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Encystment in a Dynamic Environment: Deposition of Dinoflagellate Cysts by a Frontal Convergence

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ABSTRACT: The dinoflagellate *Gyrodinium uncatenum* forms massive summer red tides in Chesapeake Bay (USA) and tributary estuaries. These blooms are delimited in the downstream direction by estuarine fronts which may serve to concentrate and recirculate the population. Toward the end of the bloom cycle, *G. uncatenum* sexual stages accumulate in the frontal convergence and are transported downward along the frontal interface. These stages are retained below the pycnocline in net upstream flowing bottom waters and settle out into the sediments along the subsurface transport pathway. Examination of sediments indicates that major deposits of cysts of *G. uncatenum* are bounded in an upstream direction by a benthic front (where the pycnocline intersects the bottom). Above this area, motile cells and sexual stages are absent from the water column and cysts are absent from the sediment. Streamflow-induced variations in the location of the estuarine front in 1979 and in 1980 result in deposition of cysts in different regions, predictable from examination of the location of the convergence. Thus, it is proposed that the convergence zone of the estuarine front and the associated pycnocline serve to transfer encysting dinoflagellate forms from surface waters to their ultimate seedbed locations.

INTRODUCTION

Many coastal and estuarine areas subject to recurring red tides are dynamic systems with complex circulation patterns which ultimately affect the location and timing of bloom formation. Periodic nearshore upwelling of nutrient-rich oceanic waters may result in the enhancement of biological production of surface populations (Blasco, 1975). Population accumulation into patches may occur when positively phototactic or buoyant organisms are entrapped in wind or flow driven convergences (Stommel, 1949; Garvine, 1977; Tyler and Seliger, 1978; Seliger et al., 1979; Tyler et al., in preparation). When these convergence velocities are high, surface accumulations may be entrained downward along the frontal interface giving rise to subsurface concentration maxima within the pycnocline (Tyler and Seliger, 1981). These populations can be carried hundreds of kilometers by subsurface currents and form surface blooms in areas of enhanced vertical transport (Tyler and Seliger, 1978).

Many of the dinoflagellates which form blooms produce a resting cyst which is linked to their sexual cycle (Huber and Nipkow, 1922, 1923; Cao Vien, 1967; Von Stosch, 1973; Pfiester, 1975, 1976, 1977; Pfiester et al. 1980; Tyler et al., in preparation). Typically, a series of mitotic divisions results in highly motile gametes which fuse into a swimming zygote. These planozygotes retain both posterior and transverse flagella of the fused gametes, a morphological marker characteristic of this stage (Von Stosch, 1973), and transform into the nonmotile cyst form, or hypnozygote.

The germination of these dormant cysts has recently been proposed as another mechanism of bloom formation. As noted by Wall (1971), Steidinger (1975), and Wall (1975), hypnozygotes may serve as a source of organisms to repopulate the same locale from one year to the next. Excystment in response to an environmental change would govern the timing of the appearance of the motile stage in the water column and thus the

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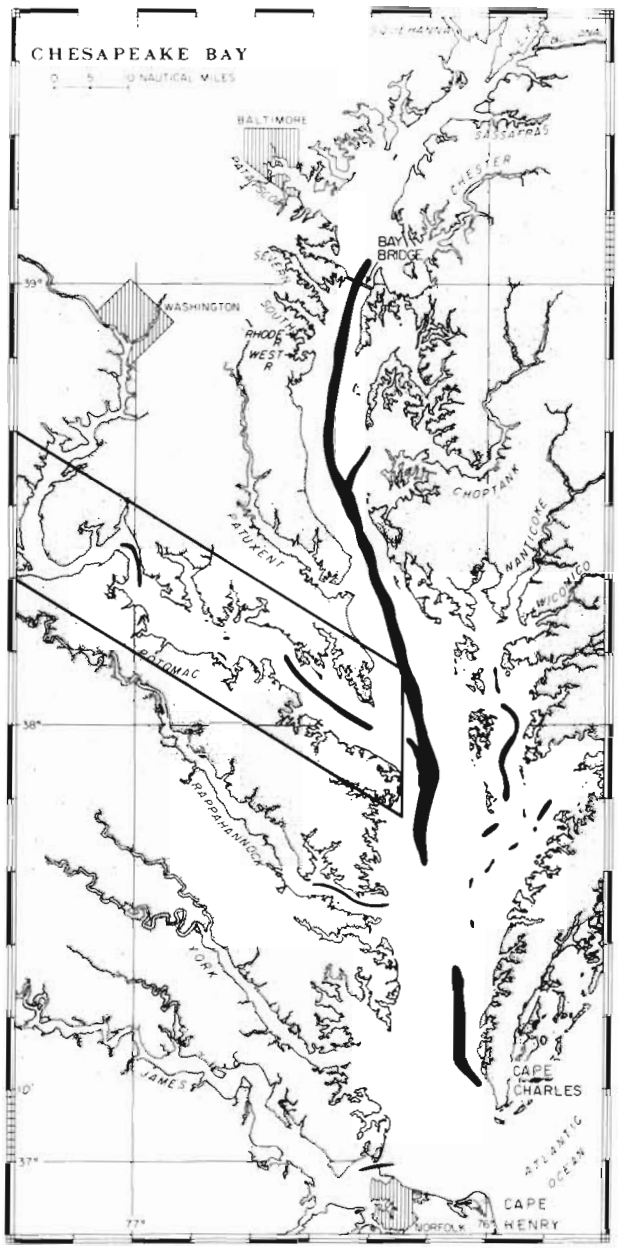

Fig. 1. Chesapeake Bay region. Location of study area. Intensive investigation covered the Potomac River from the mouth to approximately 110 km upstream (enclosed in box). Black: channel > 20 m deep

temporal distribution of the species. The functioning of the cyst as a zygote should result in an encysted population with greater genetic variability. Furthermore, the resistance of the cyst to adverse conditions ensures the success of a species and may aid in the dispersal of the organism. While cyst formation and germination have been implicated in the production and dissipation of red tides (Steidinger, 1975; Wall, 1975; Lewis et al., 1979; Yentsch and Mague, 1979) the only *in situ* demonstration of the initiation of a dinoflagellate bloom via the excystment process is the work of Anderson and Morel (1979). Their studies in shallow, restricted embayments demonstrated that seed beds for the blooms occur in the sediment in the same area as the surface bloom (Anderson and Wall, 1978). In other regions, however, observations of the sediment in areas known to be subject to recurring red tides reveal no evidence of cyst accumulations (Anderson et al., in press). It has been proposed that seed beds can lie offshore with either periodic upwelling or storms causing resuspension and onshore subsurface currents aiding in the onshore transport (Hartwell, 1975; Mulligan, 1975; Steidinger, 1975; Seliger et al., 1979; Yentsch and Mague, 1979).

Gyrodinium uncatenum (Hulburt) forms seasonal blooms in Chesapeake Bay first observed in upstream shoaling areas in the tributary estuaries of the northern and mid-bay in early summer. These blooms spread in a downstream direction in the bay proper such that by late summer Chesapeake Bay from the Potomac River northward is subject to this red tide (previously reported as *Gymnodinium nelsoni*, Loftus et al., 1972; Seliger and Loftus, 1974; Seliger et al., 1975). Dense surface patches of the organism are usually observed at the mouths of the rivers. In late summer/autumn, *G. uncatenum* rapidly disappears from the water column over the entire Chesapeake Bay region until early summer.

In the present paper, we present evidence that the disappearance of *Gyrodinium uncatenum* from the water column is the result of encystment of the organism and overwintering in the sediments. We further demonstrate that deposition of cysts is mediated by flow patterns of the estuary, particularly those at a frontal convergence. Thus, the location of the seed beds can be predicted from knowledge of system hydrography.

METHODS OF COLLECTION AND MEASUREMENT

The area investigated covered the Potomac River from 100 km upstream to its mouth, and the adjacent Chesapeake Bay region (Fig. 1). Measurements of temperature and conductivity were made with a Plessy Systems CTD, Inter-Ocean ICTI Model 513 or Chesapeake Bay Institute flow cell ICTI. Water samples for vertical profiles were taken with a submersible impeller pump (maximum flow rate: 0.4 l min^{-1}). Surface water transect samples were obtained by sampling a portion of the flow of a centrifugal pump mounted 1 m below waterline. Current velocities were measured by *in situ* moorings of Endeco and Braincon current meters.

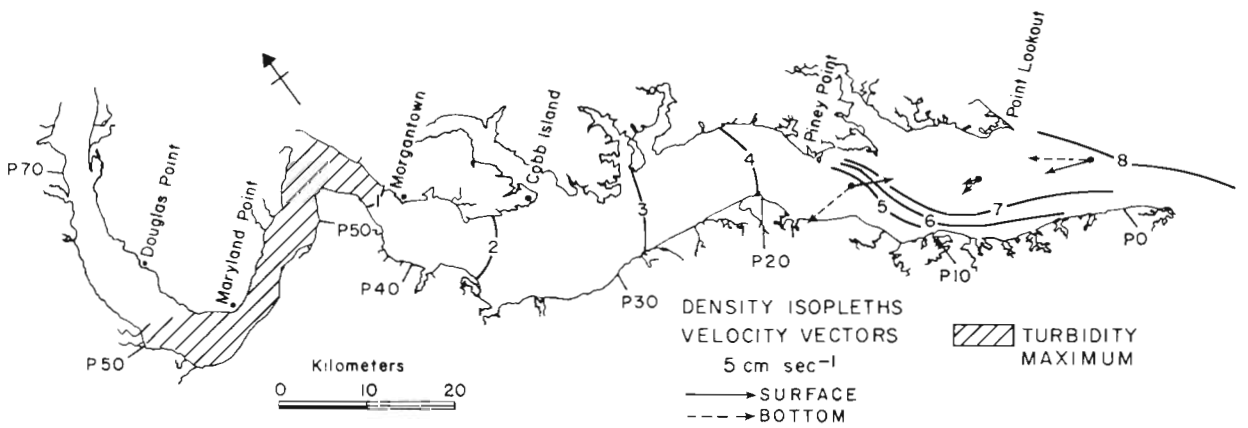

Fig. 2. Surface water contours of σ_t density isopleths from slack water runs for Potomac River; November, 1979. Velocity vectors for surface (solid arrows) and bottom water (dashed arrows) are drawn at P0, P7, and P14. Frontal region delineated by surfacing of 5, 6, and 7 σ_t isopleths. Turbidity maximum indicated by hatched region

Rhodamine dye injections were made by trailing dye from a hose at depth from the stern of the ship. Three ships monitored the dye track over a 5-d period by continuous vertical profiles of temperature, conductivity, and dye fluorescence. Turner fluorometers, Model 111 and Turner Designs fluorometer, Model 10 were used to monitor *in vivo* rhodamine fluorescence. Final dye concentrations, corrected for temperature and background fluorescence, were determined by spectrophotometric measurements.

Water samples for phytoplankton enumeration were taken from depth by Van Dorn bottle, rosette sampler or pump. Phytoplankton species as well as sexual stages were enumerated live in a Sedgewick Rafter or Palmer Maloney counting cell on board ship, immediately after collection of the water sample. In most cases, water samples were mixed with Union Carbide Resin WRS 301, Polyox, to slow the organisms (Spoon et al., 1977) allowing for enumeration and determination of sexual stages. In non-bloom areas, cells had to be concentrated on Nitex netting before enumeration such that a minimum of 50 cells were counted.

In situ growth of *Gyrodinium uncatenum* was measured using 'phytoplankton cages' fitted with 3 µm Nuclepore membranes, and suspended in surface waters from floats (Owens et al., 1977). Laboratory growth rates of *Gyrodinium uncatenum* were measured at a number of salinities in F/2 medium (Guillard and Ryther, 1962).

During the 1979 season, sediment samples for relative benthic cyst distribution were obtained using an impeller pump, which was lowered to rest on the bottom, and interstitial mud was pumped to the surface. During the 1980 season, vertical sediment profiles were taken as well to provide accurate numbers of cysts in the sediment. Sediment profiles were taken with a Benthos gravity corer and sectioned at 1 cm intervals for cyst enumeration by the methods of Anderson et al. (in review). A known volume of the sediment was sonified, presceened on a 64-µm mesh Nitex netting, concentrated and washed on a 20-µm mesh Nitex netting, and resuspended in filtered water prior to microscopic enumeration (Anderson and Wall, 1978). Samples from each core were also analyzed for percent sand, silt, and clay using standard sieving/centrifugation methods.

RESULTS AND DISCUSSION

Hydrography

The Potomac River is a partially mixed coastal estuary which represents 18% of the total freshwater runoff into the Chesapeake Bay. The tidal portion of the river begins above Washington, DC, 186 km from the mouth. Two layer estuarine circulation extends approximately 100 km from the mouth to the region between Morgantown (P42) and Douglas Point (P62) (Fig. 2). This upstream area or transition zone (hatched area) is well mixed and characterized by a turbidity maximum. Above this zone, net flow of freshwater is directed seaward at all depths (Elliott, 1976). Downstream of the transition zone, the river exhibits an internal estuarine circulation with dominant net outflow in low salinity surface waters and up-estuary flow in high salinity bottom waters (Elliott, 1978). The extent of this estuarine circulation depends upon the salinity penetration and may exist above Maryland Point during the fall salinity maximum (Elliott, 1976).

In the mouth region, another 'transition zone' delineates the downstream extent of the internal estuarine flow of the river from the bay inflow. During November 1979, 3 strings of current meters were moored for 7 d in

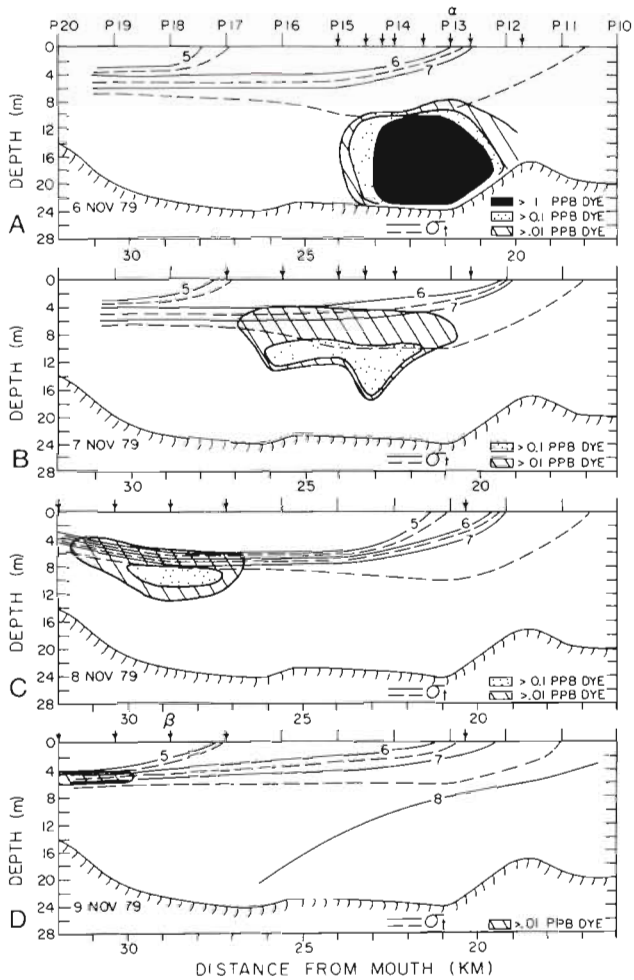

Fig. 3. Longitudinal section through Potomac River from station P10 (16 km from mouth) to P20 (32 km from mouth) showing upstream movement of rhodamine dye over a 4-d period originally injected into bottom waters (19 m) at P12 on 5 November, 1445 h. (A) 6 November, 0845 h, t = 18 h. (B) 7 November, 1300 h, t = 46 h. (C) 8 November, 1300 h, t = 70 h. (D) 9 November, 1200 h, t = 93 h. σ and β: position for cross-sections shown in Fig. 4. Station locations indicated by arrows

the central channel, at the mouth P0, at P7, and at P15. During this same time period, slack water runs were made to determine the density structure of the river. The resulting velocity vectors for surface (solid arrows) and bottom waters (dotted arrows) are shown in Fig. 2 along with surface σ_t isopleth distributions (solid lines). At the mouth of the Potomac River (P0), the net nontidal flow is upstream in both near surface and bottom waters of the central channel. Flow rates averaged 7.5 cm s^{-1} in the bottom and 5 cm s^{-1} in surface waters. The surface outflow along the southern shore of the Potomac was not monitored. At P7, surface and bottom flows are lower with net upstream surface and bottom currents of 2.4 cm s^{-1} and 0.9 cm s^{-1} respectively. At P15 a true estuarine circulation pattern was observed with net nontidal flows downstream in surface waters of 4 cm s^{-1} and net upstream flow in bottom waters of 6 cm s^{-1}.

During this period, a strong, visible front (represented in Fig. 2 by the higher density gradient centered about the 6 σ_t isopleth) persisted in an oblique cross-stream orientation and moved upstream and downstream with the tide. Vertical profiles of σ_t taken along the river axis indicate this to be the region where the major pycnocline of the river tilts and breaks the surface (see isopycnals in Fig. 11) and as such, it represents a likely area of vertical transport. This frontal convergence appears to delimit the downstream extent of the estuarine circulation.

The tilting of the pycnocline also occurs in a cross-stream direction. Isopycnals of Fig. 12, at P10, indicate that the high salinity net inflow extends surface to bottom on the north side of the river. On the south side, high salinity waters are confined below the net outflow. The pycnocline does not continue all the way to the shoreline, but intersects the bottom in shoaling areas. Thus, very close to the southern shore of the Potomac, net flow is downstream at all depths. Similar pycnocline orientation has been observed in the southern Chesapeake Bay and attributed to the Coriolis Effect (Pritchard, 1952).

There are, however, problems in interpreting the current data of Fig. 2. Such sparce Eulerian measurements often lead to oversimplification of the dynamics of the system, and vertical movements cannot be determined with certainty. Therefore, during autumn of 1979, a dye experiment was conducted near the frontal region to follow the movement of bottom waters. Fig. 3 represents a series of longitudinal sections through the Potomac River with σ_t isopleths and dye concentrations plotted for 6–9 November, 1979. The estuarine flow front with a visible foam line was observed in the region where the 6 σ_t isopleth breaks the surface. Rhodamine dye was injected as a dye trail into 19 m of water, σ_t of 7.6, at 1445 h (t_o) on 5 November, 1979 between Stations P11 and P12. At t = 18h (Fig. 3A), the center of dye was located in bottom waters at P13. At t = 46h (Fig. 3B), the dye had moved in an upstream direction and was concentrated near the pycnocline. By t = 70h (Fig. 3C), the rhodamine dye had formed a lens just below the pycnocline with the center of mass at P18. The last detectable concentrations of dye were observed at t = 93h, on 9 November (Fig. 3D). The central lens was again confined to the pycnocline and located at P19.5. This rhodamine dye time sequence demonstrates classic two-layer estuarine flow, with pronounced net nontidal upstream movement of waters below the pycnocline (approximately 3.2 km d^{-1}) in close agreement with the current meter records.

Examination of the movement of the central lens of the dye in a cross-stream direction results in the sequence shown in Fig. 4. Locations of the cross-sections are indicated in Fig. 3A and D by α and β. Fig. 4A, of cross-section α indicates the dye was distributed throughout the bottom waters and confined to the central channel. At the termination of the sampling program (Fig. 4B), the central mass of dye had moved upstream and was confined to the pycnocline on the south shore of the river. The results indicate that circulation patterns in the Potomac River may be influenced by a cross-stream flow as well as an upstream movement.

The sequence in Figs. 3 and 4 suggests that the central mass of the dye moved upward in the water column with time. Since the σ_t of injected dye was the same as waters into which it was placed, this apparent upward movement did not result from floatation of dye into a less dense layer. Rather, this distribution is likely due to a shearing off of the more rapidly flowing sub-pycnocline layer containing the dye. Alternately, the apparent accumulation in the pycnocline may possibly result from bottom waters moving upstream in a helical fashion, which would force dye laterally into shoaling areas where it would mix with less dense water and be returned within the pycnocline due to cross-stream flow. In a separate paper (Wang et al., unpubl.) the exchange rates between surface and bottom waters during the same time period are presented, indicating little advection across the pycnocline.

The results of the dye experiment are of particular interest, since the lens configuration below the pycnocline appears to be a characteristic feature of a subsurface transport of phytoplankton (Tyler and Seliger, 1978, 1981). The same resultant lens configuration of a dye patch indicates that physical mechanisms must be included when assessing the effects of organism mobility, such as positive phototaxis and buoyancy, on subsurface patch formation.

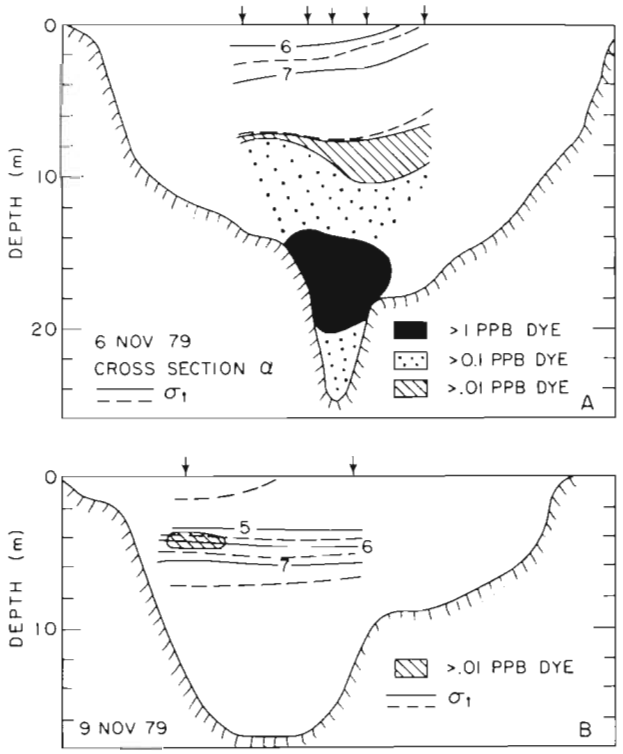

Fig. 4. Cross-section through Potomac River showing distribution of rhoadamine dye in cross-stream direction. (A) 6 November, 0845 h, t = 18 h, at P13. (B) 9 November, 1200 h, t = 93 h, at P18, cross section β

Red Tide Formation: Recirculation and Retention at a Frontal Convergence

In late summer 1979 and 1980 red tides of *Gyrodinium uncatenum* were observed in the estuarine portion of the Potomac River, their downstream extent bounded by an estuarine front. Fig. 5 represents a vertical section through the Potomac estuary for July, 1979. Samples for σ_t and cell counts were taken at 1 m intervals in the water column at designated stations (arrows). Highest concentrations of *G. uncatenum* occurred toward the mouth of the estuary in association with a visible estuarine front at P13. Bayward of this area, there was a sharp decline in organism concentration. The bloom extended upstream to P35 and observations in the Wicomico River at P30 indicate high concentrations also present. Data from the National Marine Fisheries (pers. comm.), U. S. Geological Survey, Maryland (1979), and our own observations indicate that this frontal region oscillated between P5 and P20 for most of the summer season.

The position of the 1980 bloom was significantly upstream of that observed in 1979, with concentrations of *Gyrodinium uncatenum* increasing sharply at P35 and extending to P50 (Fig. 6). While the location of the bloom differed by approximately 35 km from the 1979 bloom, the sharp delimitation of the bloom by the estuarine frontal region was again evident. During the 1980 bloom season, drought conditions resulted in low streamflow throughout the region. The run-off for the Potomac River in 1979 was 771 $m^3 s^{-1}$ as compared to the much reduced flow of 432 $m^3 s^{-1}$ in 1980. This low flow allowed high salinity bay waters to penetrate significantly up-estuary. The resulting estuarine front, delimiting Potomac outflow from bay inflow, moved in an up-estuary direction, and with it, the associated *G. uncatenum* concentrations. The summer excursion of the frontal region (from National Marine Fisheries and H. H. Seliger, pers. comm.) showed little change in position throughout the bloom season.

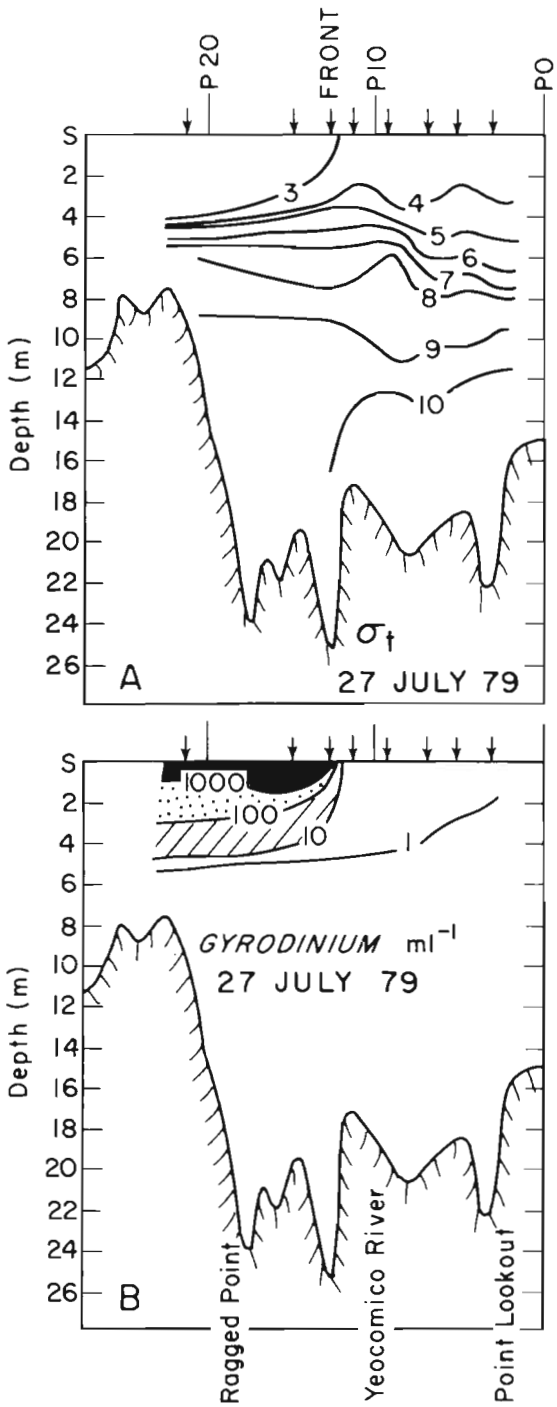

Fig. 5. Longitudinal section through spine of Potomac estuary; summer, 1979. (A) Density isopleths represented as solid lines with frontal region (foamline) evident at P15. (B) *Gyrodinium uncatenum*. Number of individuals ml^{-1} showing association with frontal region and highest concentration in surface waters

It does not appear that differential growth is responsible for the rapid changes in cell concentrations from one side of the front to the other. Growth rate isopleths for *Gyrodinium uncatenum* cultures grown at various salinities and temperatures are shown in Fig. 7 Cultures were originally isolated from bloom concentrations and acclimated to various growth conditions for approximately 1 month before determining log phase growth rates. At temperatures above 20°C, *G. uncatenum* exhibits high reproductive rates at salinities greater than 5 ‰ S. Below 5 ‰ S, growth rates are repressed with no detectable growth at 1 ‰ S. At temperatures less than 15°C, growth rates decline and below approximately 7°C, motile cells are not observed in culture. The repression of growth at very low salinities may explain the absence of *G. uncatenum* upstream of the turbidity zone, in essentially fresh water. However, it does not appear that a temperature or salinity-induced differential growth can explain the sharp changes in cell number across the front. In 1979 and 1980, the salinities across the frontal region were 7–10 ‰ S (σ_t = 2.5–3, T = 25°C) and 8–10 ‰ S (σ_t = 3–4, T = 26°C) respectively. Salinities on both sides of the front were within the range of the relatively high growth potential. Furthermore, a slightly higher growth might be expected on the downstream side of the convergence, in conflict with the observed distributions.

While the culture experiments indicate that *Gyrodinium uncatenum* may do well on both sides of the front, factors other than salinity or temperature (i.e. nutrient concentrations), may impart differential growth rates. In July 1979 samples of water containing *G. uncatenum* from upstream and downstream of the front were placed in phytoplankton cages (Owes et al., 1977) and resuspended on deck in drums containing surface waters from the location of their capture. Differential cell counts over a 24-h period indicated a growth rate of μ = .70 on the upstream side of the front and a comparable μ = .68 on the downstream side. This indicates that the decline of *G. uncatenum* downstream of the front cannot be attributed to a repression of growth rate.

Fig. 8 represents a vertical distribution of σ_t contours, over a 24-h period (24–25 July, 1979) at P13 in the estuarine portion of the river, upstream from the frontal region. Superimposed upon this plot is the normalized distribution of *Gyrodinium uncatenum* counted on an hourly basis at 1 m depth intervals. During mid-day, highest concentrations are in surface waters indicating a strong positive phototaxis. *G. uncatenum* migrates downward in the water column before sunset and remains within the pycnocline (σ_t 4–5) until sunrise. High concentrations reappear in the surface by about 0900 h. The pronounced downward migration appears limited by the pycnocline which may slow the seaward exchange of the organisms resulting in a retention within the estuary.

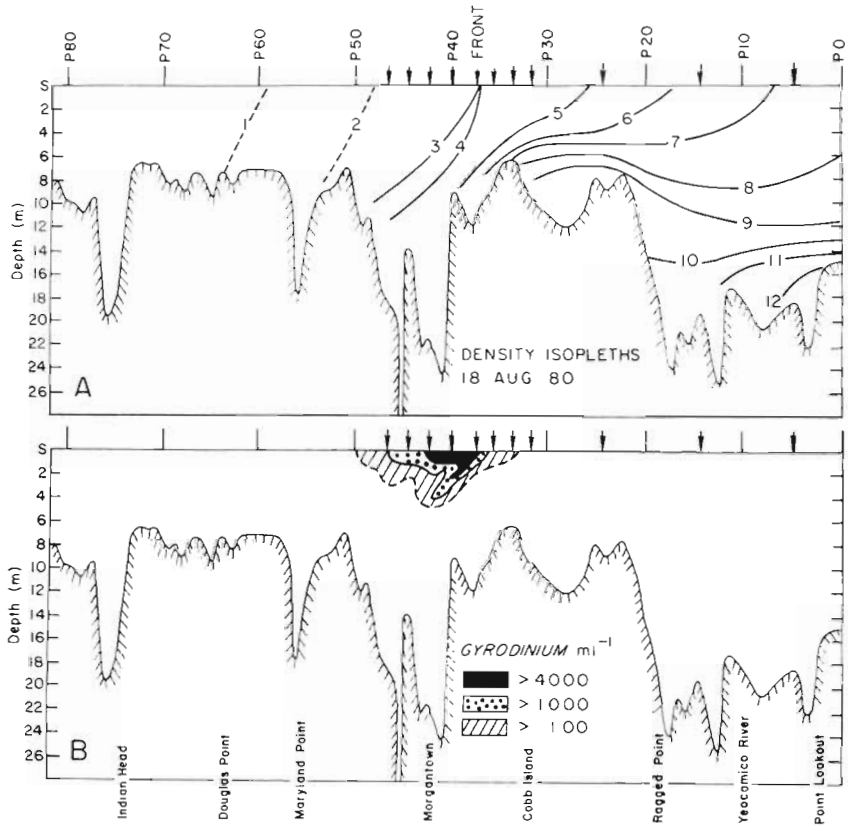

Fig. 6. Longitudinal section through spine of Potomac estuary; August, 1980. (A) Density isopleths represented as solid lines with frontal region (foamline) evident near P35. (B) *Gyrodinium uncatenum*. Individuals ml^{-1} showing association with frontal region

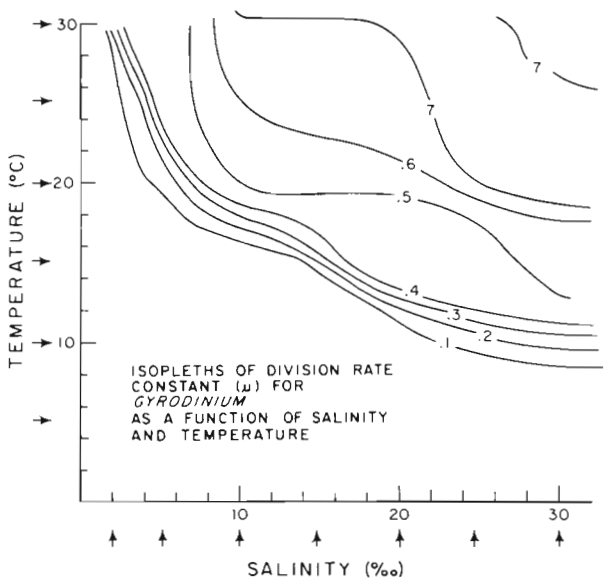

Fig. 7. *Gyrodinium uncatenum*. Isopleths of division rate constants (μd^{-1}) as a function of salinity and temperature. Experimental temperature and salinity points on matrix shown by arrows

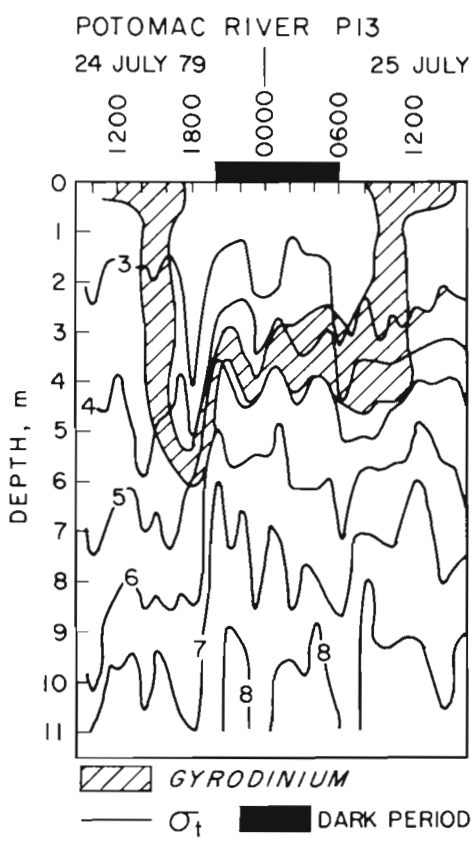

Fig. 8. *Gyrodinium uncatenum*. Diurnal migration over a 24-h period; 24–25 July, 1979. Cell enumeration on an hourly basis and at 1-m intervals. Shading: area in water column where *G. uncatenum* is most abundant (> 80 % maximum)

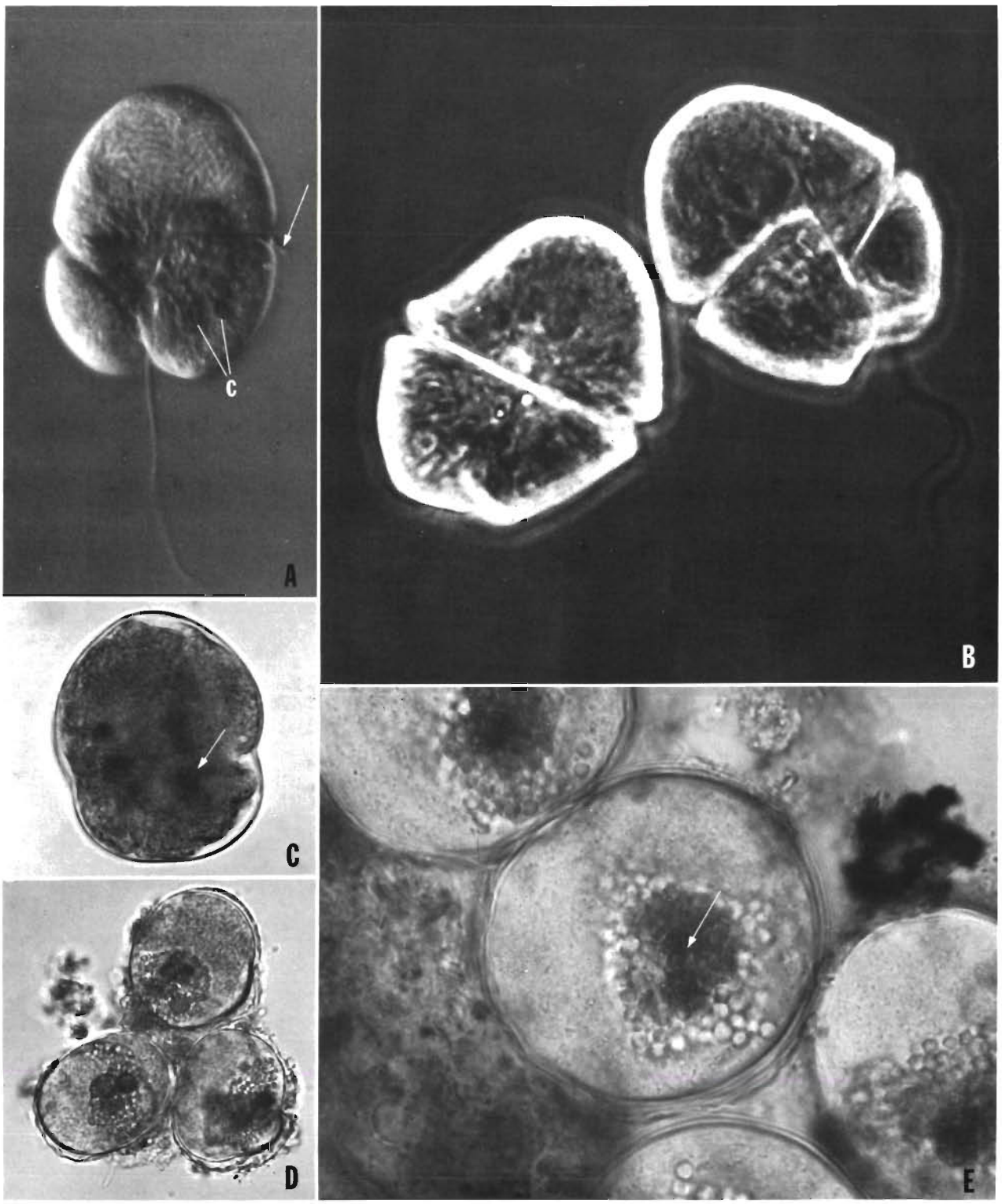

Fig. 9. *Gyrodinium uncatenum*. Major life cycle stages in individuals isolated from the Potomac River of Chesapeake Bay. (A) Nomarksi interference micrograph (× 1064) of a typical vegetative cell characterized by numerous, closely spaced chloroplasts (c), and 2 flagella (1 transverse [arrow], 1 posterior). (B) Two planozygotes characterized by 2 distinct posterior flagella in addition to 2 transverse flagella (not visible). Red pigmentation granules have developed in the hypocone (phase contrast, × 1028). (C) Late planozygote/early hypnozygote characterized by rounding of the cell, red pigmentation granules (arrow), and a decrease in motility (× 800). (D) Hypnozygote cluster showing outer membrane separated from inner smooth cell wall (× 518). (E) Hypnozygotes at higher magnification with interior of cell containing red pigmentation granules (arrow), and surrounded by numerous oil droplets (× 1332)

Further downstream at the convergence zone, the strong positive phototaxis in conjunction with the downwelling flows may result in the accumulation of the cells at the frontal interface as described elsewhere for other dinoflagellates (Tyler and Seliger, 1981). When convergence velocities exceed migrating speed, cells may become entrained along the frontal interface, giving rise to observations of cells below the surface, within the convergence. However, as the pycnocline assumes a more horizontal position, entrainment velocities decrease and it is possible for the positively phototatic *Gyrodinium uncatenum* to swim to the surface. Any cells advected across the front in a downstream direction by disruption of the frontal convergence would meet net upstream flowing waters (Fig. 2). The combination of a behavioral response and a hydrographic pattern may serve to accumulate and recirculate cells and thus retain them within the estuary.

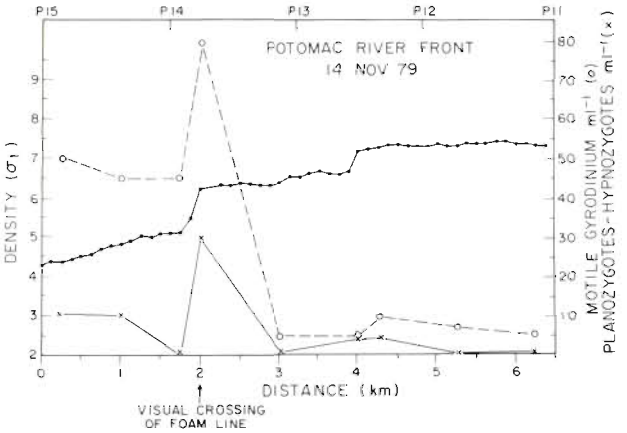

Fig. 10. Surface water transect (November, 1979) across an estuarine front at the mouth of Potomac River showing increase in motile cells and late planozygote/early hypnozygote *Gyrodinium uncatenum* within the convergence zone

Encystment: Accumulation and Entrainment of Sexual Stages at a Frontal Convergence

Gyrodinium uncatenum which usually divides asexually, possesses a sexual cycle resulting in the formation of a resting cyst capable of long periods of dormancy (Tyler et al., in preparation). The sequence in Fig. 9 shows the transition from the vegetative cell to the cyst. The asexual haploid *G. uncatenum* (Fig. 9A) is highly pigmented with closely packed chloroplasts, and 1 trailing and 1 transverse flagellum originating in a deeply offset sulcus. These cells are motile and positively phototatic. The diploid cell or planozygote (Fig. 9B) results from the fusion of 2 gametes. It is characterized by 2 distinct posterior flagella and 2 transverse flagella. The chloroplasts begin to condense around the nucleus and red pigmentation develops in the area of the sulcus. The late planozygote/early hypnozygote stage (Fig. 9C) exhibits a rounding up of the cell and a significant decrease in motility As the hypnozygote (cyst) develops, the outer wall becomes separated from the inner membrane and 'crinkles' around the smooth internal cell wall (Figs. 9D and E). Flagellation is lost, oil droplets appear, and cytoplasmic streaming is readily observed. Cells may retain a slight brownish cast.

In 1979 and 1980, planozygotes of *Gyrodinium uncatenum* were observed in the water column along with vegetative cells. Sexual stages were abundant in samples taken from the end of the bloom season in late summer-autumn. Fig. 10 shows a horizontal surface transect taken near P14 in November, 1979. *G. uncatenum* was concentrated within the convergence zone near the mouth of the river forming surface patches of late planozygote/-early hypnozygotes, and vegetative cells. Concentrations dropped off rapidly downstream of the frontal region.

Fig. 11A represents σ_t distribution for a longitudinal section through the spine of the Potomac River also for 14 November, 1979. Optical cell counts of motile *Gyrodinium uncatenum* are represented in Fig. 11B. In these counts, planozygotes were abundant but were not distinguished from vegetative cells. Highest cell concentrations were found within the convergence zone with a significant portion of the population apparently entrained along the frontal interface. Surface concentrations were high between P30 and P12, with a rapid decrease in cell numbers downstream of the frontal region, indicating the front again appears to be acting as a barrier to downstream movement. A similar distribution for late planozygote/early hypnozygotes (Fig. 9C) of *G. uncatenum* is shown in Fig. 11C. Highest concentrations (30 ml^{-1}) were located in the water column along the frontal interface of the convergence zone, representing approximately 30 % of the total *G. uncatenum* population in the water column. However, substantial concentrations were also present throughout the bottom 'raining' down from the pycnocline. *G. uncatenum* was not observed above P50.

Fig. 12A represents a south-to-north cross-section through the Potomac River at Station P10, approximately 16 km from the mouth with σ_t isopleths (solid lines) inclined toward the surface. Fig. 12B shows the concentration of late planozygote/early hypnozygotes of the same cross-stream section. Highest concentrations coincide with the maximum density gradient and follow the frontal interface to the shoaling area near the south shore, thus emphasizing the importance of cross-stream flow.

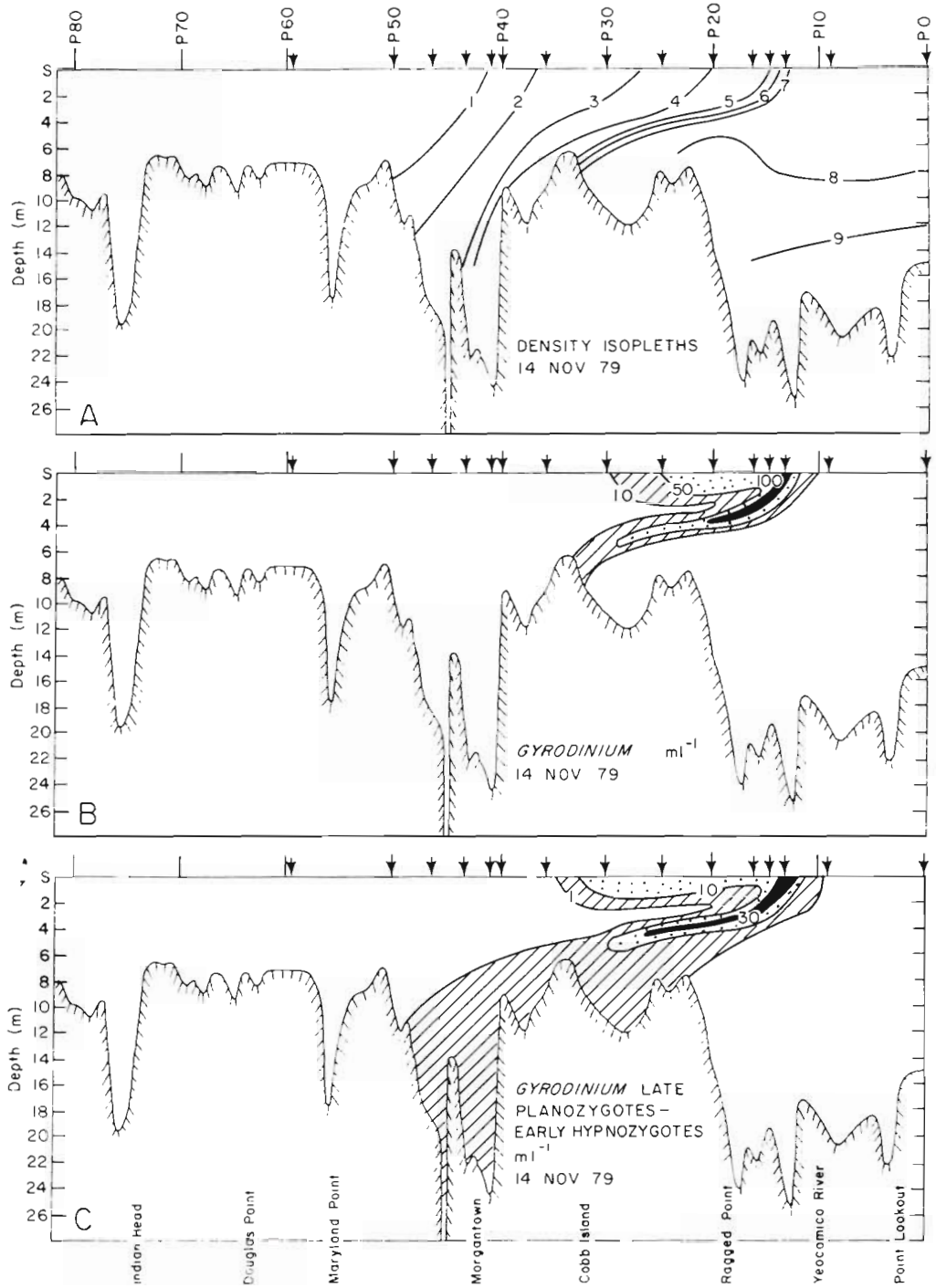

Fig. 11. Longitudinal cross-section through spine of Potomac River; 14 November, 1979. Arrows: vertical profile station locations. (A) Density isopleths indicating upstream penetration of higher-salinity bay water to Station P50. A surface frontal region exists in the region of P15 as indicated by the 5, 6 and 7 σ_t isopyncals breaking the surface. (B) *Gyrodinium uncatenum*. Optical counts of motile individuals, including planozygotes and vegetative cells. (C) Ditto. Optical counts of late planozygotes/early hypnozygotes

During this same cruise period, depth profiles of *Gyrodinium uncatenum* were taken over 24 h to determine organism distribution on a diel cycle. Fig. 13 represents an hourly depth-time contour of σ_t isopleths and the distribution *G. uncatenum* late planozygote/early hypnozygote concentrations. The ship was anchored mid-channel at the upstream excursion of the frontal region such that the front and associated foam

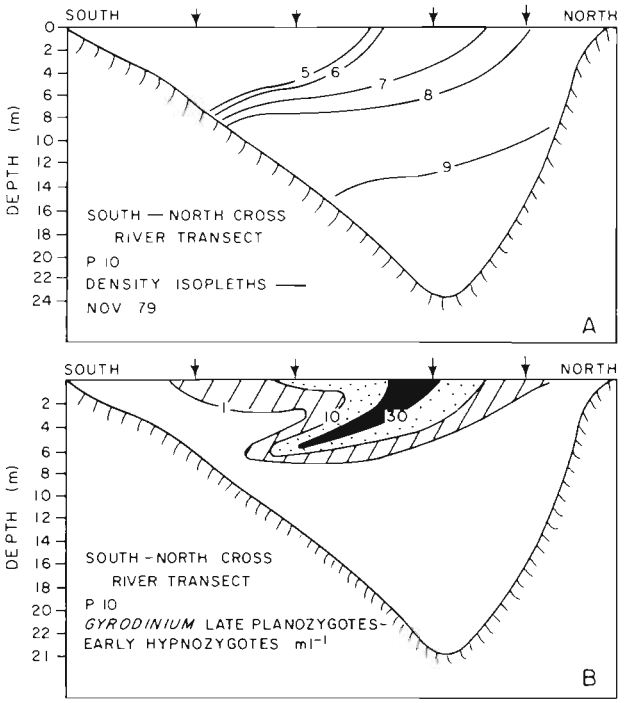

Fig. 12. South-to-north cross-section looking upstream through Potomac River; November, 1979. Arrows: vertical profile station locations. (A) Density isopleths (σ_t) indicating fresh water outflow confined to the southern shore. (B) *Gyrodinium uncatenum*. Optical counts of late planozygotes/early hypnozygotes in the water column showing an accumulation in the front and cross-stream transport in the pycnocline region

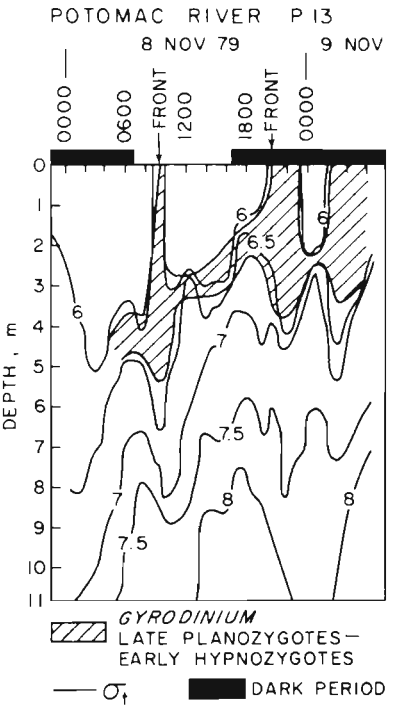

Fig. 13. Depth-time contours of σ_t isopleths (solid lines) observed for 8–9 November, 1979. Highest concentrations of *Gyrodinium uncatenum* late planozygotes/early hypnozygotes determined by microscopic observations are shown as hatched areas indicating highest concentrations (> 30 cysts ml^{-1})

line passed the ship twice daily (indicated by arrows). The shaded areas indicate highest concentrations of late planozygotes/early hypnozygotes. As the foam line passed the ship, high cell concentrations were seen in surface waters. As the front passed downstream, surface waters became less saline and the 6.0 σ_t isopleth was submerged below the outflow as the pycnocline. The area of highest cell concentration follows the pycnocline as it submerges to become the subsurface concentrations seen upstream of the front in Fig. 11C. In contrast with the data presented in Fig. 8 of summer vegetative cells, the late planozygotes/early hypnozygotes do not appear to migrate during the 24-h period. There is no evidence of a pronounced downward migration during dark hours or of a strong positively phototactic response during the photoperiod. Rather, the highest concentration of these sexual stages of *G. uncatenum* remains associated with a particular water density, the 6–6.5 isopycnal. Their upstream confinement in bottom waters (Fig. 11C) may reflect this weak motility or lack of positive phototaxis. The continued loss of motility associated with hypnozygote (cyst) formation results in the disappearance of *G. uncatenum* from the water column and appearance of cysts in the sediments.

Deposition of Hypnozygotes in Seed Beds Along the Subsurface Transport Pathway

During 1979 and 1980 sediment samples were taken from approximately 50 locations throughout the river to determine the distribution of *Gyrodinium uncatenum* hypnozygotes. Examples of vertical distributions of *G. uncatenum* cysts in the sediments of the Potomac River are represented in Table 1. During autumn 1980, 90 % of the cysts in all samples were contained in the upper 4 cm of the sediment, with peak concentrations usually occurring in the upper 2 cm. In the Potomac River, the surface flocculent layer is typically < 1 cm deep. Partitioning of the 1 cm layer into surface flocculent material and underlying layer showed no enrichment at the surface. Thus, for the Potomac River at this time, the upper 4 cm can be combined to give an estimate of the number of cysts per unit area. Pollen and ^{210}Pb indicate an average rate of sedimentation for the Potomac River of 0.5 cm yr^{-1}, slightly higher in upstream areas and slightly lower near the mouth (G. Brush, The Johns Hopkins Univ., pers. comm.). However, our observations of hypnozygotes at 4 cm in depth within a few months of their

Table 1. *Gyrodinium uncatenum*. Vertical distribution in the sediment expressed as number of cysts cm^{-3} vertical sediment interval at representative locations throughout the Potomac. Integrating the top 4 cm at each location provides 90 % of the cysts. Data can be expressed on a volume (cm^3) or area (cm^2) basis. Subdivision of surface layer at designated locations shows no enrichment of near surface (SP 26, NP 35)

	Cysts cm^{-3}/Vertical sediment slice Sediment depth (cm)											Cysts cm^{-3}	Cysts cm^{-2}
Station	1	2	3	4	5	6	7	8	9	10	% 1–4 cm	1–4 cm combined	1–4 cm combined
PO	41	17	12	17	0						100	22	87
SP5	75	110	41	6		6		0		0	97	58	232
SP10	6	0	0	0							100	1	6
SP15	81	157	41	52	6	6					97	83	331
SP20	46	29	0	0							100	19	75
SP26	298/765	133	←23→								–	279	1113
SP30	499	186	226	23	0	6	0	0			99	233	934
NP35	388/566	70	← 6→								–	140	559
P40	284	719	93	17	99	6	0				90	280	1119

deposition indicate that sediments are rapidly reworked. Hammond and Fuller (in press) have estimated depth of mixing of the sediments and rates of benthic exchange in the Potomac River by integrating the radon deficiency over depth in sediment cores. Rn/Ra ratio for the areas between P10 and P50 show large increases in this ratio occurring below 5 cm depth (often below 3 cm), indicating mixing to this depth. The distributions of *G. uncatenum* cysts to 4 cm correlate well with these findings, and may provide an additional measurement of sediment mixing.

During 1980, replicate pump and core samples were taken for comparison of the 2 methods of sediment sampling. All samples were processed indentically and normalized to dry weight for comparison. Under ideal conditions with 2 point anchoring of the ship, replicate core and pump samples taken at multiple locations showed a 1-to-1 correspondence between the pumped sample and the average value of the top 2 cm of core sediment. Under sampling conditions of variable winds and no anchor, pumped samples typically yielded values 1 to 7 times higher than numbers obtained for the top 2 cm of cores. In these cases, pumped samples often agree more closely with the top centimeter of core sediment, perhaps indicating that the pump skimmed the sediment surface as the ship drifted. Consequently, under varying weather conditions, the pumped samples must be viewed as qualitative estimates of cyst numbers which are of use in discriminating between cyst concentrations of an order of magnitude or more apart. During both 1979 and 1980, cyst concentrations in the river sediment ranged over 3 orders of magnitude. Thus both pump and core samples accurately reflect relative cyst distributions; however, only core samples appear reliable in determining absolute cyst numbers, and accounting for distributions deeper than 2 cm.

Gyrodinium uncatenum cyst distributions for 1979 and 1980 are presented in Fig. 14A–C. Fig. 14A and B provide a direct comparison between cyst distribution for the 2 years and Fig. 14B and C compare data obtained through the 2 sampling procedures. In 1979, highest cyst concentrations (blackened areas) were confined close to the southern shore and near the mouth of the river. *G. uncatenum* cysts extended in an upstream direction to P30, dropped of rapidly above this point and were not detected beyond P50. During the 1980 season, cysts were concentrated between P30 and P40, approximately 30 nautical miles (55 km) upstream from the 1979 seed bed, and were detectable in sediments to P65. Note that the relative distribution and peak locations of cysts for 1980 pumped and cored samples are identical.

In Fig. 14A–C, approximate limits of the surface frontal excursion for the bloom season are shown by solid lines. In 1979, the frontal region occurred between the mouth and P15, an area encompassing the location of highest cyst concentrations. In 1980, the frontal excursion was approximately 30 nautical miles (55 km) upstream from its 1979 location and also encompassed the region of peak cyst concentrations. In 1979, the major pycnocline associated with the front penetrated in an upstream direction to P30 (Fig. 11), while in 1980, it extended farther upstream and indicated a strong estuarine circulation to P45. In both years, cyst numbers dropped off rapidly above the upstream penetration of the major pycnocline.

From Fig. 4, cross-stream flow appears to be an important component of the circulation pattern near the mouth. In 1979 major cyst deposits appear confined toward the southern shore near the mouth, in a shoaling region where the pycnocline intersects the bottom (Fig. 12). In 1980, however, upstream deposits are more evenly distributed across the river perhaps indicating

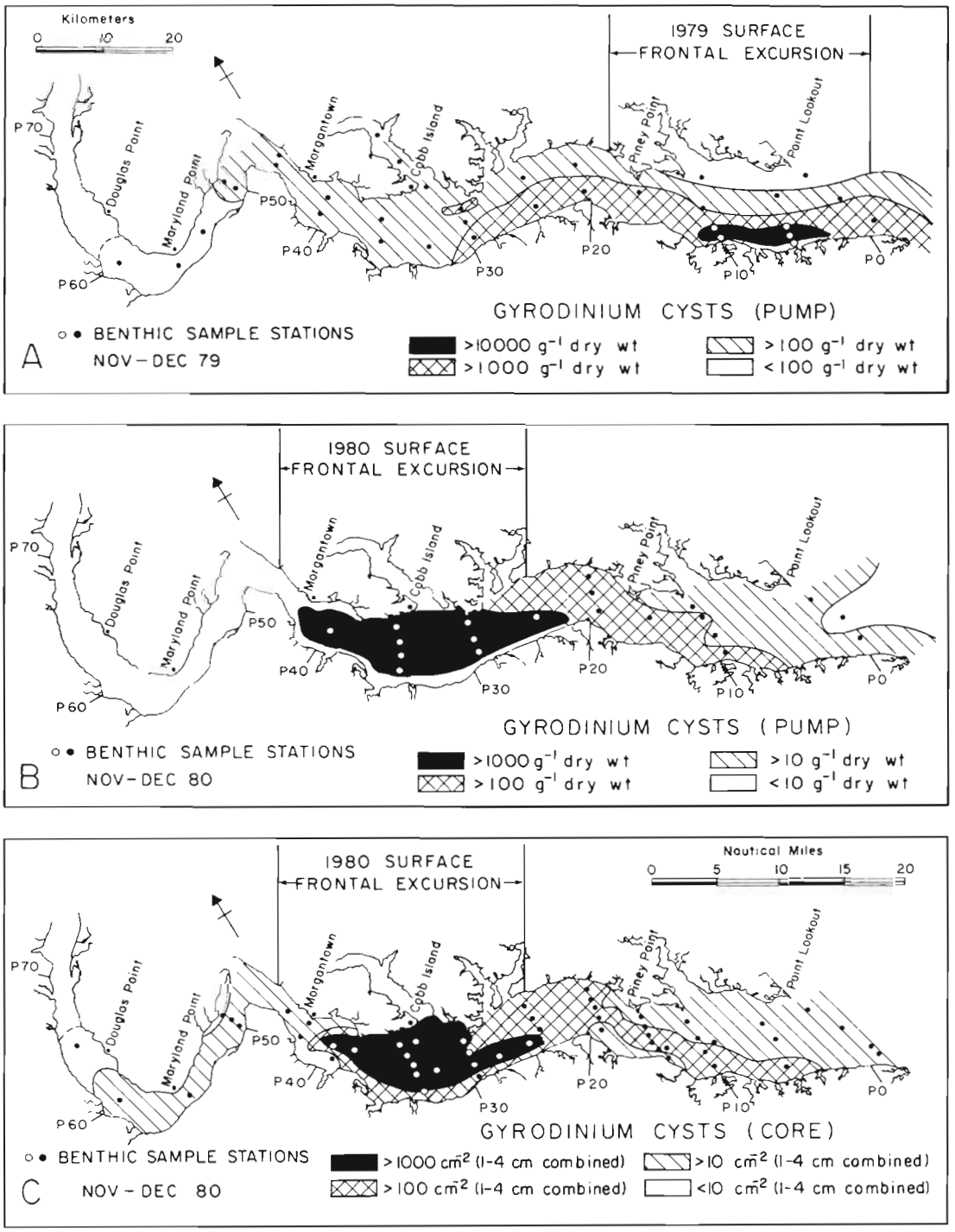

Fig. 14. *Gyrodinium uncatenum*. Distributions of benthic cysts in sediments of Potomac River. (A) November–December, 1979; pumped sediments; circles: sampling stations. (B) November–December, 1980; pumped sediments; highest cyst concentrations signficiantly upstream from 1979 location, with highest numbers between P35 and P40. (C) November–December, 1980; core samples; 1–4 cm core samples integrated and expressed per unit area (cm^{-2}); center of mass corresponds with that in B

that cross-stream flow is less important as one proceeds away from the mouth. Sediment analyses (percent sand, silt, clay) show that the mouth area near the north shore is predominantly sand suggesting that this is a highly scoured area. This provides an alternative explanation for the absence of cysts in this area.

Overview

For a bloom to occur in a given area, there must exist a seed population and conditions sufficient to support growth. In cases where the seed population consists of motile organisms translocated from one bloom area to another, it is important to understand the physical and biological mechanisms controlling population transfer, as was demonstrated for blooms of *Prorocentrum mariae-lebouriae* in Chesapeake Bay (Tyler and Seliger, 1978, 1981). For a red tide organism that is known to encyst, the location of cyst accumulations becomes an additional parameter in determining the temporal/spatial sequence of the bloom. In one restricted Cape Cod embayment subject to recurrent toxic blooms, cysts of *Gonyaulax tamarensis* were found in sediments only within the bloom area (Anderson and Morel, 1979). In this stable system with reduced circulation, cyst germination initiated blooms in the overlying water.

However, most coastal areas subject to red tides are dynamic systems with complex circulation patterns. Examination of sediments underlying these bloom areas may indicate that the cysts seeding the bloom are not within the bloom area (Anderson et al., 1982). For example, Steidinger (1975) observed that blooms of *Gymnodinium breve* (= *Ptychodiscus brevis*, Steidinger, in press 1979) which are transported onshore under certain physical conditions, appear to be initiated up to 64 km offshore, possibly the location of a seed bed. An offshore origin for cysts of *Gonyaulax tamarensis* and subsequent onshore transport has also been proposed for dinoflagellate blooms in the southern Gulf of Maine (Hartwell, 1975; Mulligan, 1979).

Where cyst accumulations are outside a bloom area, the hydrographic transport mechanisms are clearly important. Frontal regions are areas of high biomass and productivity. Surface accumulations of plankton as well as detritus, oil, and other particulates at convergences similar to those observed in the Potomac have been demonstrated in diverse systems of the world (Pingree et al., 1978; Tyler and Seliger, 1978; Holligan, 1979). However, the role of the frontal region in initiating subsurface concentrations has been more difficult to ascertain.

The picture presented in Figs. 1–14 suggests that, under favorable growth conditions, the patchy surface distributions of *Gyrodinium uncatenum* are caused by surface density discontinuities or fronts and that the persistence and magnitude of such blooms may be a function of the duration and strength of convergences. In addition, it appears that the relationship between discontinuities and organism patchiness holds in the vertical structure as well, with the pycnocline serving as the 'conveyor belt' along which surface organisms can be transported to a benthic destination. Thus, the location of the benthic distributions becomes predictable. Holligan (1979) observed high concentrations of *G. aureolum* in surface waters on the stratified side of the Ushant front and a mid-depth maximum within the frontal interface or major thermocline in the water column with a configuration similar to that shown for *G. uncatenum*. This may indicate a transport of cells (potentially sexual stages and cysts) to benthic destinations. Such frontal circulation patterns may also determine areas of meroplankton settlement – such as mollusc spat, bryozoans, etc. – providing an explanation for their patchy benthic distribution; it may explain as well the distribution of pollen in sediments. It is interesting to note that the shellfish beds in the Potomac are located in shoaling areas where the pycnocline intersects the bottom. This being the case, cysts transported in the pycnocline would be advected and deposited in mollusc harvesting areas. Potentially these same mechanisms may deliver toxic dinoflagellates, off the coast, directly to shellfish beds.

In addition to a transport there may be a physiological advantage to the accumulation at a frontal convergence. We have seen fusing gametes of *Gyrodinium uncatenum* in the water column within a frontal convergence. Such density-dependent fusion may be found at all levels of organisms and, thus, fronts should provide areas of increased sexual activity.

Frontal regions should also produce areas of enhanced trophic-level interaction. Increased concentrations of phytoplankton should be important in enhancing the growth and/or reproduction of predators. Lasker (1975) has shown that first feeding anchovy larvae requires food in excess of the average standing crop in California coastal waters for further development. In this case, only subsurface maximum layers of *Gymnodinium splendens* provided adequate concentrations. If high food concentrations are a general requirement for development, the front and associated pycnocline of the Potomac River should provide optimal feeding sites.

CONCLUSIONS

In the Potomac River, seasonal blooms of *Gyrodinium uncatenum* are spatially confined downstream by estuarine fronts. Hydrographic patterns associated with the frontal interface in conjunction with behavioral responses of the flagellate (i.e. positive phototaxis and diurnal migration) apparently retain, concentrate and recirculate the population within the estuary. Toward the end of the bloom season, sexual life history stages of *G. uncatenum* become abundant in the water column. The distribution of

weakly and/or non motile encysting organisms (late planozygote/early hypnozygotes) mimics that of the remainder of the population. The observed distributions may represent a uniform encystment of cells throughout the *G. uncatenum* population, possibly accompanied by a partial retention and entrainment of encysting organisms at the frontal interface. Cysts transferred along the frontal interface and into bottom waters settle on the sediment and form seed beds. The ultimate locations of these seed beds appear to be governed spatially by water circulation patterns at the time of encystment. If, therefore, the location of the frontal region is known during the time of the encystment, the location of the seed beds becomes predictable.

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