).

The importance of light becomes significant when evaluating the potential for benthic cysts to initiate blooms. If light is necessary for successful germination, cysts on the bottom below the photic zone may not contribute directly to seasonal blooms unless they are resuspended, as has been suggested by numerous authors (Anderson et al., 1983, 1987; Nehring, 1996; Brown et al., 2001; Townsend et al., 2001; Kirn, 2002). If the bottom is within the photic zone, benthic cysts on the sediment surface would be expected to germinate and yearly re-establish the vegetative cell population. If germination can occur in darkness, cyst

beds at greater depths can contribute, depending on other factors such as specific light levels and temperatures, consequent germination rates, and the ability of planomeiocytes to successfully transit upward to the photic zone.

Burial in bottom sediments may also prevent benthic cysts from germinating, either due to anoxia or by physically preventing the escape of the planomeiocyte. The large majority of cysts are buried in anoxic sediments, so a relatively low percentage of total benthic cysts can be expected to germinate (Anderson and Keafer, 1985; Martin and White, 1988; Wyatt and Jenkinson, 1997; Anderson et al., 1987; Brown et al., 2001). Planomeiocytes, or germling cells, are posteriorly biflagellated, diploid, and, at 40–50 μm in diameter, larger than vegetative cells (Anderson and Wall, 1978). The planomeiocytes undergo their first division within 24 h of emergence (Anderson and Wall, 1978). Although the effect of burial on the emerging planomeiocyte has not been directly investigated, there is likely a limit to the depth from which a planomeiocyte can escape (Anderson et al., 1982a, 2005). After the cells escape the sediment, they must swim or be advected upward to attain the photic zone before growth is possible.

Surveys for benthic *Alexandrium* hypnozygote cysts in the Gulf of Maine show them widely distributed along the coastal Maine shelf (Lewis et al., 1979; Anderson et al., 1982b, 2005). Benthic cyst surveys in the Bay of Fundy have shown high densities of cysts as compared with the rest of the Gulf of Maine, especially in the area north and east of Grand Manan Island (White and Lewis, 1982). A more recent gulf-wide benthic sampling project found high densities of *A. fundyense* hypnozygote cysts in the area just south of Penobscot Bay and just south of Casco Bay (over 500 cysts per cubic cm of sediment; Keller et al., 1999; Anderson et al., 2005). Keller et al. (1999) and Anderson et al. (2005) found the highest densities in offshore waters deeper than 80 m. This positive relationship between cyst abundance and depth is likely due to sediment dynamics as well as the biology and ecology of encystment and excystment. As cysts are passive, silt-sized particles, their distribution patterns in the Gulf of Maine are likely controlled, at least in part, by sediment dynamics, as was concluded in the Bay of Fundy (White and Lewis, 1982). In a study looking at PSP in the northeast United Kingdom by Joint et al. (1997) also suggested that high benthic cyst concentrations might better reflect the depositional environment of the bottom than the source for spring bloom inoculums.

While we do know that cysts are present in sediments in the Gulf of Maine and that cysts likely initiate the spring and summer populations (Anderson et al., 2005), we do not know where this critical germination occurs. Three possibilities exist: deep benthic cyst beds, shallow benthic cyst beds (within the euphotic zone), or suspended cysts in the water column. Aside from a single *A. fundyense* cyst found by Yentsch and Mague (1979) in the surface plankton tow at an unspecified location, one other study briefly looked for cysts suspended in the western Gulf of Maine. Anderson and Keafer (1985) reported abundances of suspended cysts from 0 to 2000 cysts m^{-3}, with the vast majority of samples containing no cysts. This compares with cyst concentrations on the bottom that were 3–4 orders of magnitude greater. Anderson and Keafer thus concluded that the numbers of suspended cysts were insignificant to bloom inoculation. We suspect that because suspended cysts have ample oxygen, possibly higher temperatures and higher light than their benthic counterparts, they might make a significant contribution to the planktonic population as is likely the case for other dinoflagellate species. Reid (1978) found a wide variety and distribution of dinoflagellate cysts in the top 30 m of water around Great Britain and especially in the North Sea. Nehring (1996) found dinoflagellate cysts (25 known species and 8 unidentified types) present throughout the German and Kiel Bights, with the relative abundance of each species consistent between water column and bottom sediments. Nehring (1996) commented that while only 10% of total benthic cysts are in oxygenated sediment close enough to the sediment surface to allow planomeiocyte escape (citing Anderson et al., 1983), 100% of cysts suspended in the water column are in germination-favorable conditions, making these water column cysts 10 times as important as the benthic population of cysts. The

purpose of this study was to provide a first-order estimate of the abundances of suspended *A. fundyense* hypnozygote cysts in the Gulf of Maine and to assess the relative role these cysts play in establishing the seasonal vegetative cell populations.

2. Methods

Samples for the enumeration of suspended cyst densities in the Gulf of Maine were collected on three survey cruises in 2000: 20–25 February on the R/V *Delaware II*, 24 April to 2 May and 5–15 June aboard the R/V *Cape Hatteras*. These cruises allowed us to examine the cyst abundances prior to excystment (the February cruise), at the time of germination and establishment of the springtime *A. fundyense* population (the April cruise) and after the vegetative cell bloom when cyst formation might be occurring (the June cruise) (Townsend et al., 2005). Water samples were collected from three depths: 2 m below the surface, 5 m above the bottom, and, in all months but June, the top of the bottom nepheloid layer as located by transmissometer. These depths were chosen because the surface waters would be where cysts would be assumed to have the greatest likelihood of contributing to the spring vegetative cell bloom and because the nepheloid layer and near bottom are known to be the most likely place to find suspended particles such as cysts.

In February, two casts were necessary at each station. First, a CTD package (CTD, transmissometer, fluorometer) was deployed followed by a hydrocast with 30-L Niskin bottles. Bottles were positioned on the wire to be as close as possible to the hydro weight, at the approximate depth of the top of the bottom nepheloid layer as indicated by the transmissometer on the CTD cast, and 2–3 m below the surface. On the April and June cruises the 30-l bottles were deployed using a SeaBird carousel sampler as part of the CTD package.

The entire contents of the 30-l bottles were poured through a 20-μm mesh sieve, backwashed into a 50-ml centrifuge tube using filtered seawater to a total volume of 45–48 ml; to preserve the samples, 2.5 ml of buffered formalin were added to the each, the sample was inverted three times, then refrigerated.

Laboratory analyses were based on those of Yamaguchi et al. (1995). Samples were sonified for 60 s with a Branson 250 probe-style sonifier to free debris from the mucilage surrounding the cysts. After sonification samples were centrifuged (this and all subsequent centrifugations were at 700*g* for 15 min and 18 °C) and the supernatant aspirated to leave 5 ml in the 50-ml centrifuge tubes. Using deionized distilled water (DDW), samples were then transferred into 15-ml centrifuge tubes to facilitate precise volume measurements. To reduce their volume, samples were again centrifuged and aspirated to 1 ml. To prepare the cysts for staining, 10 ml of methanol were added, and the tubes were refrigerated. After 48 h, the methanol was removed by centrifugation and aspiration. To remove additional methanol, the sample was resuspended in 10 ml DDW and centrifuged. Again, the supernatant was withdrawn until 1 ml remained in the tube. Two ml of filtered primulin staining solution (0.067 g primulin powder/50 ml DDW) were added to each tube, samples were inverted three times, and refrigerated. Primulin is a fluorochrome stain that targets the cellulose wall, starches, and cell membranes in the cysts (Yamaguchi et al., 1995), thus facilitating the identification of cysts against the background of other detrital materials in the samples. After 2 h the primulin was removed with two consecutive washings with 10 ml DDW, centrifugation and aspiration. Finally, the sample was resuspended in 1–5 ml DDW to allow for ease of microscopic examination. Each sample was counted in its entirety, 1 ml at a time in a Sedgwick–Rafter counting cell with a Nikon Optiphot-2 epifluorescence microscope (using 330–380 nm excitation and 435–485 nm emission filters). Identification of individual cysts was checked by examination with transmitted light. Final cyst concentrations were determined relative to the original seawater volume sampled at sea, and reported as cysts m^{-3}.

Although intact *A. fundyense* cysts were the primary targets of counting, empty *A. fundyense* cysts also were enumerated. These were likely both dead cysts and the empty cyst walls left behind by germinating planomeiocytes. In most cases it was

impossible to determine which, as observation of the archeopyle was only possible if the cyst wall was oriented optimally in the Sedgwick–Rafter counting cell. The reported abundances of empty *A. fundyense* cysts should be regarded as minimum densities, as the protocol and counting did not specifically address empty cysts.

We distinguished in our counts between recently formed cysts, mature cysts, and cysts ready to germinate. Mature cysts are not noticeably dark, and their cell contents are somewhat withdrawn from the cyst walls. Recently formed cysts and cysts ready to germinate are both characteristically dark in transmitted light; however, they can be distinguished from one another by the appearance of their contents. Newly formed cysts are distinctly dark and full of round starch granules, as compared with mature cysts or cysts preparing for germination, whose cellular contents are similarly dark but are withdrawn from the cell wall (Anderson, 1980 and Fig. 4). This distinction between newly formed and mature cysts was made using transmitted light microscopy and was based on morphological characteristics described in Anderson (1980).

Surface water samples from our February cruise also were subsampled for vegetative *A. fundyense* cell counts. Five-milliter subsamples were taken from the approximately 48 ml of concentrated surface samples (see protocol in Townsend et al., 2001). Although these subsamples were only 5 ml, they corresponded with an unconcentrated water sample of approximately 2 l, the usual sample size for *A. fundyense* vegetative cell counts.

3. Results

Intact and empty cysts were present in the water column in February, April, and June in concentrations up to 8000 cysts m^{-3} (Figs. 2 and 3). In February, cysts were present in water samples from the surface, top of the bottom nepheloid layer, and near bottom. Although distribution of cysts with depth varied between stations, the highest concentrations were generally found in the near-bottom sample. A small fraction of the total cysts observed in February samples (19 of the 575 total cysts observed, or 3%) were autofluorescent under epifluorescent light and showed condensed cell contents. Empty cysts were present in all but two surface water samples. Vegetative cells were also found in surface samples from February, but their densities were low (maximum densities 1400 cells m^{-3}) compared with summer values (e.g., Townsend et al., 2001).

The April cruise revealed cysts to be widely distributed in the samples from the top of the nepheloid layer and near bottom throughout the Gulf of Maine, from the Bay of Fundy to the waters off the Massachusetts coast. Neither empty nor intact cysts were found in surface samples from April. Of the 55 cysts observed in April samples, 12 exhibited autofluorescence, all but two of which were from near-bottom samples. The highest empty cyst concentration was found well outside of the Bay of Fundy. Samples from several April cruise stations in the Gulf of Maine proper had no empty cysts, whereas all near-bottom samples from February had empty cysts.

The June cruise sampled only the surface and near-bottom depths. Like the April survey, no surface samples from June contained cysts. Far more of the June near-bottom samples than those of April contained intact cysts; 20 of 28 (71%) near-bottom samples had cysts in June, while only 11 of 32 (34%) near-bottom samples had cysts in April. Of the 109 cysts counted from June samples, 18 showed evidence of recent formation. Autofluorescent cysts also were observed in the June samples, although their abundance was lower than that observed in April.

4. Discussion

This research project produced a number of key findings. Significant numbers of *A. fundyense* cysts were observed in suspension over wide temporal and spatial scales in the Gulf of Maine. Autofluorescent cysts were found in deep-water samples, providing field-verification of laboratory experiments that show germination of cysts in darkness is possible. Cysts that appeared newly formed (round and full of dark starch globules) were observed in near-bottom water samples from

the June cruise, documenting cyst formation and settling in the Bay of Fundy, west of the entrance to Penobscot Bay, and in the southern vicinity of Jordan Basin. Lastly, *A. fundyense* vegetative cells were found in surface waters in February. These findings are discussed in more detail in the sections that follow.

4.1. Temporal distribution of *A. fundyense* hypnozygote cysts

We observed intact suspended *A. fundyense* hypnozygote cysts to be one to two orders of magnitude more abundant in February than in April. In February intact cysts were nearly twice as abundant as empty cyst walls at that depth, while in April empty cysts were nearly four times as abundant as intact cysts in near-bottom samples. Because we would expect that vertical turbulence would have decreased or remained the same from February to April due to the development of the pycnocline and decreased intensity and frequency of storms, the drop in relative concentration of intact versus empty cysts could be explained physically or biologically. If less energy were available to keep cysts in suspension, then denser intact cysts might preferentially settle while lighter, empty cysts remain in suspension. Endogenous clock germination experiments have shown that germination is possible in February (Anderson and Keafer, 1987; Thompson et al., 2000; Matrai et al., 2005). Observations of autofluorescent cysts from bottom and top of the bottom nepheloid layer samples support this laboratory finding. Thus, the relative decrease in intact cysts relative to empty cysts is likely a combination of both germination and the decreasing energy of the environment. It must be noted that empty cysts may persist for years without degradation; empty cysts observed may have accumulated over many years, not only from the most recent germination.

Water samples containing no cysts were observed in all months and at all depths. This lack of cysts could have resulted from insufficient sample size, an actual absence of cysts in certain areas, or patchy distribution. It is unknown whether cyst formation occurs in specific and isolated areas or throughout the Gulf of Maine, but the presumed dependence of sexual reproduction on a high concentration of gametes implies that sexual fusion is limited to areas of very high cell density. Soon after hypnozygote cysts develop from planozygotes they become negatively buoyant, passive particles and begin settling through the water column unless held in suspension by vertical turbulence and advected by currents. By February planktonic cysts formed the previous summer could be advected far from where they were formed. The planktonic distribution of cysts in winter and spring months would thus be expected to reflect physical transport more than the actual biological production of cysts.

Strongly pigmented cysts were observed shortly after the bloom decline in June. The appearance of these cysts is consistent with that of newly formed cysts (Anderson, 1980; appearance of cysts in sample of recently formed cysts provided to these authors by D. Anderson's lab). These cysts were observed in samples from the coastal station between Penobscot and Casco Bays, from stations southwest and east of Jordan Basin, and numerous stations inside the Bay of Fundy. We believe this is the first documentation of sexual reproduction by *A. fundyense* in the Gulf of Maine, and it may in part explain the apparent increase in concentration of intact cysts in near-bottom samples from April to June.

The increase in empty cysts in the near-bottom waters from April to June may be explained by the settling of the empty cyst walls from the water column. As empty cysts have low densities, it is feasible that they might take a long time to reach the bottom. According to evidence from endogenous clock studies, germination continues into July, so there is likely a supply of newly emptied cyst walls throughout the summer. Again, it is unknown how long empty cyst walls persist in the environment, so it is impossible to say where the cyst walls originated or when they were abandoned. An alternative explanation for the increase in empty cysts is resuspension of older cyst walls, although there is no increase in energy from April to June to cause more resuspension. Bioturbation also might resuspend cysts along with other particles, although we are uncertain how such activity might change with season. Vertical

stratification of the water column is weakest in winter in the Gulf of Maine, thus the mixing energy from storms penetrate deeper in winter, potentially resuspending sediments and cysts and keeping them in suspension. As stratification develops in spring, the depth to which surface mixing extends is reduced. With lower energy in the deep-water column, cysts that were held in suspension during the energetic winter months may settle to the bottom. The elevated concentrations of cysts in February in the Bay of Fundy likely reflect both the energetic environment of the Bay of Fundy and the persistence of the *A. fundyense* population there.

4.2. Spatial distribution of *A. fundyense* hypnozygote cysts

With the exception of the Jordan Basin station in February, the highest concentrations of cysts were found near the bottom in the Bay of Fundy for all three sampling periods (Kirn, 2002). This pattern is less clear for our February results because of the limited number of stations, but it is strengthened in subsequent cruise results. The only location that consistently had cysts in the water column at multiple depths was northeast of Grand Manan Island in the mouth of the Bay of Fundy directly over the dense benthic cyst deposits mapped by White and Lewis (1982). Another area that consistently had cysts was in the western Gulf of Maine directly south of Penobscot Bay, although this is poorly resolved as only the April and June cruises sampled here. Notably, this area has some of the highest benthic cyst concentrations in the gulf (Keller et al., 1999; Anderson et al., 2005).

The highest density of suspended cysts ($>8000\,m^{-3}$) documented by this study was in the near-bottom sample taken in Jordan Basin in February (Fig. 3). The relative concentration of empty cysts was not as high in this sample; the intact cyst concentration was an order of magnitude greater than that of empty cysts. Elsewhere, the ratio of intact to empty cysts was much smaller, with empty cysts commonly exceeding the number of intact ones. What this means is uncertain, as suspended particles are likely a result of both biological and physical factors, but it is noteworthy. A portion of the cysts in this sample may have formed in the 1999 summer and been entrained in the gyre circulation around Jordan Basin and therefore prevented from settling.

When in suspension, cysts may be advected throughout the Gulf of Maine with the residual circulation. As an example, a cyst found in a surface water sample collected in Jordan Basin in February is evidence of such transport in suspension. This cyst is unlikely to have been brought to the surface by resuspension from the bottom of Jordan Basin because the water here is strongly stratified at depth even in winter.

Cysts were widely distributed and occurred in bottom and top of nepheloid layer samples throughout the Gulf of Maine, especially over known concentrations of benthic cysts in the Bay of Fundy and offshore from Penobscot Bay (White and Lewis, 1982; Keller et al., 1999; Anderson et al., 2005). There appears to be general east–west gradient of decreasing cyst abundances across the Gulf of Maine (Figs. 1 and 2). The concentrations of cysts found in the offshore transect in February were higher than any found in subsequent months, with the exception of stations offshore from Penobscot Bay sampled at near-bottom depths in April and June. All cysts sampled in April and the majority of the cysts found in June were mature cysts and were not newly formed; rather, they must have remained in the water column for the six to eight interceding months, or, more likely, they were resuspended, perhaps from the high concentration of benthic cysts south of Penobscot Bay (Keller et al., 1999; Anderson et al., 2005) (Figs. 3 and 4).

There are a number of possible explanations for the greater concentration of planktonic cysts in the Bay of Fundy than elsewhere in the Gulf of Maine, the most obvious of which is the relatively great concentration of benthic cysts (Anderson et al., 2005). Biological factors, of course, are at the root of both these distribution patterns. Because they are sexually produced, cysts will only be formed where gamete concentrations are sufficient for encounter and fusion. The threshold of gamete density necessary to assure encounters and fusion may be reached in areas of bloom formation, or in

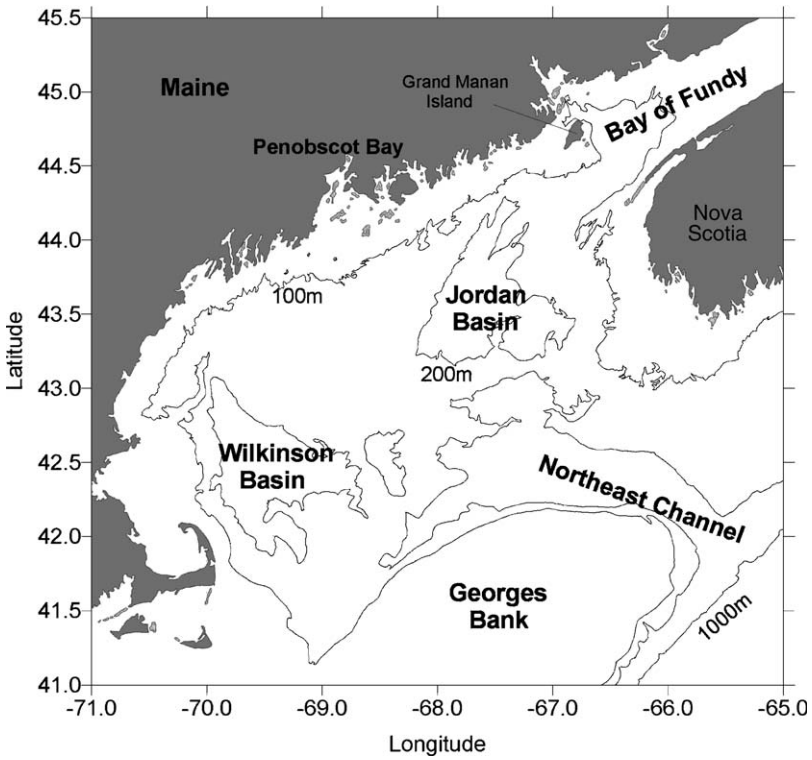

Fig. 1. Map of the Gulf of Maine study site and features referred to in the text. The 100, 200, and 1000 m isobaths are indicated.

isolated thin layers or patches. We know that the Bay of Fundy experiences dense blooms of *A. fundyense* (Martin and White, 1988; Townsend et al., 2001). Proximity to areas where sexual reproduction may occur could explain the greater abundances of cysts in these areas, however, sediment dynamics and physical oceanography likely play as important a role in cyst distribution. As has been noted by several authors, cyclonic circulation to the east of Grand Manan retains and supports a high concentration of *A. fundyense* vegetative cells (McGillicuddy et al., 2005; Martin and White, 1988).

4.3. Potential contribution of benthic cysts

In February suspended cyst concentrations in the Bay of Fundy were on the order of 10^2 cysts m^{-3} in surface waters and between 10^2 and 10^3 cysts m^{-3} in near-bottom waters. Anderson et al. (2005) report maximum concentrations of $2–20 \times 10^6$ cysts m^{-2} in the upper cm of sediment in the Bay of Fundy. If 100% of those benthic cysts germinated and escaped the sediment, their average density in 100 m deep-water column would be $2 \times 10^4–2 \times 10^5$ cysts m^{-3}. Observations in salt ponds found that not all cysts in bottom sediments germinate, in part due to oxygen limitation (Anderson et al., 1983). However, in the numerical germination model reported by Anderson et al. (2005) and bloom dynamics models of Stock et al. (2005) and McGillicuddy et al. (2005), 100% of the cysts in the top cm were allowed to germinate from the benthic cyst seedbed.

Oxygen has never been measured in sediment cores taken for the purpose of benthic cyst enumeration, but is thought to be present to the depth of 1–2 cm in sediments in the Gulf of Maine (Watling, pers. comm.). If there were enough oxygen to support germination, a 35 μm-long *A. fundyense* planomeiocyte that emerges from a cyst buried beneath 1 cm of bottom

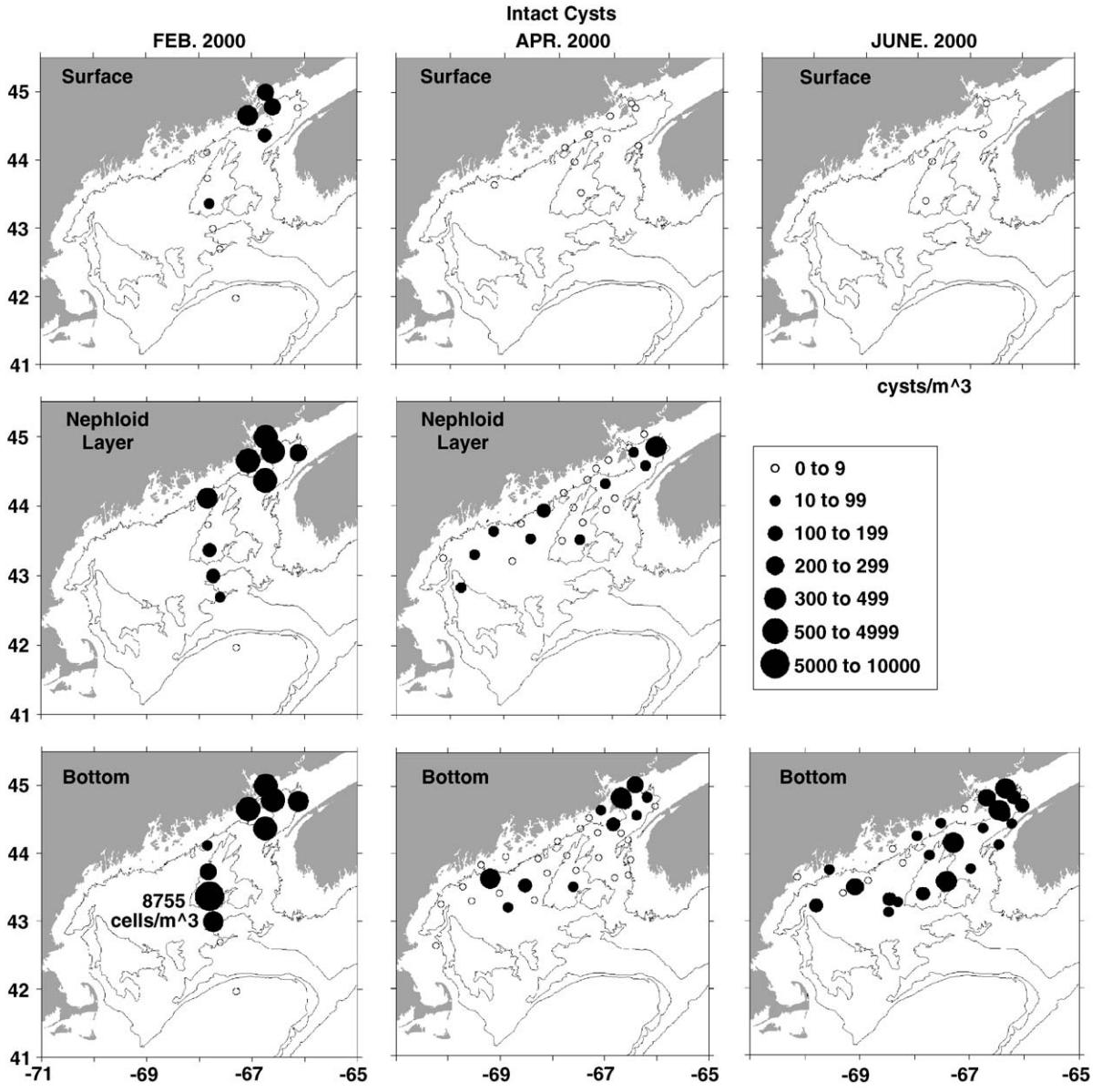

Fig. 2. Abundances and distributions of intact *Alexandrium fundyense* cysts in the Gulf of Maine region during surveys in February, April–May and June, 2000, at the surface (ca. 2 m depth), at a depth near the top of the bottom nepheloid layer, and just off the bottom.

sediment must swim a distance equal to 250–300 body lengths through interstitial water to reach the sediment–water interface, which seems problematic, even if this sediment is relatively uncompacted. Nonetheless, in our calculations we are assuming that the uppermost 1 cm layer of cysts will excyst, in keeping with the modeling approaches reported by Anderson et al. (2005), Stock et al. (2005), and McGillicuddy et al. (2005).

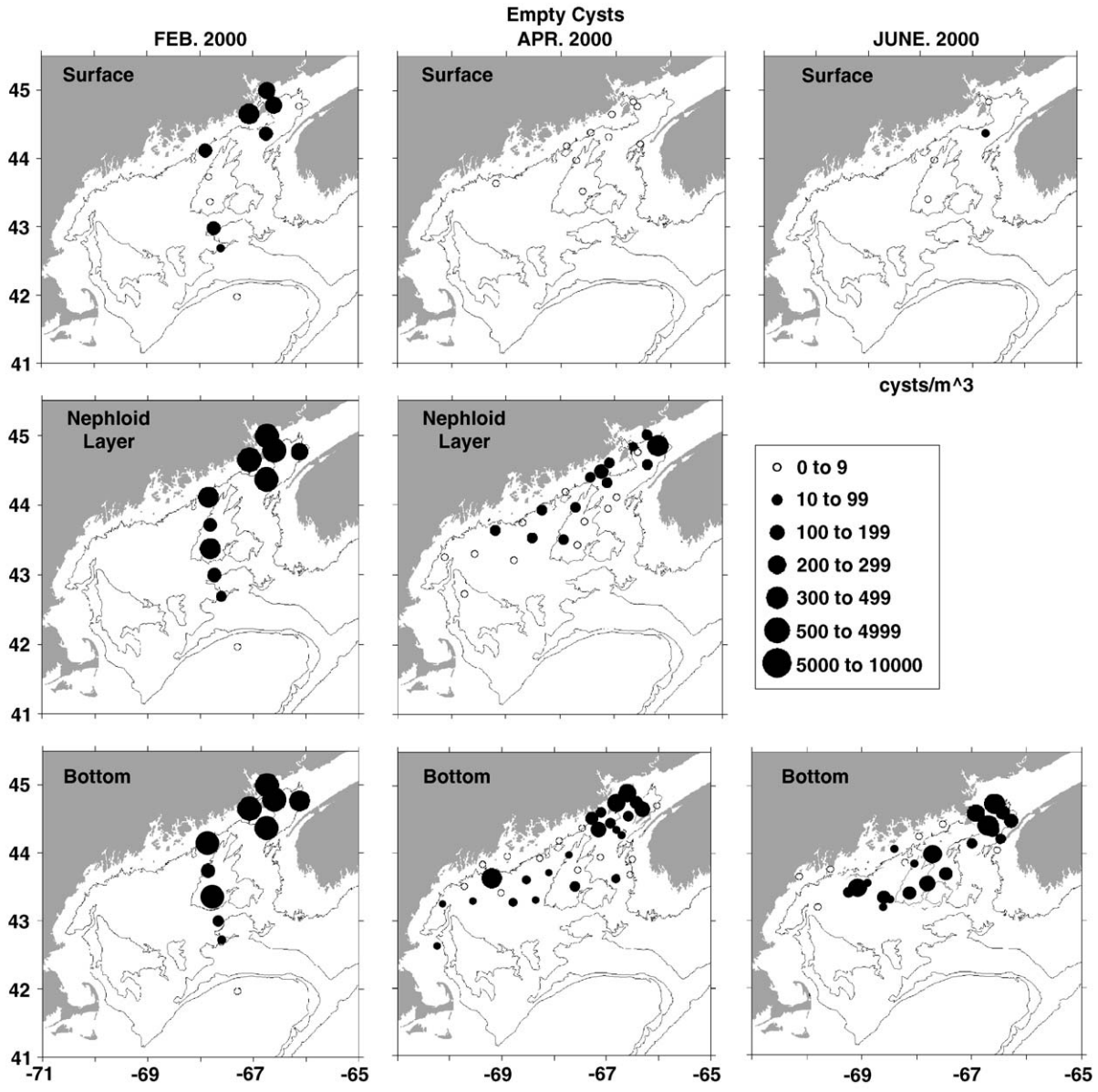

Fig. 3. Abundances and distributions of empty *Alexandrium fundyense* cysts in the Gulf of Maine region during surveys in February, April–May and June, 2000, at the surface (ca. 2 m depth), at a depth near the top of the bottom nepheloid layer, and just off the bottom.

We observed suspended cyst concentrations of 10^2–$10^3\,m^{-3}$ in the Bay of Fundy in February, which is approximately two orders of magnitude less than estimates of benthic cyst densities. However, should only the top mm of sediment release its cysts, and not the top cm, and/or if 10% survive rather than 100%, the relative contribution of cysts in sediments and in the water column begin to converge.

In addition to anoxia, germination rates also can be limited by light and temperature; germination rates in light are enhanced by a factor of as

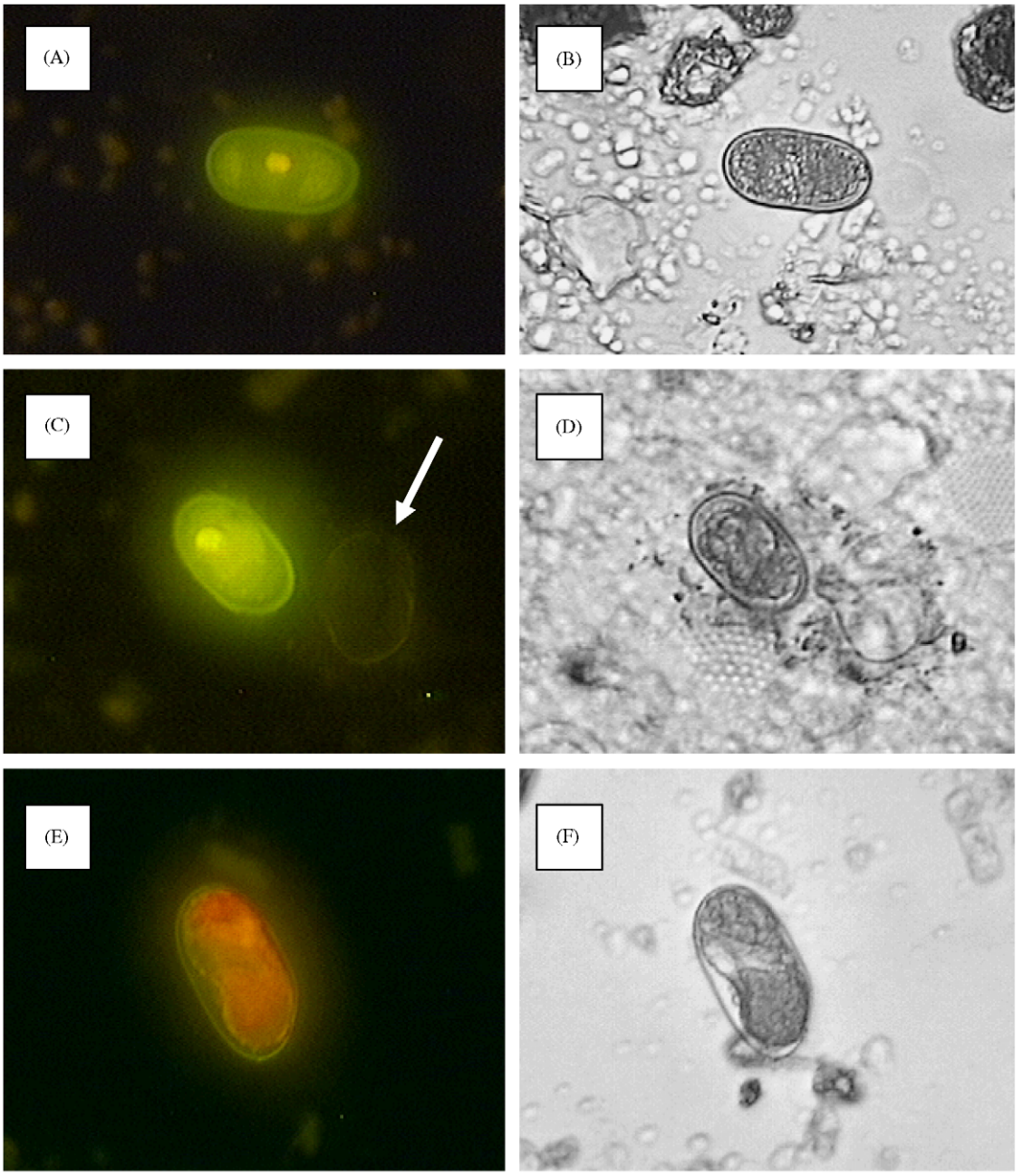

Fig. 4. Photomicrographs (200 ×) of *A. fundyense* cysts collected at near-bottom depths in the Gulf of Maine in June 2000. Panel A: epifluorescence image of newly formed *A. fundyense* hypnozygote cyst collected in the Bay of Fundy; sample depth = 97 m. Cyst length is approximately 56 μm. Panel B: same as A, but transmitted light. Panel C: epifluorescence image of a mature *A. fundyense* cyst collected in Jordan Basin, and an empty cyst (shown by arrow); sample depth = 212 m. Cyst length is approximately 48 μm. Panel D: same as C but transmitted light. Panel E: epifluorescence image of a mature *A. fundyense* cyst collected in the Bay of Fundy; sample depth 105 m. Note chlorophyll autofluorescence (red). Cyst length is approximately 50 μm. Panel F: same as E but transmitted light.

much as 2.5 at temperatures found in the deep Gulf of Maine (Anderson et al., 2005). Temperatures in the Gulf of Maine at the onset of the germination period are around 5 °C, which is near the minimum required. Given the significant abundances of suspended cysts and the more favorable conditions in the water column versus the sediment, we conclude that suspended cysts might contribute significantly to the spring inoculum of *A. fundyense* vegetative cell population in the Bay of Fundy and in the EMCC.

4.4. Spatial and temporal resuspension potential

For benthic cysts to contribute to the springtime vegetative cell population, cysts or planomeiocytes must be released from bottom sediments and reach the euphotic zone. Release from bottom sediments can be driven by physical resuspension produced by benthic shear stresses associated with waves and currents, or mechanical disturbance of sediment by benthic or pelagic organisms, bottom trawling, or dredging activities. Once cells or cysts are off the bottom, they must reach the euphotic zone in order to be a viable inoculum for the spring bloom. Even for the mobile planomeiocytes and vegetative cells, which are believed to be capable of swimming on the order of $10\,\mathrm{m\,d^{-1}}$ (Eppley et al., 1968), physical assistance may be required to reach the upper waters from deep (order 100 m) cyst beds in the Gulf of Maine. Clearly cysts are almost entirely dependent on physical transport via turbulent mixing or advection to keep them in or bring them to the euphotic zone.

Density stratification, which generally develops in the spring as coastal surface waters warm and freshen is an impediment to vertical transport. However, if tidal currents are sufficiently strong and water depth sufficiently shallow, coastal waters may remain unstratified throughout the summer. In the Gulf of Maine regions that remain vertically well mixed throughout the year include the general region at the entrance to the Bay of Fundy, Georges Bank, portions of the southwestern Scotian Shelf, and a nearly continuous band adjacent to the coast of eastern Maine and New Brunswick (Loder and Greenberg, 1986). In these regions without seasonal stratification, the critical factor for benthic cyst initiation of the spring *Alexandrium* bloom would be the shear stress required for cyst or planomeiocyte cell resuspension.

To determine the shear stress (τ) necessary to resuspend a hypnozygote cyst, we used a version of the Shields diagram (Middleton and Southard, 1984) representing the empirically derived relationship between the Yalin parameter and shear stress. The dimensionless Yalin parameter is given by

$$\Lambda = \left[\frac{(\rho_s - \rho)gD^3}{\rho\nu^2}\right]^{1/2}, \quad (1)$$

where ρ_s is the particle density ($\sim 1300\,\mathrm{kg\,m^{-3}}$), ρ is the density of the water ($\sim 1030\,\mathrm{kg\,m^{-3}}$), g is the acceleration of gravity ($9.8\,\mathrm{m\,s^{-2}}$), D is the spherical equivalent diameter of a cyst ($3.4 \times 10^{-6}\,\mathrm{m}$), and ν is the kinematic viscosity ($1.46 \times 10^{-6}\,\mathrm{m^2\,s^{-1}}$). Using the above values yields a Yalin parameter value of 0.2 from which the critical shear stress for resuspension of the cyst is estimated from the Shields Diagram to be $2.0 \times 10^{-2}\,\mathrm{kg\,m^{-1}\,s^{-2}}$.

Benthic shear stress is a nonlinear function of current velocity (tidal or otherwise) and orbital velocities from surface waves (Grant and Madsen, 1979). Waves and swell can generate higher bottom stresses than tidal current because they produce near-bed oscillations rather than steady flow. Under oscillatory conditions, benthic boundary layers do not have time to develop to the same thickness that they do under a steady current condition, thus shear and shear stress may be several orders of magnitude larger than for steady currents of a magnitude similar to the orbital velocities. On the other hand, the shear stress and turbulence associated with the tidal currents may be necessary to keep the suspended material from resettling as the wave orbital velocities periodically go through zero.

The largest waves and swells commonly observed in winter in the eastern Gulf of Maine or Bay of Fundy region are typically 5 m with periods of 6 and 12 s, respectively (data from National Data Buoy Center, buoy 44005, available on the web at www.ndbc.noaa.gov). Wave energy is

roughly a factor of ten lower during summer months. The wavelengths of the 6 and 12 s waves were estimated from small amplitude wave theory (Ippen, 1966) as, $\lambda_6 = 56\,\mathrm{m}$ and $\lambda_{12} = 225\,\mathrm{m}$ with corresponding wave numbers $(2\pi/\lambda)$ of $k_6 = 0.112\,\mathrm{m}^{-1}$ and $k_{12} = 0.028\,\mathrm{m}^{-1}$. Once the wave numbers are known, the orbital velocity of the wave as a function of depth can be determined from the relationship:

$$u_b = \frac{agk}{\sigma}\frac{\cosh k(h+z)}{\cosh kh}, \tag{2}$$

where a is the wave amplitude (1/2 the wave height), g is the acceleration of gravity, σ is the radial frequency of the wave $(2\pi/T)$, h is the total water depth, and z is the depth coordinate measured negative downward from the surface (Ippen, 1966). Using Eq. (2), surface orbital velocities for a 5 m wave height are easily found to be $2.68\,\mathrm{m\,s}^{-1}$ for the 6 s wave and $1.33\,\mathrm{m\,s}^{-1}$ for the 12 s wave. Assuming a nominal bottom depth of 100 m for cyst beds in the Gulf of Maine, the corresponding bottom orbital velocity is found to be $0.00\,\mathrm{m\,s}^{-1}$ for a 6 s wave, and $0.16\,\mathrm{m\,s}^{-1}$ for a 12 s wave. In a 50-m water column, a 6-s wave produces a $0.02\,\mathrm{m\,s}^{-1}$ bottom orbital velocity, while a 12-s wave produces a $0.66\,\mathrm{m\,s}^{-1}$ bottom velocity. Only the swells penetrate deep enough to have a significant impact at the depths at which cyst beds occur in the gulf.

The maximum bottom stress from the nonlinear wave/current interaction is given by Grant and Madsen (1979) as:

$$|\tau_{cw}| = \left(\frac{\rho}{2} f_{cw}\alpha\right) u_b^2$$

$$\text{where } \alpha = 1 + \left(\frac{|u_a|}{|u_b|}\right)^2 + 2\left(\frac{|u_a|}{|u_b|}\right)\cos\varphi_c \tag{3}$$

and u_a is the current speed, u_b is the wave orbital speed, φ_c is the angle between the current and wave velocity, and f_{cw} is an empirical friction factor estimated as 0.02. The friction factor depends upon u_a, u_b, the wave frequency, and the ripple height (estimated as 10^{-2} m on smooth mud, the likely topography of a depositional environment with high cyst concentration). For the present calculations a representative tidal current (u_a) of $0.25\,\mathrm{m\,s}^{-1}$, a wave-generated bottom orbital velocity (u_b) of $0.16\,\mathrm{m\,s}^{-1}$ (corresponding to a 5 m, 12 s swell at 100 m depth) , and φ_c of $\pi/4$ are assumed. Substituting these values into Eqs. (3) yields a wave-current stress value of $1.5\,\mathrm{kg\,m}^{-1}\,\mathrm{s}^{-2}$, which is nearly 2 orders of magnitude in excess of our critical benthic shear of $2.0\times10^{-2}\,\mathrm{kg\,m}^{-1}\,\mathrm{s}^{-2}$. One concludes that all cysts, or cyst-like particles, on the bottom in water 100 m deep or less will be readily resuspended by 5 m swells that commonly occur in the Gulf of Maine in winter. When this occurs in the absence of any stratification, cysts may easily mix to the surface where the conditions are favorable to germination.

While winter swell is a dominant source of shear stress capable of resuspending cysts in the Gulf, the strong tidal currents, in the absence of wave activity, are themselves capable of cyst resuspension. Following the approach of Souza et al. (2001), we calculate that in regions where the near-bottom tidal currents reach approximately $0.2\,\mathrm{m\,s}^{-1}$, stresses exceed the critical value for resuspension. Thus, in many regions in the eastern Gulf, swells may not be needed to resuspend *A. fundyense* cysts.

This analysis of the potential for resuspension in the Gulf of Maine helps to explain the apparent relationship between depth and benthic cyst distribution and suggests that the distribution of benthic cysts in the Gulf of Maine is strongly affected by resuspension. One can be almost certain that cysts are regularly delivered from the bottom sediment to the water column. But to what height in the water column they are raised depends in part on the strength of stratification. If stratification is strong, the pycnocline will be the upper limit to which cysts may be resuspended. If the water column is well-mixed top to bottom, cysts may be resuspended into the surface waters. Thus, there is likely a seasonal component to the delivery of cysts to the surface waters; not only is storm swell more common in winter months, the lack of stratification ensures that, if turbulent energy is sufficient, cysts may be delivered to the surface. Likewise, areas where cysts reach the surface may be limited by water depth and tidal velocity. The water over Jordan Basin is always well-stratified (between deep and intermediate depths), but the near-shore waters in the eastern

Gulf are always well-mixed. These lines of reasoning are equally applicable to hypnozygote cysts and to swimming planomeiocytes or vegetative cells moving toward the photic zone. This physical mechanism by which motile cells may be delivered to the surface in no way negates the fact that cysts in the water column, ensured of oxygen and perhaps exposed to light, will be more likely to germinate than those on the bottom.

4.5. Vegetative cell observations in February surface samples

We believe this study provides the first documentation of vegetative cell population of *A. fundyense* in winter months in the Gulf of Maine. Although the densities of these cells, which ranged from less than one per liter to little over one per liter, were patchier and far lower than summer populations, which are on the order of 100 s per liter (e.g., Townsend et al., 2001), this finding is of significant interest. These cells might represent over-wintering survivors from the previous summer or cells from early germinating cysts (consistent with observations in Matrai et al., 2005).

5. Conclusions

One important goal of the ECOHAB-GOM program was to understand *A. fundyense* dynamics sufficiently to be able to make accurate predictions of the onset of Paralytic Shellfish Poisoning (PSP) toxicity in the Gulf of Maine. This work has established the potential of suspended hypnozygote cysts to initiate, or contribute to initiating, the *A. fundyense* vegetative cell population. In particular, our study has established that *A. fundyense* cysts are not confined to the bottom sediments but that they can be found suspended in the water column.

We present calculations that show the likelihood that cysts found in the water column have been resuspended by bottom stresses resulting from the interaction of currents and winter storm waves. When this resuspension occurs in the absence of stratification, cysts (or planomeiocytes) may be brought to the surface where conditions may be favorable for germination and growth, contributing to the seasonal vegetative cell population. Our observation of vegetative cells in surface waters in the Gulf of Maine in February is consistent with this hypothesis. Although our study showed that cyst densities in the water column are orders of magnitude less than reported densities in bottom sediments, cysts in the water column are already in the water column and therefore exposed to oxygen, light, and temperatures that may enhance excystment rates. These cyst densities are sufficient to contribute significantly to the vegetative cell densities observed in spring and summer.

Whether the suspended cysts sampled in this study represent cysts that were resuspended or had not yet settled is unknown. We did observe in our study both newly formed cysts and mature cysts, which may suggest that both resuspension and sustained suspension of hypnozygote cysts occurs. While our results do not allow us to reject the possibility that the *A. fundyense* motile cell populations that persist through the winter months contribute to the initiation of the spring–summer population, the vegetative cells population that we observed was sparse and patchy. It remains likely that the seasonal growth cycle starts with germination from the hypnozygote cyst stage, in accordance with numerous earlier reports. This paper contributes evidence that the source of germinating cysts may be those suspended in the water column or those in the benthos, or both. Previous work has suggested that *A. fundyense* blooms develop not from mass germination of hypnozygote cysts, but from the mitotic division of vegetative cells (Anderson et al., 1987, 2005; Townsend et al., 2001, 2005). Recent work using numerical modeling suggests that the numbers of cysts that germinate may be comparable with observed early spring vegetative cells (Anderson et al., 2005). However, the model used did not explicitly include transit from benthos to photic zone; in effect it modeled water column-germination. It is most likely that some combination of benthic and water column germination is responsible for re-establishing the springtime *A. fundyense* vegetative population. Germination of even low densities of cysts in February and sub-optimal

growth rates are consistent with the vegetative cell densities observed later in the spring.

Much work remains to be done before the *A. fundyense* germination dynamics in the Gulf of Maine can be confidently explained. A time history of benthic and suspended cyst concentrations within the context of time series monitoring of the physical factors germane to resuspension and vertical transport (including near-bottom currents, waves, winds, and density stratification) would go a long way toward clarifying the time-and-space dependent potentials of benthic and water-column germination. In addition, an evaluation of the relative germination rates of suspended cysts and benthic cysts, and the survival and fate of these early planomeiocytes should be undertaken. Differential germination and success of benthic and water-column cysts may prove to be a key factor in the population dynamics of *A. fundyense* in the Gulf of Maine, and thus a key factor determining factor in the skill of predictive models that are presently under development.

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