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The effects of darkness and anaerobiosis on dinoflagellate cyst germination[1,2]

Donald M. Anderson, Craig D. Taylor, and E. Virginia Armbrust
Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Abstract

The effects of light and anaerobiosis on germination of marine dinoflagellate resting cysts were examined. Germination of all species was completely inhibited during 7 weeks of anaerobic incubation, although the cysts remained viable. Light requirements were more variable. Germination rates in the light varied 20-fold between species; dark rates varied by a similar factor but were generally much lower. One species (*Gonyaulax polyedra*) required light to germinate, three germinated faster in the light than in the dark (*Gonyaulax tamarensis, Scrippsiella* sp., and *Gonyaulax verior*), and one germinated at comparable rates in the light and in the dark (*Gonyaulax rugosum*). Brief exposure to light at the beginning of the experiments made it impossible to say whether germination is possible in constant darkness. It is clear, however, that prolonged light exposure is a requirement only for *G. polyedra,* although it can significantly accelerate germination of the other species. Germination inhibition by darkness and anaerobiosis helps to explain the subsurface accumulation of dinoflagellate cysts in marine sediments and the persistence of benthic cyst populations at times when temperatures favor germination. These constraints are sufficiently common that many cysts will never germinate once they reach bottom sediments.

Many aspects of the timing, location, and duration of dinoflagellate blooms have been linked to the encystment/excystment cycle of those species that form resting cysts during their life histories (Huber and Nipkow 1922; Anderson and Morel 1979; Heaney et al. 1983; Anderson et al. 1983; Lewis et al. 1985). One of several roles attributed to cysts is that of seeding or inoculating the water column with vegetative cells to initiate blooms (Steidinger 1975). Cysts are also important to paleontologists who use fossilized remains to date rock strata (Evitt 1970). In both cases, the abundance, distribution, and fate of deposited cysts are important variables.

Several factors determine whether a cyst formed in the water column will eventually germinate and resume a motile existence. The most important factor is the requirement for a period of development or maturation during which the cyst cannot germinate (true dormancy). The shortest mandatory dormancy period observed thus far for a marine dinoflagellate is 25 d for *Scrippsiella trochoidea* (Binder 1986); the longest is nearly 5 months for *Gonyaulax* (=*Protogonyaulax*) *tamarensis* (Anderson 1980). Given the relatively fast sinking rates of dinoflagellate cysts (Anderson et al. 1985), it is thus likely that most will fall from the water column and reach the sediments in coastal waters before their dormancy period is complete and germination is possible.

Once the cyst is mature, the resting state is maintained until external conditions are suitable for germination. Temperature is often cited as the major environmental factor regulating germination (e.g. Huber and Nipkow 1923; Wall and Dale 1968; Fukuyo et al. 1982; Endo and Nagata 1984). Both low and high temperatures have been shown to retard excystment (Anderson 1980), with germination occurring only within a permissive temperature range or "window."

It is evident from several observations that many viable cysts remain in the sediments at times when temperatures are within optimal ranges for germination (Huber and Nipkow 1922; Anderson and Morel 1979; Heaney et al. 1983; Anderson et al. 1983; Lewis et al. 1985). Some other factor or factors can obviously override a favorable temperature regime. Many deposited cysts are buried well below the sediment surface by bioturbation or sedimentation (Anderson et al. 1982). Sediments in regions

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of high organic matter deposition are anoxic except for the top few millimeters (Hutchinson 1957; Jørgensen 1982), so the bulk of a benthic cyst population would be without light or oxygen. Past studies of the effect of light on cyst germination have generally shown that the ultimate germination frequency does not differ significantly between light and dark treatments (Huber and Nipkow 1922, 1923; Anderson and Wall 1978; Endo and Nagata 1984). One study documented a decrease in germination rate (but not germination frequency) with dark incubation (Endo and Nagata 1984). The absence of a similar effect in other studies could reflect species-specific differences, but the variability in cyst isolation, inoculation, and observation methods among these studies makes such comparisons difficult. One of the objectives of the experiments reported here was to examine light effects on the cysts of six dinoflagellate species simultaneously. Specifically, the kinetics of germination with and without light were determined, as were the ultimate germination frequencies over a range of irradiances.

A second objective was to determine whether germination could occur in the absence of oxygen. There is no doubt that cysts can survive for years in anoxic sediments (Huber and Nipkow 1922; Dale 1983), but their oxygen requirements during germination are unknown. An early series of experiments by Huber and Nipkow (1923) suggested that germination of the freshwater dinoflagellate *Ceratium hirundinella* was possible without oxygen. This was confirmed for the same species by Krupa (1981), but Endo and Nagata (1984) found that germination of *Peridinium* sp. cysts was inhibited under anoxic conditions. As the incubations in the latter study were only 1 week long, germination may have been delayed rather than completely inhibited by the lack of oxygen. Furthermore, in all three of these studies, oxygen concentrations were not measured, so anaerobic conditions are not assured.

The contradictory and incomplete nature of these earlier studies emphasizes the need for a quantitative investigation of the effects of light and anaerobiosis on dinoflagellate cyst germination.

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Methods

Cyst collection and identification—Cysts were recovered from laboratory cultures and from natural sediments collected in two locations. The first was Perch Pond, a shallow estuarine embayment in Falmouth, Massachusetts, where sediments were collected in February 1984 using a hand-held coring device (Anderson et al. 1982). The second was a 160-m-deep station in the southern Gulf of Maine near Cape Ann, Massachusetts (43°N, 70°19′W), where a Craib corer was used (Craib 1965), also in February 1984. These latter cores were extruded on board ship, whereas Perch Pond cores were stored in darkness and cold during transport and extruded in the laboratory. In both cases, oxygenated surface sediments (upper 1–2 cm) were removed with overlying water. The outer layer of extruded sediment was also discarded because it had been exposed to light during coring. The extrusion process was conducted in near darkness. Most cysts were also shielded from light by the mass of sediment surrounding them. Sediment was then stored in the dark at 2°C in filtered seawater until needed.

All species except *Gonyaulax polyedra* were studied using these sediment slurries. Natural abundance of *G. polyedra* cysts was low in core samples, so cysts of this species were formed in nitrogen-limited laboratory cultures grown at 20°C on a 14 : 10 h L/D cycle (Anderson et al. 1984). After formation, the medium overlying the settled cysts was removed from the culture tubes and replaced by filtered seawater enriched with f/2 levels of trace metals, chelator, and vitamins (Guillard and Ryther 1962). Tubes were stored in a dark box at 15°C for 6 months. During this time, the cysts were occasionally exposed to light (5–10 s at about 100 μEinst m^{-2} s^{-1}) when tubes were removed for other experiments.

Several of the cyst types used in this study are well described in the literature. These include *G. tamarensis* (=*Protogonyaulax*

tamarensis Taylor; Dale 1977; Anderson and Wall 1978), *Gyrodinium uncatenum* (Tyler et al. 1982), and *G. polyedra* (Evitt and Davidson 1964). Three other Perch Pond cysts were used whose motile stage affinities will be described elsewhere. These include *Gonyaulax verior, Gonyaulax rugosum,* and *Scrippsiella* sp. Cape Ann sediments were used to obtain data on *G. tamarensis* in the time-course study of light and dark germination. For the irradiance and anoxia experiments, the low number of *G. tamarensis* cysts in the Perch Pond sample made it necessary to combine mud from the two sampling sites. *Gonyaulax tamarensis* was the only type of cyst common to both sediments. The final slurry contained about equal numbers of cysts from Cape Ann and Perch Pond.

Cyst processing and enumeration—A common approach to studies of cyst germination has been to sonicate sediment and to isolate individual cysts under the microscope. Cysts are necessarily exposed to intense light several times during such processing and isolation. Furthermore, sonication may affect germination (Dale 1983). To avoid these potential artifacts, we determined cyst germination from the disappearance of cysts from a sediment slurry that was not sonicated or processed until the end of the experiment. A well-mixed slurry of sediment and filtered seawater was dispensed in 5-ml portions into 125-ml Erlenmeyer flasks. Forty-five milliliters of sterilized medium (f/2 levels of chelator, metals, and vitamins, and 3 μM NO_3^- and 0.3 μM PO_4^{3-}) were added to each flask. The nitrate and phosphate additions were low to ensure that new cysts did not form in large numbers following the growth of germinated cells. On the basis of previous culture experiments, any new cyst formation with these low concentrations of nutrients would constitute <5% of the initial cysts present. All inoculations and manipulations were carried out either in near darkness or in a dark room with low levels of red light. In both cases, the light levels were below detection limits (0.02 μEinst m^{-2} s^{-1}) of a Biospherical Instruments QSL-100P probe and QSP-200 irradiance meter.

Cysts in the sediment slurries were counted as follows. The flasks were emptied, rinsed well, and the contents sonicated (Wall and Dale 1968) and sieved to retain the 20–80-μm size fraction with precision metal sieves (Buckbee Mears Co.). The material retained on the 20-μm sieve was resuspended into 5 ml of filtered seawater and dispensed into a 1-ml Sedgwick-Rafter slide. The slide was scanned in horizontal transects at 200× total magnification. Half the slide was typically examined, but when cysts were numerous, a smaller volume was scanned.

Irradiance study—Experimental flasks containing a sediment slurry were randomly divided into three groups. Six flasks were processed immediately and the cysts counted as described above. The other two groups were incubated at either 15° or 20°C. Small "tents" of window screening completely surrounded six replicate flasks for each light level, with the flasks illuminated from below. Six flasks were enclosed in a dark box. The entire box was carried to a dark room when flasks were removed for harvesting and counting. Several times each week during the incubations, the flasks were gently swirled to equalize light exposure within the sediment. Some flasks were harvested after 3 weeks of incubation, with the remainder processed and counted after 7 weeks. Data are reported for the temperature at which each species germinated at the highest frequency (15°C for *G. tamarensis* and *G. verior*; 20°C for *G. uncatenum, G. rugosum,* and *Scrippsiella* sp.). By adjusting the distance between the flasks or the box and the light source ("cool white" fluorescent), temperature differences between treatments were minimized but were still about 2°C.

When provided, light was on a 14 : 10 L/D cycle. Irradiance was measured with a submersible probe (Biospherical Instr. QSL-100P). One reading was taken in a flask containing medium but no mud and another holding the probe above the thin layer of sediment used in the experiment. The average of these two readings is reported; differences were typically within 10–15% of their average.

Germination time-course—Experimental flasks were divided between a saturating level of irradiance (150 μEinst m^{-2} s^{-1},

based on the excystment results in the irradiance study: Table 1) and a dark box in the same incubator. Flasks were inoculated as described above, placed in the light or the dark at 15° or 20°C depending on the species, and removed from these treatments at intervals and harvested in duplicate. Results were compared to the mean of six replicate flasks processed at the beginning of the experiment. Sediment was collected from Perch Pond on 21 February 1984.

The time-course of *G. polyedra* germination was determined with cysts from laboratory cultures. The cysts were formed and stored as described above and dispensed in 1-ml portions into small culture tubes. The tubes were sealed with parafilm, divided into two groups, and incubated at 26°C either in the dark or the light (150 μEinst m^{-2} s^{-1}; 14:10 L/D cycle). This entire operation and any subsequent sampling from the dark box were conducted in total darkness. Germination success was monitored by counts of ungerminated cysts and empty cyst walls.

Anoxia study—Anaerobic medium was prepared according to a modification (Wolfe 1971) of the Hungate technique (Hungate 1950). Filtered seawater (31‰) was enriched with f/2 nutrients, vitamins, and trace metals, and buffered with Hepes to a final concentration of 10 mM, placed in a round-bottom glass flask and the pH lowered to 4.5 with concentrated HCl to prevent formation of precipitates during boiling. Argon, stripped of trace levels of oxygen by passage through a heated copper furnace, was introduced into the flask via a gassing cannula made from a glass syringe and a 10-cm, 18-gauge needle. The medium was boiled for 2 min under a blanket of argon, allowed to cool on ice, and sealed with a gas-impermeable butyl rubber stopper.

Distilled water used for reagent preparation was treated similarly, but no Hepes buffer or acid was added. Solid chemicals were added to the anaerobic liquid to produce oxygen-free reagents. Reagents, media, or sediment were dispersed under a continuous flow of oxygen-free argon gas to maintain strict anaerobiosis. Liquid additions were made via a glass pipette and pipette bulb attached to a source of oxygen-free argon. Anaerobic Na_2CO_3 (400 mM) was

Table 1. Germination success of cysts (concn ml^{-1}, SE in parentheses) incubated for 3 and 7 weeks at different irradiances and under anaerobic conditions. (Not measured—NM.)

	G. tamarensis		*G. verior*		*G. nygosum*		*G. uncatenum*		*Scrippsiella* sp.	
	3	7	3	7	3	7	3	7	3	7
Initial counts	286 (26)	286 (26)	1,007 (121)	1,007 (121)	5,734 (267)	5,734 (267)	192 (9)	192 (9)	5,886 (268)	5,886 (268)
Irradiance exp (μEinst m^{-2} s^{-1})										
0	273 (64)	225 (7)	659 (83)	391*(23)	4,555 (474)	2,857*(62)	119*(8)	100*(19)	5,845 (329)	5,926 (504)
40	97*(16)	112*(26)	35*(16)	58*(18)	1,781*(64)	391*(77)	75*(4)	0*(0)	2,752*(162)	2,117*(81)
84	NM	66*(15)	NM	25*(13)	NM	906*(116)	NM	0*(0)	NM	2,646*(220)
120	NM	75*(14)	NM	46*(23)	NM	564*(159)	NM	9*(9)	NM	2,469*(221)
213	NM	21*(4)	NM	20*(8)	NM	295*(86)	NM	0*(0)	NM	2,011*(186)
435	93*(20)	16*(4)	53*(20)	37*(7)	1,393*(64)	420*(6)	31*(12)	0*(0)	2,680*(442)	1,270*(191)
Anoxia exp										
Aerobic controls	123*(22)	102*(31)	94*(26)	67*(17)	3,503*(462)	2,349*(463)	121*(19)	98*(15)	4,815*(289)	3,545*(237)
No O_2; Na_2S added	251 (33)	290 (31)	1,078 (111)	899 (62)	6,244 (279)	5,842 (176)	151 (17)	152 (13)	6,646 (182)	6,406 (170)
No O_2	223 (38)	311 (33)	912 (145)	1,129 (282)	5,962 (263)	5,662 (53)	172 (26)	190 (18)	5,785 (93)	6,261 (410)

* Significantly different from initial cyst concentration ($P < 0.05$).

added to the anaerobic f/2 medium to a final concentration of 2 mM and pH adjusted to 7 with anaerobic 1 M NaOH. In one group of flasks, 50 mM NaS was added to a final concentration of 500 μM. A subsample of the same sediment used for the irradiance experiment was bubbled overnight with nitrogen gas and then added with the pipette system described above (10 ml of sediment slurry to 50 ml anaerobic medium in each flask).

An aerobic control series of flasks was prepared with medium identical to that described above, without boiling or sulfide addition. Flasks were divided into two groups, placed in two dark boxes, and incubated at either 15° or 20°C. At weekly intervals, flasks were removed from the boxes to monitor pH, O_2, and sulfide. Cysts were counted after 3 and 7 weeks of incubation. Special precautions were not taken to prevent occasional light exposure during the weekly sampling.

Oxygen concentrations were measured with a Radiometer E5046 PO_2 polarographic electrode in a D616 thermostatted cell, connected to a Strathkelvin model 781 meter. The unit was calibrated with air-saturated seawater and Radiometer PO_2 Zero Solution, with adjustments for barometric pressure. Aerobic control flasks were analyzed first with a 5-ml glass syringe which was filled once, emptied, and filled again with medium. Two milliliters were flushed through the cell, and the volume retained was allowed to stir for 60 s. The sample was displaced with a 1-ml injection from the same syringe and an oxygen measurement made after 60 s.

Separate syringes were used for measurements of medium in the anaerobic flasks. The syringes were flushed with nitrogen gas (all connecting tubing was metal) before sampling. During sample withdrawal, the headspace above the medium was continuously purged with oxygen-free nitrogen gas. Five-milliliter samples were taken and injected into the O_2 electrode twice as described above. Samples for sulfide analysis were taken before the O_2 measurements. Five-milliliter samples were withdrawn with N_2-flushed glass syringes and introduced through the butyl rubber stopper of an anaerobic culture tube (Bellco Glass Inc., No. 2047) containing 5 ml of sulfide antioxidant buffer (SAOB; Orion Res. Corp. 1976). Samples, which may be stored for at least 3 d in this buffer, were analyzed for total sulfide ion concentration with an Orion model 94-16 silver/sulfide electrode and model 90-02 double-junction reference electrode. Anaerobic solutions of sodium sulfide served as standards. SAOB consisted of an anaerobic solution of 2 M NaOH, 200 mM ascorbic acid, and 230 mM disodium ethylene diaminetetraacetic acid (EDTA). Cyst germination data are reported only from those flasks in the anoxia experiment in which sulfides were detectable or oxygen concentrations were near or below detection limits (3 μM) at the time of harvest.

Results

Light effects—Two different experiments examined the effects of light on cyst germination. The first was an end-point determination of the number of cysts that germinated after 3 and 7 weeks of exposure to a range of irradiances (the irradiance experiment). The second experiment was a time-course of the germination of cysts incubated either in complete darkness or at a saturating level of irradiance (the time-course experiment).

Results of the irradiance experiment for five species are shown in Table 1 and representative curves of germination frequency vs. irradiance are presented for two species in Fig. 1. After 3 weeks of incubation the mean cyst concentrations in the illuminated flasks were significantly lower than initial counts ($P < 0.05$) for all five species, even at 40 μEinst m^{-2} s^{-1}. Concentrations in dark flasks were also lower than initial levels, but the difference was significant only for *G. uncatenum*. After 7 weeks of incubation, all five species once again showed significant germination at all irradiance levels except complete darkness. Germination was evidenced in the dark for three species (*G. verior, G. uncatenum,* and *G. rugosum*; $P < 0.05$), but the final counts for *G. tamarensis* and *Scrippsiella* sp. were not significantly different from starting concentrations ($P > 0.05$). Many of the ungerminated *G. tamarensis* cysts in the dark flasks exhibited visible red autofluorescence under blue-light excitation.

Even when light was saturating, some cysts never germinated during this experiment. This residual population of ungerminated cysts was, however, largest in the dark flasks, and often represented 40–60% of the initial concentrations (Table 1). These cells appeared fully viable under the light microscope.

Time-course experiment—A consistent observation for all species except *G. rugosum* is that the rate of germination in the light was considerably faster than in the dark (Fig. 2). To quantify these differences, we fitted the time-course of germination frequency for each species to a simple first-order model similar to that used to describe activation of bacterial or fungal spores (Vary and McCormick 1965; Sussman and Halvorson 1966). For an initial concentration of cysts N_0:

$$N_t = N_0 \exp(-kt) \qquad (1)$$

where N_t is the concentration of cysts remaining at time t and k is the specific germination rate with units of time^{-1}.

The concentration of *G. rugosum* and *G. verior* cysts in the flasks decreased through time until a small residual population remained that did not germinate with continued incubation (Fig. 2A, B). To improve the fit to the model, we subtracted these remaining cysts from each data point before the regression.

Germination rates varied between species in both the light and the dark (Table 2). Light germination rates ranged from 0.035 d^{-1} for *Scrippsiella* sp. to 0.69 for *G. polyedra*. Dark rates were much lower, ranging from 0.007 d^{-1} for *Scrippsiella* sp. and 0.018 for *G. tamarensis*. *Gonyaulax polyedra* did not germinate in the dark during 64 d of incubation. These cysts were still viable and germinated quickly when placed in the light. *Gonyaulax rugosum* germinated equally fast in the light and the dark. Note that dark germination occurred to some extent in four of the five species examined during this experiment.

Anoxia effects—Cyst concentrations of the five species monitored in this experiment were significantly different from initial counts in the aerobic control flasks after both 3 and 7 weeks of incubation ($P < 0.05$). Due to the oxygen demand of the sediment

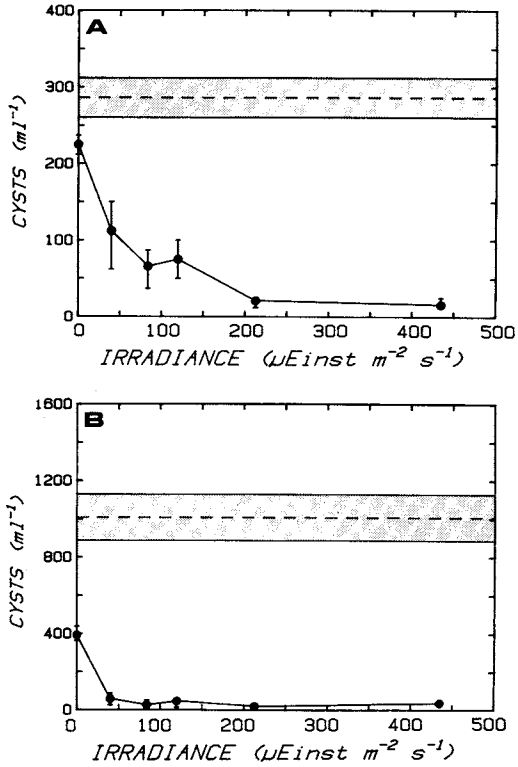

Fig. 1. Germination response to variations in irradiance after 7 weeks of incubation. A—*Gonyaulax tamarensis*; B—*Gonyaulax verior*. The means of three replicate counts of ungerminated cysts are plotted, and error bars indicate the range of those replicates. Dashed lines and shaded areas represent mean of initial counts ±1 SE. The difference between the initial mean and the final counts is assumed to represent germinated cysts.

slurry and limited air exchange in the dark box, the O_2 concentrations at the time of harvest averaged 150 μM, or 65% of saturation in these flasks. Sulfide was undetectable in the aerobic controls.

Cyst concentrations in those experimental flasks containing anaerobic media and additional sulfides were not significantly different ($P > 0.05$) from starting conditions after 3 and 7 weeks of incubation for all species examined. The pH and final sulfide concentration of these flasks averaged 7.1 and 24 μM respectively. In a second series of flasks, sulfide was not added to the anaerobic media. Thus, the redox potential of the system was poorly buffered if an O_2 leak occurred. The average pH in these flasks was 6.3 and the final sulfide concentration

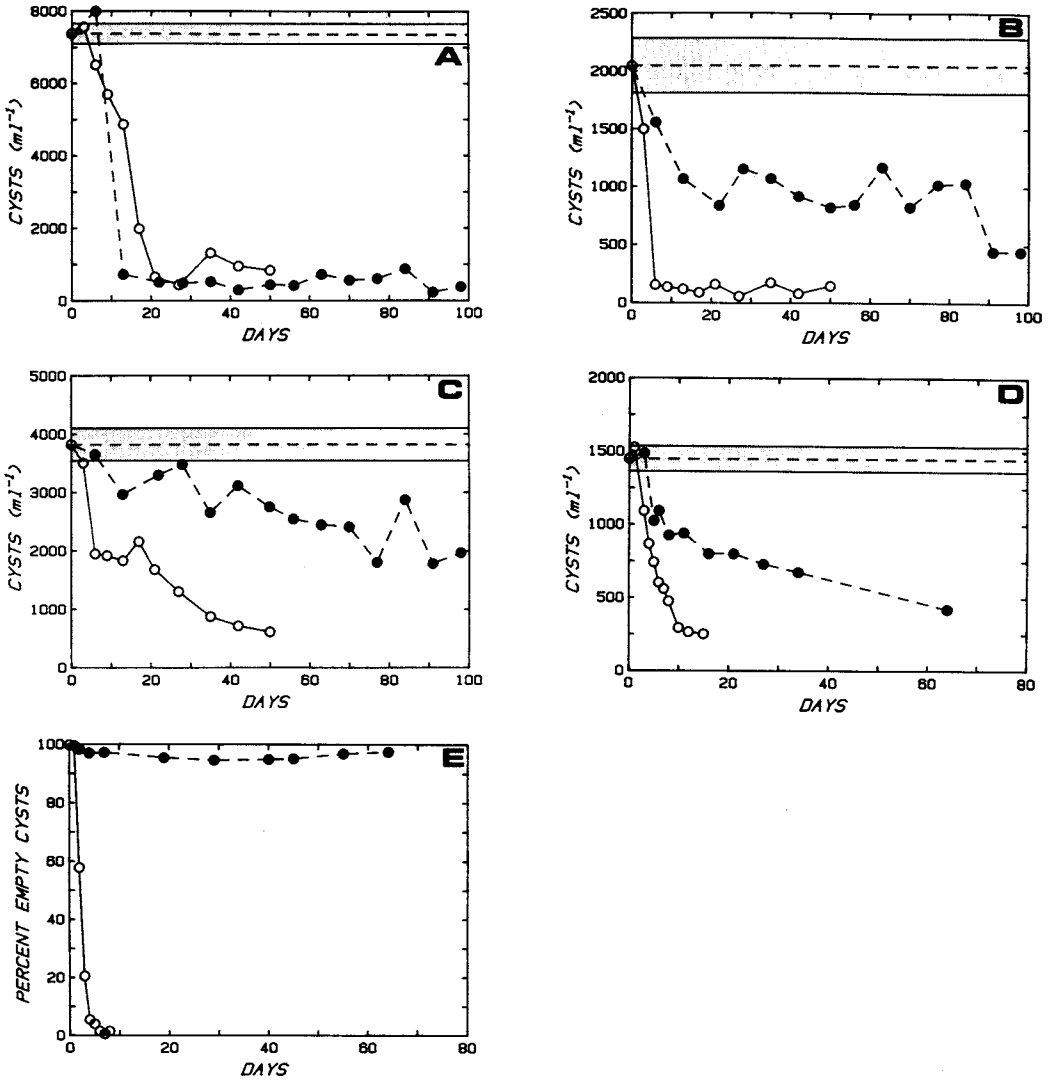

Fig. 2. Germination time-course with light (O) and dark (●) incubation. Dashed lines and shaded areas represent mean of initial counts ±1 SE. The difference between the initial mean and the final counts is assumed to represent germinated cysts. A—*Gonyaulax rugosum*; B—*Gonyaulax verior*; C—*Scrippsiella* sp.; D—*Gonyaulax tamarensis*; E—*Gonyaulax polyedra*.

averaged 2.9 μM. Once again, final cyst concentrations for the five species in these anaerobic flasks were not significantly different from starting values.

Discussion

The experiments described here demonstrate that darkness and anaerobic conditions can be critical factors in the germination of marine dinoflagellate cysts, affecting both the rate of germination or the final germination frequency. Anoxia inhibited all species equally, but distinct differences were seen in their responses to darkness or different light levels. The inhibitory effects of darkness and anoxia reported here help to explain the large numbers of living cysts which accumulate below the surface of marine sediments (Anderson et al. 1982; Tyler et al. 1982; White and Lewis 1982)

Table 2. Cyst germination rates in the light and dark.

	Origin	Specific germination rate* d^{-1} (SE) Light	Dark
Gonyaulax tamarensis	Cape Ann	0.14 (0.01)	0.018 (0.002)
Gonyaulax verior	Perch Pond	0.19 †(0.05)	0.009 †(0.002)
Gonyaulax rugosum	Perch Pond	0.10 †(0.01)	0.13 †(0.04)
Scrippsiella sp.	Perch Pond	0.035 (0.003)	0.007 (0.001)
Gonyaulax polyedra	Culture, Perch Pond strain	0.69 (0.08)	0.001 ‡(0.0)

* Derived from a least-squares regression of ln-transformed data using Eq. 1.
† Residual, ungerminated population not included in regression.
‡ Slope not significantly different from 0 ($P > 0.05$).

and the persistence of benthic cyst populations at times when temperatures have passed through permissive temperature "windows" on a seasonal basis (Anderson and Morel 1979; Fukuyo et al. 1982; Heaney et al. 1983; Endo and Nagata 1984; Lewis et al. 1985). Environmental constraints on germination of dinoflagellate cysts are thus more severe than previously thought, suggesting that relatively few cysts can ever germinate to resume a motile existence once they sink from the euphotic zone.

Anoxia effects—Excystment of the five marine species examined was completely inhibited by anoxia. For 7 weeks there was no germination of any type of cyst in either of the two anaerobic treatments (with or without added sulfides), whereas 50–95% germination occurred in flasks with oxygen (Table 2). The appearance of cysts incubated under anaerobic conditions did not change throughout the experiment, and the cells remained fully viable as evidenced by their ability to germinate when resuspended in oxygenated medium. This is fully consistent with field studies where viable cysts are routinely isolated from black, sulfide-rich sediments (Anderson et al. 1982; Dale 1983).

Although sulfide was not added to one series of flasks, the sediment slurry produced an average sulfide concentration of 2.9 μM. We thus cannot state unequivocally that it was the absence of oxygen and not the presence of sulfides that inhibited germination.

Oxygen concentrations could not be monitored on a continuous basis due to the high probability of atmospheric contamination. Measurements made immediately before cyst harvesting indicated that some flasks in the anoxia series had leaked, giving final O_2 concentrations near 20% of saturation. All species germinated as well in these flasks as they did in the oxygen controls. The data are not tabulated in the results, however, since it was not possible to determine when contamination occurred. Nevertheless, they are useful in demonstrating that excystment was possible at subsaturating concentrations of O_2.

Huber and Nipkow (1923) studied the effects of anoxia on cyst germination and concluded that the germination frequency of *C. hirundinella* cysts was normal in medium stripped of oxygen and carbon dioxide, although the process was delayed (4 vs. 2 d). The newly germinated cells were also small and deformed. This same species was examined by Krupa (1981) who reported normal germination following additions of "hydrogen sulfide water" to tightly sealed flasks.

Endo and Nagata (1984) showed that anoxia inhibited germination of marine *Peridinium* sp. cysts. Incubations lasted only 1 week, however, so delayed germination could not be distinguished from complete inhibition. Our data extend these results and indicate that anoxia causes complete inhibition of excystment and not simply a retardation of the process. The appearance of the cysts when they were harvested (i.e. the distribution of storage products, the absence of developing chloroplasts, the motion of granular cytoplasm) suggests to us that cyst morphology was unchanged and thus that germination would not have occurred with longer incubations.

There are thus two studies that report normal germination under anóxic conditions and two that describe inhibition. Species-specific oxygen requirements might explain this discrepancy, and such differences have in fact been documented with the seeds of higher plants (Bewley and Black 1982). Another possibility is that the two studies on *C. hirundinella* did not totally exclude atmospheric oxygen. Unfortunately, the anaerobic techniques used in those studies were not described in detail, and no measurements of oxygen or sulfides were made.

Light effects—In contrast to several published reports on other species, light clearly affected germination in five of the six dinoflagellates examined. When light was available, the maximum specific germination rate varied by a factor of 20 between species. Germination frequency increased with increasing irradiance for three species (Table 1), providing evidence for light limitation of germination rate at low photon flux densities.

The two light experiments revealed three responses to total darkness: normal germination, no germination, and delayed germination. In the irradiance experiment, three species (*G. uncatenum, G. verior,* and *G. rugosum*) germinated in the dark, but germination frequency was lower than in flasks illuminated for the same amount of time. Two species (*G. tamarensis* and *Scrippsiella* sp.) showed no significant germination in the dark. However, many *G. tamarensis* cysts showed chlorophyll autofluorescence during the final counts, implying that germination had begun but was not yet complete (Yentsch et al. 1980; Anderson and Keafer 1985). Thus except for *Scrippsiella* sp., the irradiance experiment demonstrates that darkness can retard germination but not totally prevent it for four species. *Gonyaulax polyedra* cysts were not monitored in that experiment.

The time-course experiment also demonstrated that darkness can slow germination. For three species (*G. tamarensis, Scrippsiella* sp., and *G. verior*) germination in the light was 5–20 times faster than in the dark. The other two responses to dark incubation during the time-course are represented by *G. polyedra,* which did not germinate in the dark at all, and *G. rugosum,* which germinated equally fast in the light and the dark (Fig. 2A, E). The dark germination data should be qualified somewhat since our use of natural sediment assemblages necessitated a small amount of light exposure at the beginning of the experiment during core processing and flask inoculations. Likewise, cysts in the *G. polyedra* cultures were exposed to light during storage before the experiment. These exposures were brief and of lower intensity than those of other studies (Huber and Nipkow 1923; Anderson and Wall 1978; Endo and Nagata 1984), but we still cannot claim constant darkness at all stages of storage and incubation. When our study began, we thought this was a minor concern, but the recent report of an extremely low intensity, short duration pulse of light as a sufficient photomorphogenic trigger for germination of *S. trochoidea* cysts (Binder and Anderson 1986) emphasizes the potential importance of exposure to any light, no matter how dim.

Given this unexpected discovery, we must now consider both long- and short-term light exposures. The dark germination observed for five species did follow brief exposures to dim light at the start of the experiment (<0.02 μEinst m^{-2} s^{-1}), but it is not known whether those exposures were a factor in the germination. We thus cannot state unequivocally that germination can occur in constant darkness, but instead we must consider two hypotheses. The first is that the brief light exposure at the beginning of our experiments did act as a photomorphogenic trigger to initiate germination, which could then proceed only at a slow rate in the absence of additional energy input from photosynthesis. Until we can repeat this study using cysts from laboratory cultures, species-specific requirements for a pulse of light remain unknown. We can address the second part of this hypothesis, however, and ask whether germination requires sustained light exposure. In this regard, the germination observed in the dark for five species clearly indicates that long-term light exposure (presumably acting via photosynthesis) is not an absolute requirement. Only *G. polyedra* seems to require sustained illumination. On the other hand, the difference between light and dark germination rates for all species

except *G. rugosum* demonstrates that light can significantly enhance germination kinetics. This is also evident in the increased germination success with increasing light exposure in the irradiance experiment (Table 1). The implication is that most species can proceed through the metabolic steps needed in the transformation from dormancy to a motile, vegetative existence without an external supply of energy. The process can be very slow, however, compared to the rapid excystment with sustained light exposure. Not all species follow this pattern, as *S. trochoidea* (Binder 1986) and *G. rugosum* (Fig. 2A) germinated equally fast in the light and the dark.

A second hypothesis is that our species are physiologically different from *S. trochoidea* and do not require a light pulse. One important physiological difference is that *S. trochoidea* cysts contain chlorophyll (Binder unpubl. data) and maintain a continuous red autofluorescence throughout dormancy, whereas our six species develop the pigmentation for fluorescence only in the late stages of germination. *Scrippsiella trochoidea* also has the shortest dormancy period of all marine dinoflagellates studied to date (25 d: Binder 1986), so one could argue that its physiology is optimized to cycle rapidly between dormancy and motility. Except for *G. rugosum,* the cysts we studied have longer maturation periods (Anderson unpubl. data) and are thus less likely to maintain the same physiological state of competency. In a sense, they are more deeply dormant and require either more light or longer incubations to germinate. Interestingly, only *G. rugosum* germinated as rapidly in the dark time-course as in the light and, like *S. trochoidea,* also has a short maturation period (28 d: Anderson unpubl. data).

Certain apparent inconsistencies in the data warrant discussion. The first is that several species germinated in the oxygenated (but dark) control flasks in the anoxia experiment but did not germinate after the same length of time in the dark flasks of the irradiance experiment. We attribute this difference to the exposure of the anoxia flasks to light several times each week when the box covering the flasks was removed for stirring and sampling. No special precautions were taken to exclude light during these sampling episodes as the objective of the dark box was only to prevent long-term photosynthetic oxygen evolution in these treatments, and the possible effects of short pulses of light were not appreciated at the time.

Another discrepancy is that *G. rugosum* and *Scrippsiella* sp. cysts germinated faster in the dark during the time-course than they did during the irradiance experiment (Table 1; Fig. 2A, C). One potentially important difference between these experiments is that an incubator with better air circulation was used for the time-course studies so that temperatures were virtually constant between light and dark treatments. The dark box used in the irradiance experiment was several degrees cooler at night than during the day, so germination was probably retarded for warm-water species like *G. rugosum* and *Scrippsiella* sp.

Ecological implications—Where temperature was once thought to be the major factor controlling the germination of mature dinoflagellate cysts, we now see that darkness and anoxia can completely inhibit or substantially retard this process as well. This is important to the ecology of these organisms because cysts have relatively high specific gravities and sinking rates (Anderson et al. 1985) and mandatory dormancy periods after formation lasting several weeks to several months. Thus most cysts will not remain long in the water column or in the oxygenated surface sediments where excystment can occur. Those that accumulate in shallow water sediments where light reaches the bottom can germinate rapidly; for some species, germination will be slow if irradiance is low. Many that fall too deep or that are shaded by other material at the sediment surface can germinate if oxygen is present, but only at a slow rate that can add months to the optimal germination time of a week or less. This could lengthen the bloom "inoculum" phase from germinating cysts, but it also could delay germination to the point where surface water temperatures are no longer suitable for rapid vegetative growth.

We note also a significant difference between species in maximum germination rates in both the light and the dark. The "seeding" process at the start of bloom will

vary accordingly, with one species entering the water column all at once while others spread the inoculum phase out over time.

In most marine environments, burial due to deposition or bioturbation will expose many cysts to anaerobic conditions once they reach the bottom. Buried cysts can survive without oxygen but cannot germinate. This represents a more severe constraint than that imposed by darkness, as inhibition by anoxia is complete, not just delayed. Bioturbation or resuspension events can also bring buried cysts back to the surface, of course, but our experience has been that this upward flux is small relative to burial.

It is now clear that the vast majority of viable cysts in most sediments will not germinate in a given year, or perhaps ever, because of either darkness or anaerobic inhibition. Species that are able to cycle between dormancy and motility quickly enough to avoid excessive burial (i.e. those with short dormancy periods and minimal light requirements such as *S. trochoidea* and *G. rugosum*) would maximize the numerical contribution of cysts to the bloom initiation phase. Those with longer maturation times and a need for light to achieve rapid germination run a greater risk of burial. Their contribution to the bloom initiation phase might be smaller or more gradual (as has been observed for *G. tamarensis*: Anderson et al. 1983; Anderson and Keafer 1985).

The effects of anoxia may be even more severe in those regions where anoxia occurs either continuously or periodically in bottom waters. In this context, it is interesting to note that sediments are rich in dinoflagellate cysts in the Black Sea, a restricted basin where bottom waters are anoxic (Wall and Dale 1973). Dinosterol, a unique sterol found only in dinoflagellates, has been isolated from these sediments and linked to the remains of vegetative cells from dinoflagellate blooms (Boon et al. 1979). Our data suggest a variation on this concept—that dinoflagellate cysts may have also been a source of dinosterol since they can accumulate without germination in the anoxic bottom waters and are more resistant to predation and degradation in the water column than motile cells. An important unknown in this hypothesis is the proportion of dinoflagellate bloom biomass that reaches the sediment in the form of cysts rather than as general detritus or in fecal pellets. Blooms of cyst-forming dinoflagellates can decline very rapidly, often with population losses much larger than can be attributed to grazing pressure (Anderson et al. 1983; Watras et al. 1985). The deposition of this biomass in the form of resistant cysts that can remain intact for decades may have important geochemical implications.

In summary, the germination of dinoflagellate cysts has been shown to be a complex process regulated by several environmental factors. The variability in these factors between regions or within the sediments at specific sites can markedly affect the germination success of the "seed" population and, in the long term, determine the suitability of a given water body for a particular species. In the absence of major resuspension events or other advective processes, it would appear that relatively few cysts ever germinate to complete the encystment/excystment cycle, presumably because of darkness or anaerobic conditions. Despite this potentially low germination rate, species can persist because they produce large numbers of cysts as blooms terminate. This has obviously been a successful strategy, as fossilized dinoflagellate cysts virtually identical to those formed by some species living today have been recovered from sedimentary material that is several hundred million years old (Evitt and Davidson 1964; Wall 1966).

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