DINOFLAGELLATE CYST DYNAMICS IN COASTAL AND ESTUARINE WATERS

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ABSTRACT

Gonyaulax tamarensis cyst dynamics were studied in a shallow estuarine embayment and in deeper nearshore waters. In these two locations, the cysts accumulate in basins with other fine particulate materials, they are buried below the sediment surface such that the majority are found in anoxic sediments which can inhibit germination, their total abundance is very high, and their germination is restricted to surface sediments and therefore is small relative to the number of cells in a bloom. The major differences are in the timing and duration of germination. A tight temporal coupling between excystment and bloom initiation is apparent in the estuary where cysts germinate during a one month interval coinciding with the motile cell bloom. Germination begins at a lower temperature in the deeper coastal waters, precedes the motile cell bloom by several months and lasts eight months in total. The Gulf of Maine is thus seeded by the gradual germination of cysts over many months, providing multiple opportunities for bloom development. In the estuary, bloom success hinges on favorable conditions during a short period of active germination.

INTRODUCTION

In southern New England, the toxic dinoflagellate Gonyaulax tamarensis blooms within estuaries and small embayments, with essentially no input of cells from coastal waters [1-3]. Further north, coastal blooms are common in the Gulf of Maine [4]. Whereas G. tamarensis resting cysts are clearly important as "seed populations" for bloom initiation in certain estuaries, [1,5], their role in nearshore waters remains unknown despite the presence of extensive accumulations in coastal sediments [2,6,7]. This paper compares the accumulation and germination patterns of G. tamarensis cysts in both shallow and deep waters. Detailed analysis of the cyst and motile cell dynamics at each location will be published separately.

METHODS

The Cape Ann site spans a 2500 km^2 region in the coastal waters of northern Massachusetts and has a maximum depth of 150 m (Fig. 1). The other site is Perch Pond, a 0.07 km^2 embayment in Falmouth, MA, average depth 1.5 m. Sediments at approximately 20 stations were sampled at each site, using a hand-held coring device in Perch Pond [8] and a Craib corer [9] near Cape Ann. Cores were extruded and sectioned immediately after collection and subsamples stored at 2-4°C until analysis [2]. Core sections 1-cm thick were either examined individually or were combined with other sections to give average cyst concentrations over a specific depth interval. Changes in cyst vertical distribution were monitored through time using 4-5 replicate cores from one station. An estimate of G. tamarensis motile population size was obtained at each site through time. At two Cape Ann stations, water was pumped through 20 μm Nitex at a constant rate as a hose was raised over a 30 m depth. In Perch Pond, a 3-m plastic pipe was used to collect a surface-to-bottom water sample at each of several stations. These were combined and a subsample

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taken and fixed with 3% formalin.

Gonyaulax tamarensis cyst fluorescence changes during development [10], so the red auto-fluorescence from developing chloroplasts can be used as a marker of impending germination. Core sections were sonicated, sieved, and resuspended in filtered seawater [11] and examined within 24 hours of collection using a Zeiss 487706 filter combination in an inverted epifluorescence microscope. Cysts were scored as "ripe" if chlorophyll fluorescence was visible at 400X total magnification.

RESULTS

Vertical and Horizontal Distribution. Most of the cysts in both study areas were buried below the sediment surface, often peaking 3-4 cm deep with only 10-20% of the total in the top cm. Horizontal surveys were thus based on average cyst concentrations over 0-2, 3-6, and 0-6 cm depth intervals. This does not account for all cysts at a given location, but does include the bulk of those available as an inoculum. Cyst abundance was remarkably similar in the two study areas, peaking near 1000 cysts cm^{-3} (averaged over the top 6 cm) or 6 x 10^7 cysts m^{-2} (Fig. 1). The bathymetry of the Cape Ann area is dominated by deep basins surrounded by shallower, heavily-scoured terrain. Cyst abundance was highest in the northern basins, lowest in shallow waters, and decreased from north to south even though the southern region has basins that are deposition sites for fine material. The Perch Pond cyst distribution coincided with bathymetry as well, with highest concentrations along the western side of the embayment where the water is deepest and where clay and silts predominate.

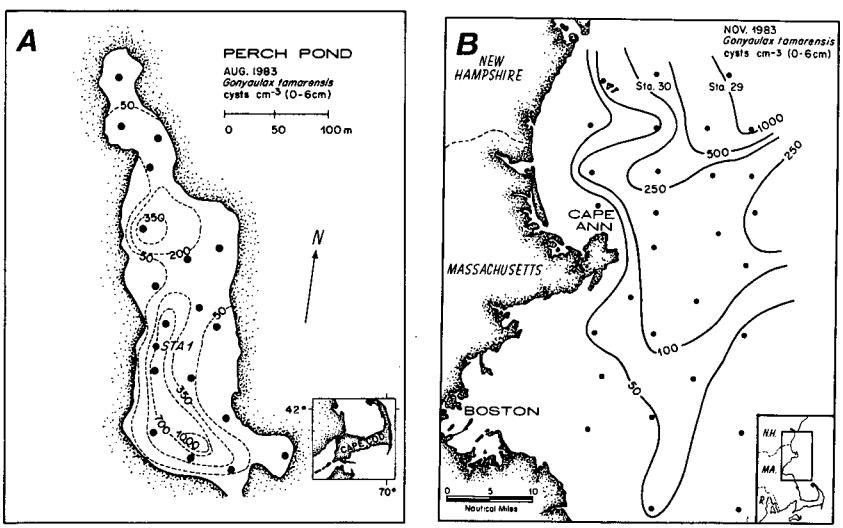

Figure 1. Horizontal G. tamarensis cyst distribution in the two study areas. Darkened circles represent stations for cyst sampling. Contours are cysts cm^{-3} averaged over the top 6 cm of sediment. (A) Perch Pond, Famouth, MA; (B) Cape Ann site in the Gulf of Maine.

Germination. A major G. tamarensis motile cell bloom occurred off Cape Ann in 1984, resulting in shellfish closures in New Hampshire and Massachusetts in June and July. Several months before motile cells appeared, there was a steady increase in the number of cysts in the top cm of sediment at Sta. 29 that visibly fluoresced (Figs. 3A, 3B). This exceeded 80% of those examined by mid-June, decreasing thereafter to 3% in December. Throughout this time, bottom temperatures increased from 4.5 to 6°C. Cysts from 5-6 cm depths showed essentially no fluorescence.

In Perch Pond, motile cells appeared as the first fluoresence was observed in cysts. No shellfish toxicity was detected during this bloom. Perch Pond cysts fluoresced for one month, beginning when the bottom temperature was 7°C and ceasing at 12°C (Fig. 2A,2B). Cysts from 5-6 cm core sections never fluoresced.

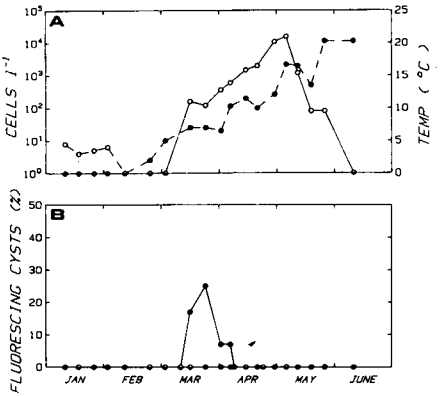

Figure 2. Motile cell and cyst dynamics in Perch Pond, 1983. (A) Motile cells (O) and temperature (O); (B) Cyst fluorescence in the top cm of sediment (O) and at 5-6 cm depths (O).

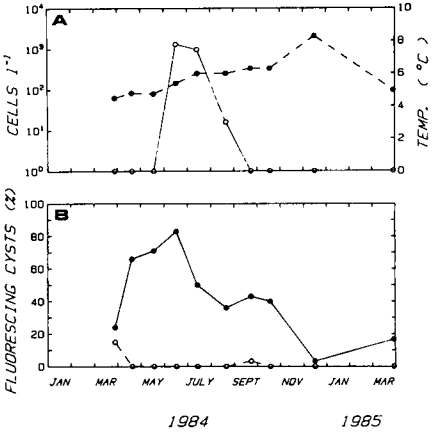

Figure 3. Motile cell and cyst dynamics at Sta. 29 near Cape Ann in 1984. (A) Motile cells (O) and temperature (O); (B) Cyst fluorescence in the top cm of sediment (O) and at 5-6 cm depths (O).

DISCUSSION

Cyst Distribution. The large concentrations of Gonyaulax tamarensis cysts in both locations represent a sizeable potential for bloom initiation. The horizontal distributions are consistent with the deposition of cysts in areas where fine clay and silt particles accumulate, typically in basins and depressions [7]. Shallower, more scoured terrain had low cyst abundance. Cyst concentrations are high within Perch Pond but decrease rapidly to low levels a short distance from the inlet [8]. Perch Pond thus represents a "point source" or localized seedbed in contrast to the widespread Cape Ann distribution. The isolation of G. tamarensis within embayments in this manner is typical of the species' motile cell distribution in southern New England

and Long Island [2,3] and is consistent with the decrease in cyst abundance from north to south near Cape Ann (Fig. 1A). Since G. tamarensis was rarely observed in Massachusetts waters prior to a massive bloom in 1972 [2], this north/south trend may be indicative of a recent "colonization" of the region by the species which has long thrived in eastern Canada to the north. Alternatively, some aspect of the physical or chemical environment may limit G. tamarensis growth and accumulation in the south which would in turn be reflected in low cyst abundance.

The Cape Ann cyst accumulations represent the southern edge of a massive deep water "seedbed" stretching north to the Bay of Fundy in Nova Scotia [6,7]. Since greater than 90% germination of cysts from all sediment depths can occur under favorable laboratory conditions [12], the potential inoculum for bloom initiation is astounding. However, the factors that inhibit or trigger cyst germination in situ must be evaluated to gauge the actual level of excystment.

Perch Pond sediments are typically high in carbon (5%) and are dominated by fine clay or silt (57% and 31%). Cape Ann sediments are more variable, but the areas where cysts are most numerous are approximately 1, 68, and 30% carbon, clay, and silt respectively. At both sites, the vertical distribution of cysts often shows low surface abundance, peak concentrations 3-4 cm deep, and high concentrations to 12 cm depths or greater [8]. (This is true whether the data are expressed as cysts per cm^3 or cysts per gram dry weight.) Two burial mechanisms are possible - bioturbation and sediment deposition. We feel that bioturbation is the most probable mechanism since unreasonably high deposition rates exceeding 1 cm yr^{-1} would be required in both locations to explain the presence of cysts at 12 cm depths. Gonyaulax tamarensis was presumably introduced to the Cape Ann and Cape Cod region with the 1972 New England red tide [2,15].

Germination. Recent work emphasizes the importance of temperature, oxygen, and light in dinoflagellate excystment [1,12]. Over-wintering G. tamarensis cysts germinate as waters warm in the spring, but the low temperature threshold for excystment is not well-defined. This is partly because it has been inferred from field data with insufficiently frequent measurements and partly because of artifacts from sonication or microscopic isolation of cysts. Studies on Cape Cod suggest that germination begins when temperatures rise to 6-8°C [1,5]. This is substantiated by Perch Pond cyst fluorescence (Fig. 3B) which increased sharply as the bottom temperature reached 7°C. Cape Ann cysts began fluorescing at lower temperatures, typically 4.5-5°C. This 2°C discrepancy may either represent a fundamental difference between the populations from these two regions or the different warming rates of their waters.

Cyst fluorescence preceeded the subsequent motile cell bloom by several months and lasted eight months near Cape Ann whereas in Perch Pond the fluorescence increase coincided with the appearance of motile cells and only lasted one month (Figs. 2B, 3B). This may be because the germination process takes longer at the colder Cape Ann temperatures. Alternatively, since high temperatures can maintain quiescence and prevent cyst germination [12], the rapid warming of Perch Pond may provide a shorter "window" of permissive germination temperatures. From a shellfish management or bloom prediction standpoint, the temporal coupling between cyst fluorescence and the subsequent bloom is tighter and potentially more useful in the estuary than in the coastal waters.

Figures 2B and 3B demonstrate that cysts in surface sediments can germinate while those a few cm lower cannot. This is probably a result of inhibition by anoxia since laboratory studies show that cysts can survive anoxia for years but require oxygen for germination [12]. A comparison between profiles of cyst fluorescence, Eh, denitrification, and ferrous iron concentrations within the same core from Cape Ann showed visible fluorescence only for cysts within top cm while the chemical measurements all indicated anoxia below that level [13]. Most marine sediments are anoxic a few mm below the sediment surface, so we conclude that in the absence of a major resuspension event, the vast majority of cysts in the Gulf of Maine and in Perch Pond will not germinate due to a lack of oxygen. In vertical profiles at nine stations near Cape Ann, an average of 22% of all cysts were within the top cm. Thirty liter samples collected from various near-bottom depths off Cape Ann throughout this study had resuspended cyst concentrations ranging from 0-2000 cysts m^{-3}, fully 3 to 4 orders of magnitude lower than the inoculum expected from germinated surface layer cysts.

Excystment of G. tamarensis cysts can occur in darkness [14], but more recent data suggest that light can significantly increase the germination rate [12]. A hierarchy of regulatory factors can thus be described: certain temperatures are inhibitory and others favorable; if temperatures are favorable, light can enhance the germination rate; the positive effects of light and temperature can in turn be overridden by anoxia following burial. The net result in Perch Pond is that the small number of cysts in surface sediments can only germinate for a short time each spring (or fall). In Cape Ann waters, excystment is also restricted to surface sediments but is more protracted in time due to low temperatures and darkness. In both areas, the input of new cells from cysts is probably <10% of the total. Thus the magnitude of the resulting G. tamarensis blooms appears to depend on the growth and accumulation of the motile populations.

Despite our focus on one deep station, these conclusions may apply to much of the Cape Ann G. tamarensis cyst population. For example, cysts in 20-40 m deep sediments may be more important than their low abundance (<50 cm^{-3}) would imply since light would still be present and the high energy hydrographic environment can increase the depth of oxygen penetration into the sediments. This might lead to a greater germination success, but the number of motile cells supplied to the overlying waters would still be small compared to the cell densities associated with major blooms.

There are several remaining uncertainties in our understanding of G. tamarensis cyst dynamics. For example, the 1984 bloom caused shellfish closures near Cape Ann, but vertical profiles at Sta. 29 and 30 showed no obvious change in surface cyst abundance through time due to the deposition of new cysts. Likewise, a survey conducted one year after the one pictured in Fig. 1B showed no significant change in cyst abundance across the entire study area. Uncertainties in coring and cyst enumeration might obscure small decreases in cyst abundance due to germination, but we are surprised that the post-bloom data do not reveal a major input of new cysts. Perhaps the small number of germinated cysts was approximately balanced by the number deposited. Although reasonable, this explanation cannot be invoked year after year since such a steady state would fail to account for the high sediment cyst abundance. Alternatively, the number of new cysts formed may be highly variable

between years and not directly proportional to the magnitude of motile cell blooms. There may also be a major cyst accumulation site that lies outside our 2500 km^2 study area.

Another mystery concerns the germination success of individual cysts isolated from Cape Ann sediments. Starting with 90% germination during spring and early summer (regardless of the core depth from which they were isolated) the success rate decreased to 20, 10, 0 and 0% in August, September, October, and December respectively, increasing thereafter to 50% in March [12]. This would appear to be a maturation phenomenon reflecting the mandatory dormancy of newly-formed G. tamarensis cysts [16], but the lack of fluorescence at depth in monthly cores (Fig. 2B) and the consistent shape of the vertical cyst profiles at Sta. 29 and 30 through time argue that buried cysts did not germinate nor were they resuspended and replaced by new cysts. One intriging explanation is that cyst germination is regulated by yet another factor - an annual endogenous clock. Several experiments are underway to test this hypothesis.

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REFERENCES

1. D.M. Anderson and F.M.M. Morel, Est Coast and Mar Sci 8, 279-93 (1979).
2. D.M. Anderson, D.M. Kulis, J.A. Orphanos, and A.R. Ceurvels, Est, Coast, and Shelf Sci 14, 447-458 (1982).
3. S.E. Schrey, E.J. Carpenter, and D.M. Anderson, Est 7 (1984).
4. C.M. Yentsch and F.C. Mague in: Proc Int Conf (2nd), D.L. Taylor and H.H. Seliger, eds. (Elsevier/North Holland, 1979) pp. 127-130.
5. D.M. Anderson, S.W. Chisholm, and C.J. Watras, Mar Bio 76, 179-90 (1983).
6. C.M. Lewis, C.M. Yentsch, and B. Dale in: Proc Int Conf (2nd) D. L. Taylor and H. H. Seliger, eds. (Elsevier/North Holland, N.Y. 1979) pp. 235-38.
7. A.W. White and C.M. Lewis, Can J Fish Aquat Sci 39, 1185-1194 (1982).
8. D.M. Anderson, D.G. Aubrey, M.A. Tyler, and D.W. Coats, Limnol and Oceanogr 27, 757-765 (1982).
9. M.S. Baxter, J.G. Farmer, I.G. McKinley, D.S. Swan, and W. Jack, Environ Sci Tech 15, 843-846 (1981).
10. C.W. Yentsch, C.M. Lewis, and C.S. Yentsch, Biosci 30, 251-254 (1980).
11. D. Wall and B. Dale, Micropaleontology 14, 265-304 (1968).
12. D.M. Anderson, unpublished data.
13. M. Hines, personal communication.
14. D.M. Anderson and D. Wall, J Phycol 14, 224-234 (1978).
15. A.D. Hartwell in: Proc Int Conf (1st) Mass Sci and Tech Found, V.R. LoCicero, ed. (Wakefield, MA 1975) pp. 47-68.
16. D.M. Anderson, J Phycol 16, 166-172 (1980).