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Sinking characteristics of dinoflagellate cysts[1]

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Abstract

This study reports the first direct measurements of the density and sinking rates of marine dinoflagellate cysts. *Gyrodinium uncatenum, Gonyaulax tamarensis,* and *Scrippsiella trochoidea* cysts had densities of 1.14, 1.24, and 1.32 g cm^{-3}. No significant difference between cultured and natural cyst density was observed. Measured settling velocities ranged from 0.008 to 0.013 cm s^{-1} (6–11 m d^{-1}). Settling rates calculated using the measured densities and reasonable assumptions about shape and orientation during descent were within 10–20% of measured sinking rates, confirming that cyst sinking can be described by a modification of Stokes' law for nonspherical particles in a viscous medium. The three types of cysts examined are more dense and fall faster than most vegetative phytoplankton cells. Removal of an outer layer of short calcite spines from *S. trochoidea* cysts reduced both cell density and radius by 7% and the sinking rate by 37%. The faster settling velocity of the spiny cyst is not considered a general effect common to all ornamented cysts but presumably applies only to those with numerous short spines or processes. Morphological features can thus markedly affect the rate of cyst deposition, but the adaptive significance of surface ornamentation remains unknown.

About 10% of the 2,000 species of living dinoflagellates are known to include a dormant cyst stage in their life histories (Dale 1982). As might be expected, this percentage is higher in coastal and estuarine regions where shallow waters restrict the vertical range of travel and where environmental fluctuations are large. Dale (1976) found cyst stages associated with 20% of the dinoflagellate community in Trondheimsfjord, a percentage similar to that observed in the Woods Hole region (Anderson unpubl. data).

The variety of functions attributed to cysts includes roles in species dispersal, bloom initiation, bloom termination, and survival through adverse conditions (Wall 1971; Anderson 1984). Consideration of the importance of these factors in dinoflagellate population dynamics requires an understanding of the physical characteristics of cysts, especially their behavior as passive particles that can settle to sediments to seed recurrent blooms, disperse to new areas through advection, and be resuspended from the bottom into environments favorable for germination. The transport properties of cysts depend on their size, density, and settling velocity, yet little is known of these. Sarjeant et al. (in press) provide the only data on density and sinking rate, but their values are theoretical calculations based on estimates of the density and volume of various cellular constituents. They also used plastic models of cysts and glycerol as a medium (for dynamic similarity) to investigate the effects of morphological features such as spines or flanges on sinking rates. Their results suggest that spines increase form resistance more than they increase particle density and thus that ornamented cysts would fall more slowly than smooth forms. One of the objectives of our study was to test this hypothesis using living cysts with and without short calcitic spines.

We also wanted to measure the settling velocities and densities of several morphologically distinct types of cysts using living specimens from cultures and field samples. The sinking rates reported here give dinoflagellate cysts particle Reynolds numbers $\ll 0.5$. Their sinking should thus be de-

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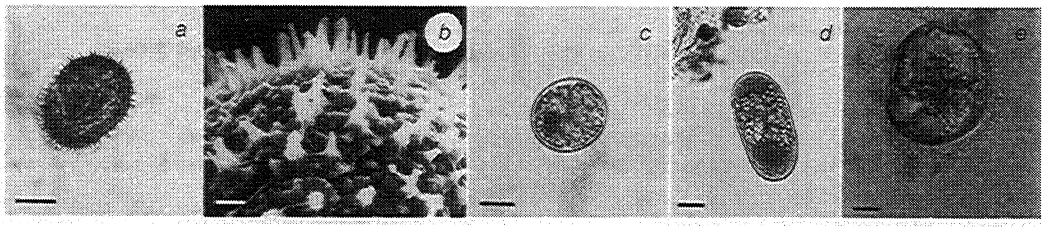

Fig. 1. Dinoflagellate cysts used. Scale bar equals 10 μm unless otherwise indicated. a—*Scrippsiella trochoidea*; b—scanning electron micrograph of air-dried *S. trochoidea* cyst (scale bar, 1 μm); c—*S. trochoidea* cyst following dissolution of spines in mild acid; d—*Gonyaulax tamarensis*; e—*Gyrodinium uncatenum*.

scribed by Stokes' law for spherical forms in a viscous medium:

$$V_s = \frac{2gr^2(\rho - \rho_0)}{9\mu} \quad (1)$$

where V_s is the sinking velocity of a sphere (cm s^{-1}), g is the acceleration due to gravity (980.7 cm s^{-2}), μ is the viscosity of the medium (poise), r is the radius of the particle (cm), ρ is the density of the particle (g cm^{-3}), and ρ_0 is the density of the medium (g cm^{-3}). Since the sinking velocity of particles of the same density and volume can vary significantly with shape, Eq. 1 has been modified for nonspherical particles (McNown and Malaika 1950)

$$V = \frac{2gr_n^2(\rho - \rho_0)}{9\mu\phi} \quad (2)$$

where V is the observed terminal velocity of the particle, and ϕ is the coefficient of form resistance. The nominal radius of the nonspherical body is r_n, defined as the radius of a sphere with volume equal to that of the body. Komar (1980) derived empirical equations similar to the one above for ellipsoidal and cylindrical particles using glass shapes in glycerine solutions, again relying on dynamic similarity for hydrodynamic equivalence.

The unknowns in Eq. 2 are the particle density, the settling velocity, and ϕ, the coefficient of form resistance (which has been well characterized by McNown and Malaika for ellipsoids of various shapes). Using a recently developed protocol for producing cysts in marine dinoflagellate cultures (Anderson et al. 1984) and a density gradient medium made from Metrizamide (Rickwood and Birnie 1975) with the correct density and osmotic balance for meaningful isopycnic banding of relatively dense particles in seawater (Taghon et al. 1984), we were able to obtain direct measurements of the density of living dinoflagellate cysts. In conjunction with measurements of sinking velocities, we can now compare the sinking characteristics of several cyst morphotypes and separate and quantify the effects of spine formation on cyst density and form resistance for one species.

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Methods

Cysts—Cultured cysts of three species of dinoflagellate were examined: *Scrippsiella trochoidea* (Perch Pond strain SA10), *Gonyaulax tamarensis* (var. *excavata*; strain GTMP from Cape Cod), and *Gyrodinium uncatenum* (strain GYRO from Baltimore Harbor). The cyst of *S. trochoidea* (Fig. 1a, b) is slightly ellipsoidal (29 × 27 μm) and is covered with hundreds of short, calcite spines (Wall et al. 1970). *Gonyaulax tamarensis* (Fig. 1d) is also ellipsoidal but is larger and more elongate (47 × 30 μm), with a smooth, unornamented cell wall (Anderson and Wall 1978). *Gyrodinium uncatenum* (Fig. 1e) is large (48 × 39 μm) with a more irregular ellipsoidal shape and a smooth cell wall (Tyler et al. 1982). Cysts of all three species were produced in NH_4^+-limited batch cultures (Anderson et al. 1984). The contents of one or two 25-ml cultures in 25- × 150-mm tubes were generally suf-

ficient for each experiment. *Scrippsiella trochoidea* cysts were agitated lightly before use, while *G. tamarensis* and *G. uncatenum* cysts were sonicated for 30–45 s at 2 A (Branson Sonifer, model S75) to break up clumps caused by mucilage production; this separated individual cysts and removed most adhering mucilage. The sample used for each run consisted of approximately equal concentrations of the three types of cyst mixed in a volume of 5–10 ml.

Gonyaulax tamarensis and *G. uncatenum* cysts in natural sediments were also used. Sediment samples were sonicated, sieved, and resuspended (Wall and Dale 1968). Samples so processed contained sand grains and living or dead organic material in suspension with the cysts, but the sonication procedure effectively eliminated aggregation or clumping. Cysts were stored at 2°–4°C to prevent germination but all gradient experiments were conducted at room temperature after equilibration.

Cysts were counted in Palmer-Maloney chambers with a light microscope and measured with an ocular micrometer ($n \geq 50$). Viability was tested in two ways. Since *S. trochoidea* produces calcitic cyst walls that are intact following excystment, germination success was easily monitored by counting empty cyst walls 2 weeks after inoculation of cysts into f/2-Si medium (Guillard and Ryther 1962) and incubation at 15°C with 300 μEinst m^{-2} s^{-1} irradiance on a 14 : 10 L/D cycle. The less-rigid cyst wall of *G. uncatenum* made it necessary to isolate individual cysts into tissue culture wells containing 130 μl of f/2-Si medium and to monitor germination success after 2 weeks of incubation in the light at 20°C.

To test the effects of spine formation on cyst density and sinking rate, we removed the calcite spines of *S. trochoidea* with mild acid. Cyst suspensions kept overnight in seawater brought to pH 6.5 with dilute HCl lost their spines without any other morphological changes (Fig. 1c). To ensure that this treatment did not significantly alter cyst physiology, we compared the viability of treated cysts with that of cysts from the parent culture. All measurements of density or sinking velocity were made within 48 h of spine removal.

Sinking velocities—Terminal sinking velocities were measured in a temperature-controlled room with a Zeiss inverted microscope and a modification of the settling tube technique of Smayda and Boleyn (1965). After equilibration at 22°C, filtered seawater (32‰) was added to a 50-ml Zeiss settling tube (total depth 100 mm). The top of the cylinder was covered with a thin plastic sheet and another settling chamber (10 ml, total depth 15 mm) placed directly on top. About 8 ml of a cyst suspension in 29‰ seawater was poured into the top cylinder and covered with a glass coverslip. The plastic sheet was then carefully pulled out, allowing the liquid in the two chambers to come into contact. With sufficient silicone vacuum grease on the cylinder bases, this procedure could be performed without leakage or mixing of the two layers. This was verified with dye solutions.

Once the separation between the two chambers was removed, cysts were enumerated at the bottom of the settling tube at 1-min intervals using an inverted microscope with no condenser and extremely low light levels (turned off between counts). At 100× total magnification, all cysts were counted in a continuous sweep across the widest portion of the settling chamber, representing about 8% of the slide area. By the end of each experiment, this typically included 250–500 cysts. On the assumption that the cyst suspension in the top chamber was uniformly distributed, we determined the time for half the cyst population to descend 107.5 mm (half the length of the top chamber plus the entire bottom chamber) and used that to calculate a mean settling velocity. Each settling experiment was repeated twice.

Density measurements—We tried to collect cysts by isopycnic centrifugation into density gradients by the Percoll method (Price et al. 1978). The maximum density of the Percoll-sorbitol-seawater mixture (1.15 g cm^{-3}) was not sufficient to float most dinoflagellate cysts, however, so we tried concentrating the sol by ultrafiltration through XM300 membranes (Amicon Corp.). One liter of Percoll could be concentrated through a 142-mm membrane in a stirred cell (HI-FLUX U-F Cell, Millipore

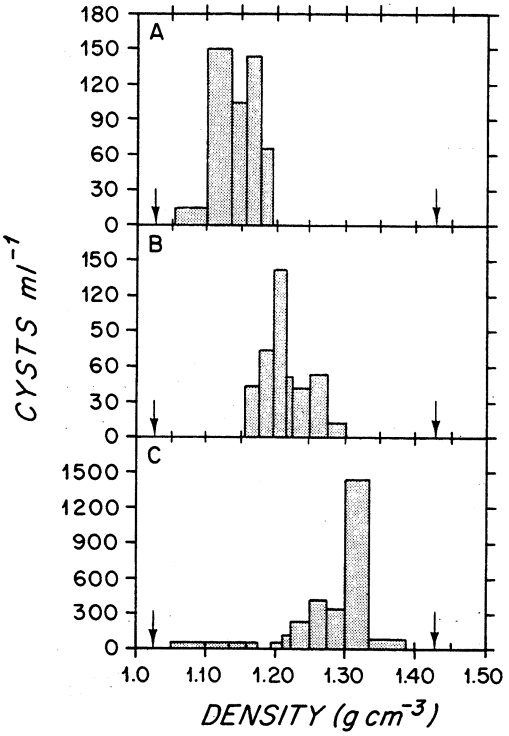

Fig. 2. Typical frequency distributions of cyst density from a single gradient. Each histogram bar represents all cysts in a gradient subsample spanning the indicated density range. A—*Gyrodinium uncatenum*; B—*Gonyaulax tamarensis*; C—*Scrippsiella trochoidea* with spines. Arrows indicate density range of the gradient.

Corp.) in about 8 h. Although the maximum density of a concentrated Percoll-sorbitol seawater mixture was 1.22 g cm^{-3}, most dinoflagellate cysts continued to pellet at the bottom of the centrifuge tubes.

Metrizamide (2-[3-acetamido-5-(*N*-methylacetamido)-2,4,6-triiodobenzamido]-2-deoxy-D-glucose), a chemically inert, nonionic derivative of benzoic acid was used in all subsequent experiments (Rickwood and Birnie 1975). We prepared continuous Metrizamide-seawater density gradients of 1.03–1.5 g cm^{-3} (3–4 ml total vol) in 10-ml polycarbonate culture tubes using a starting solution of filtered seawater (31‰), a limiting solution of 60% (wt/vol) Metrizamide in diluted seawater (Taghon et al. 1984), and a stirred, double-cone gradient mixer (Haake/Buchler Corp.) and a peristaltic pump. A 0.5–1.0-ml cushion of 60% Metrizamide was added at the bottom of each tube to facilitate separation of the pellet (sand, etc.) from the remainder of the gradient. The cyst/seawater suspension (1.0–1.5 ml per tube) was gently added to the top of the gradient with a glass 1.0-ml pipet with a rubber bulb. The tubes were then centrifuged at 200 *g* for 10 min (IEC model PR-2 centrifuge with swinging buckets) with additional acceleration and deceleration times of 3 min. After centrifugation, the gradients were fractionated into about 20 aliquants (0.2–0.4 ml) by withdrawing sample continuously through a needle inserted into the gradient to the top of the cushion. A peristaltic pump was used for withdrawal, each sample representing 30 s of flow. We then determined the cyst concentration and refractive index of each fraction (triplicate 0.1-ml cyst counts; sucrose refractometer) and converted the refractive index to density using a standard curve derived from five Metrizamide-seawater solutions (20–60% wt/vol; Mettler/Parr DMA 45 density meter).

Results

Density measurements—Each of the three types of dinoflagellate cyst banded in a consistent, reproducible manner in the density-gradient medium. Figure 2 shows the distributions in one run and Table 1 lists the means and standard errors for replicate analyses. *Scrippsiella trochoidea* was the most dense, followed by *G. tamarensis* and *G. uncatenum*. The banding patterns generally followed normal distributions with standard deviations at 3–4% of the mean for each gradient. Cysts from sediment samples had the same densities as cysts from laboratory cultures (Table 1). This was true even for *G. tamarensis* cysts, which varied considerably in size; those from Gulf of Maine sediments had 44% less cell volume than those from a small Cape Cod salt pond, yet their densities were not significantly different ($P > 0.05$).

Removal of the calcite spines from *S. trochoidea* reduced the cyst density by 0.09 g cm^{-3} (7%) and the radius by about 1 μm (7%; Table 2). Except for the obvious lack of spines, the acid-treated cysts (Fig. 1c) were similar in morphology and physiology to

Table 1. Cyst densities for three dinoflagellate species determined with density gradient centrifugation.

Source	No. of gradients	Total cysts	Cyst density (g cm^{-3})	
			Mean	Grand mean (SE)
Gonyaulax tamarensis				
Gulf of Maine sediment	3	369	1.24	
Perch Pond sediment	4	327	1.23	1.24(0.020)
Lab culture	2	108	1.23	
Gyrodinium uncatenum				
Potomac River sediment	3	474	1.13	1.14(0.02)
Lab culture	2	158	1.15	
Scrippsiella trochoidea (with spines)				
Lab culture	4	1,125	1.32	1.32(0.01)
S. trochoidea (no spines)				
Lab culture	2	434	1.23	1.23(0.02)

those with spines (Fig. 1a). Germination success after 2 weeks of incubation was high (85%) and not significantly different for the two types ($P > 0.01$).

To test the effects of the Metrizamide solution on cyst viability, we isolated individual *G. uncatenum* cysts into tissue culture wells before and after centrifugation and incubated them in the light at 20°C for 2 weeks. There was no significant difference ($P > 0.05$) in the percentage of successful germinations between cysts isolated before exposure to the gradient medium (80%, $n = 20$) and those taken from the gradient itself (88%, $n = 24$). Some slight mortality (20% more than controls) was observed in cysts left in their Metrizamide banding solutions for 4 days.

Cyst recovery varied with species. The two larger cysts (*G. tamarensis* and *G. uncatenum*) were more difficult to process quantitatively as recoveries ranged between

Table 2. Sinking parameters for dinoflagellate cysts. Two sets of calculated data are presented for each type of cyst to indicate the range of sinking velocities due to vertical or horizontal orientation of the long axis of the cell during descent. Ellipsoidal shapes assumed in all cases. Form resistance calculated from McNown and Malaika (1950). Not measured—NM. Measured sinking rate is the mean of two experiments. In parentheses—SE.

	Mean length of semiaxes (μm)		r_n (μm)	Form resistance (ϕ)	Measured cyst density (g cm^{-3})	Sinking velocity (cm s^{-1})	
	a (vertical)	*b* and *c*				Meas	Calc
Scrippsiella trochoidea	14.71(0.32)	13.59(0.23)	13.95	0.97	1.32(0.01)		0.013
(with spines)	13.59(0.23)	14.71(0.32)	13.95	1.06	1.32(0.01)		0.011
						0.013	
S. trochoidea	13.36(0.28)	12.71(0.25)	12.91	1.04	1.23(0.02)		0.007
(no spines)	12.71(0.25)	13.36(0.28)	12.91	1.02	1.23(0.02)		0.007
						0.008	
Gonyaulax tamarensis	23.60(0.40)	15.15(0.09)	17.56	0.96	1.24(0.02)		0.015
(Cape Cod)	15.15(0.09)	23.60(0.40)	17.56	1.18	1.24(0.02)		0.012
						0.011	
G. tamarensis	19.55(0.61)	12.05(0.21)	14.55	0.96	1.24(0.02)		0.010
(Gulf of Maine)	12.05(0.21)	19.55(0.61)	14.55	1.18	1.24(0.02)		0.008
						NM	
Gyrodinium uncatenum	24.2(0.36)	19.5(0.30)	20.96	0.97	1.14(0.02)		0.009
	19.5(0.30)	24.2(0.36)	20.96	1.11	1.14(0.02)		0.008
						NM	

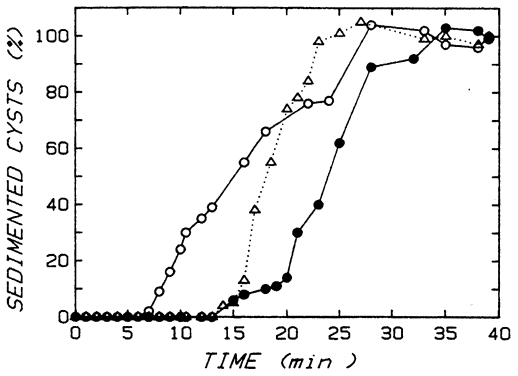

Fig. 3. Example sedimentation time-courses for three types of cyst. Each point represents the percentage of all sedimented cysts observed at that time. *Scrippsiella trochoidea* with spines (○), *S. trochoidea* without spines (●), and *Gonyaulax tamarensis* (△) had calculated settling rates of 0.013, 0.007, and 0.010 cm s^{-1}.

60 and 100%. When a cyst suspension of *G. tamarensis* was simply pumped through the tubing and counted, an average of 18% of the cysts were lost, presumably due to adhesion to the tubing itself. This loss was not observed with *S. trochoidea*; recovery efficiencies averaged 98%. *Scrippsiella trochoidea* does not produce the mucilaginous coating often associated with the other two types of cyst.

Sinking velocities—The sinking velocities of the cultured cysts varied between 0.008 and 0.013 cm s^{-1} at 22°C in 31‰ seawater. *Scrippsiella trochoidea* with spines fell the fastest, *S. trochoidea* without spines the slowest, and *G. tamarensis* at intermediate rates (Table 2). The loss of our *G. uncatenum* culture prevented measurement of cyst sinking rate for that species.

Figure 3 shows the variability in sinking rates within each population. *Gonyaulax tamarensis* was the most uniform, with the first cysts arriving at the bottom of the chamber <10 min before the last (40% of the total settling time). Some of this variability (2.5 min) is simply a result of the protocol requiring a uniform cyst suspension in the 15-mm-deep top chamber. The *S. trochoidea* populations (both with and without spines) were more variable, with the interval between the fastest and slowest cysts averaging 20 min or 55–75% of the total settling time (Fig. 2).

Sinking velocities were calculated for each species from the average cyst dimensions, mean cyst density, and calculated values of ϕ—the coefficient of form resistance in Eq. 2 (Table 2). Since the cysts generally have ellipsoidal cross sections, their volume was calculated with the equation for a prolate spheroid and equated to a sphere of radius r_n. The coefficient of form resistance ϕ was calculated (McNown and Malaika 1950) for the most- and least-streamlined orientation of each type of cyst and used in separate calculations of sinking velocity (Table 2); this provides a range of expected settling rates, with the highest value for each type of cyst as much as 20% greater than the lowest.

In general, calculated sinking rates for cysts of different species varied by a factor of nearly two, between 0.013 cm s^{-1} for *G. tamarensis* and 0.007 for *S. trochoidea* cysts without spines. This rapid settling rate for *G. tamarensis* is for cysts from Cape Cod; the smaller cysts produced in the Gulf of Maine coastal waters would theoretically fall 32% more slowly. Although the cyst of *S. trochoidea* is relatively small, its covering of calcitic spines increased the cell density sufficiently to result in a rapid settling rate (0.012 cm s^{-1}). In contrast, calculated rates for the large *G. uncatenum* cysts are low (0.008 cm s^{-1}) because of its relatively low density.

Discussion

Density measurements—The encystment process in dinoflagellates produces resting cysts with densities considerably higher than those of most free-swimming or floating vegetative phytoplankters. Direct measurements of phytoplankton cell densities are rare, but those few that have been made (Oliver et al. 1981) and other values calculated from measured settling velocities (Eppley et al. 1967) suggest a broad range of 1.03–1.20 g cm^{-3}, with most species that are not heavily silicified or calcareous near 1.05 g cm^{-3}. Only three vegetative dinoflagellates have been examined. *Gonyaulax polyedra* (a thecate species similar in general motile form to *G. tamarensis*) has a calculated density of 1.05 g cm^{-3} (Eppley et al. 1967), while *Gymnodinium nelsoni* (=*san-*

guineum) and *Peridinium* sp. have densities of 1.05 and 1.08 g cm^{-3}, measured directly by isopycnic sedimentation in gradients of Percoll (Price unpubl. data). Our measured cyst densities ranged between 1.14 and 1.32 g cm^{-3}. The validity of these density measurements clearly rests on the assumption that the gradient medium did not alter cyst physiology. The good agreement between direct measurements and calculated estimates of sinking rate (*see below*) and our success in germinating cysts isolated from gradient media support this assumption.

Several mechanisms could account for the increase in density upon encystment. Many cysts have a thick, multilayered wall composed of cellulose or of sporopollenin, a poorly defined, highly resistant organic polymer (Bibby and Dodge 1972; Durr 1979; Chapman et al. 1982). The combined thickness of these walls is generally 0.5–1.5 μm, although TEM measurements are scarce due to problems with fixation and resin penetration. Since cellulose and sporopollenin both have densities near 1.5 g cm^{-3} (Chaloner and Orbell 1971), a vegetative dinoflagellate 30 μm in diameter with an initial density of 1.05 g cm^{-3} would increase in density by 8% to 1.13 g cm^{-3} after formation of a 1-μm-thick wall. These calculations are approximate but indicate that cyst densities near 1.24 g cm^{-3} cannot be explained solely on the basis of an added cell wall. Other possible mechanisms for increasing density include a decrease in cellular water content (as is often the case with other dormant cell forms: Sussman and Halvorson 1966; Warth 1978) or the accumulation of relatively dense starch that overcomes the buoyant effect of lipids. Both lipid and starch accumulate in cells before encystment (Bibby and Dodge 1972; Anderson and Wall 1978; Durr 1979; Chapman et al. 1982). If we assume an initial cytoplasmic density for a vegetative cell near that of seawater (1.025 g cm^{-3}) and a 1-μm-thick cyst wall, the cytoplasmic density would have to increase 16% to 1.18 g cm^{-3} to yield an overall cyst density of 1.24 g cm^{-3}.

Scrippsiella trochoidea and other species that produce calcareous cysts (e.g. *Ensiculifera* sp.: Dale 1978) have an additional dense component—a layer of calcite spines (Fig. 1b; Wall et al. 1970) which adds significantly to the mass of a cyst that otherwise closely resembles its unornamented relatives. At the other extreme, *G. uncatenum* has the lowest cyst density (1.14 g cm^{-3}), possibly because of its relatively thin wall or large size (Tyler et al. 1982; Coats et al. 1984). Our observations of *G. uncatenum* cyst mortality in natural sediments and in nonaxenic cultures suggest that this species is not highly resistant to predation or environmental stress (Anderson unpubl. data). Furthermore, *G. uncatenum* is a naked dinoflagellate (i.e. the motile cell has no rigid cell wall), and Wall and Dale (1968) indicated that despite the abundance of naked dinoflagellate cysts in natural sediments, few if any fossilize, and none of the living cyst specimens they examined withstood even mild chemical treatment.

Sinking velocity—The measured sinking velocities of living dinoflagellate cysts ranged from 0.008 to 0.013 cm s^{-1}. They thus have particle Reynolds numbers between 0.002 and 0.004, so that their sinking behavior can theoretically be described by Stokes' law (Eq. 1). However, since the cysts were nonspherical, comparisons between measured and calculated sinking rates must include consideration of the independent effects of shape and orientation on settling velocity.

One way to account for shape in the Stokes' equation was given by McNown and Malaika (1950) who provided equations for the coefficient of form resistance (ϕ) for ellipsoidal shapes. This coefficient varies with orientation during descent, and, as seen in Table 2, the calculated settling rates for the fastest- and slowest-falling orientations differed by over 20% for elongate cysts like those of *G. tamarensis*. McNown and Malaika (1950) demonstrated that symmetrically weighted bodies will orient so as to provide maximum form resistance and minimum sinking speed—in effect to maximize the cross-sectional area normal to the direction of motion. This only occurred at Reynolds numbers >0.1, however—a value nearly two orders of magnitude higher than those calculated for dinoflagellate cysts. We do not know if all cysts are symmetrically weighted, but observations of the cysts as

they reached the bottom of the settling chamber suggest that they kept the orientation in which they started their descent. A population sinking rate is probably best described by the average of calculations based on the fastest- and slowest-sinking orientations.

For the three cases where measured sinking rates can be compared with calculated values, the agreement is quite good (Table 2). Estimates for *S. trochoidea* with and without spines are within 10% of measured rates, while *G. tamarensis* cysts fell within 18% of the predicted rate. Since there are alternative formulations to account for shape effects (e.g. Davies 1947; Orr 1966) and the cyst volume calculations (and hence r_n) assume a perfect ellipsoidal shape, agreement within 10–20% seems sufficient to validate the independent measurements of cyst density and sinking rate.

Ecological implications—Most vegetative phytoplankton cells are not neutrally buoyant (Eppley et al. 1967; Smayda 1970). The optimal strategy for a nonmotile phytoplankter might be one that allows it to sink with respect to the surrounding (presumably nutrient-depleted) water, but not so fast or so far that it would permanently leave the euphotic zone (Smayda 1970). In contrast, dinoflagellate cysts sink rapidly at rates that would carry them to coastal sediments in 2 weeks or less in the absence of mixing. Most phytoplankton cells and diatom spores can resume vegetative growth rapidly when they encounter a favorable environment, but nearly all dinoflagellate cysts have an endogenously controlled mandatory resting period (true dormancy) lasting from weeks to months during which germination is not possible (Pfiester and Anderson 1986). The shortest mandatory dormancy period reported to date is that of *S. trochoidea* (3 weeks: B. Binder unpubl. data) yet that species has a calcareous cyst with the highest sinking rate of those we examined. In general, the disparity between sinking rates and mandatory dormancy intervals makes it likely that many coastal and estuarine dinoflagellate cysts would not germinate before being deposited in nearshore sediments. The dense cyst wall thus decreases travel time to the bottom and provides protection from predators and from environmental stresses in the benthos. Clearly a rapid descent to the sediments underlying a bloom region would also facilitate future blooms in waters that had supported a successful bloom population.

It is generally accepted that sinking rate is closely coupled to a cell's surface-to-volume ratio (Smayda 1970). One way to increase this ratio and to decrease sinking rate is to form spines or protuberances, but only if the added density from ornamentation is exceeded by the corresponding increase in drag. In two studies in which the spines of algae were removed experimentally, spineless cells settled more rapidly (Conway and Trainor 1972; Walsby and Xypolyta 1977). A similar experiment in our study had the opposite effect: removal of *S. trochoidea* spines decreased the cyst sinking rate by 37%. Cysts with and without spines were physiologically the same in all other respects, so the difference in sinking was clearly related to ornamentation.

This is an exception to the widely held view of the effect of protuberances, but it is probably a special case that applies only to dinoflagellate cysts with dense outcroppings of short spines (e.g. *Protoperidinium claudicans, Protoceratium reticulatum, Peridinium faeroense, Ensiculifera* sp.: Wall and Dale 1968; Dale 1978). The spines of *S. trochoidea* are very short (only 7% of the cell radius), and McNown and Malaika (1950) showed with physical models that such small irregularities on the surface do not appreciably alter an object's form resistance. The spines of the diatom *Thalassiosira fluviatilis* do affect sinking rate because their length is several times the cell radius (Walsby and Xypolyta 1977). In this context, it is noteworthy that newly formed *S. trochoidea* cysts are covered with a membrane that can partially offset the potential drag from protruding spines (Wall et al. 1970). Although we do not yet know how this membrane spans the individual spines and thus limits water flow between them, the data in Table 2 show that any increase in drag from the spine/membrane configuration is small relative to the increase in density associated with spine formation.

Other cyst morphotypes with fewer and

larger protuberances made of organic compounds such as sporopollenin (e.g. *G. polyedra, Gonyaulax scrippsae, Protoperidinium conicum, Gonyaulax spinifera*: Wall and Dale 1968) may fall slowly as a result of the increased surface area from their ornamentation. Sarjeant et al. (in press) used plastic models in a viscous medium to simulate the Reynolds environment of cysts of this type. A moderate number of spines (14) did reduce sinking rates, but the effect was modest and represented only a 25% decrease over a smooth cyst of the same size. They assumed a spine length 9% of the cyst radius and no membrane covering.

There are clearly no simple generalizations on the relationship between cyst morphology and sinking rate. Cysts in coastal waters can be perfectly smooth, or covered with hundreds of small spines that make them sink rapidly, or they can have a smaller number of larger protuberances that can increase drag or decrease density depending on membrane characteristics. The existence of the last morphotype implies either an advantage to a prolonged residence in the plankton or another function for the ornamentation. Since many dinoflagellate cysts are formed within the parent cell, ornamentation may simply reduce cytoplasmic volume while maintaining a structural connection to the cell wall.

Practical considerations—Our study shows that cyst densities and sinking rates vary significantly among dinoflagellate species; the values reported here should be extrapolated to other species with caution. Futhermore, the variability in size and sinking rate (but not density) of *G. tamarensis* cysts from separate geographic regions indicates that differences among strains of the same species may also be large.

It should also be emphasized that the sinking rates are valid only at the experimental temperature and salinity (22°C, 32‰). Since the viscosity of seawater varies significantly with temperature, a 10° decrease from 22° to 12°C would decrease the sinking rates in Table 2 by about 22%. The effect of salinity is much smaller: a 50% decrease would only increase sinking rate by 3%.

Our main objective was to measure density directly, but our technique can also be used to separate cysts from sediment samples or from cultures containing vegetative cells. The variable recovery efficiencies (especially for the cysts that secrete mucilaginous material) imply that quantitative analyses may not be possible unless the gradient can be unloaded without a peristaltic pump. Since cysts in the 1–1.5-ml initial suspension were distributed in about the same total volume within the gradient, our protocol does not concentrate particles to any significant degree. A larger initial suspension could be used, but the concentration factor would still be small, and for some species would be offset by poor recovery.

As discussed above, it is likely that most dinoflagellate cysts will reach coastal or estuarine sediments before they are sufficiently mature to germinate. Once in the sediments, they are exposed to a variety of chemical and biological factors that can affect germination and reinoculation of the overlying waters. A major unknown is the impact of resuspension on deposited cysts. Cysts are not only buried well below the more easily eroded surface sediments by benthic biological activity, but they also adhere to other particles in the sediment due to mucilage production or entanglement. Although our study provides useful data on the sinking characteristics of cysts as newly formed individual particles, it has limited applicability to cysts following deposition. Studies of cyst/sediment associations are necessary to enhance our understanding of the dynamics of the dinoflagellate resting stage.

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