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PHYSIOLOGICAL AND ENVIRONMENTAL CONTROL OF GERMINATION IN *SCRIPPSIELLA TROCHOIDEA* (DINOPHYCEAE) RESTING CYSTS[1]

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ABSTRACT

The effects of aging, temperature, and growth medium on germination in culture-produced resting cysts of the marine dinoflagellate Scrippsiella trochoidea *(Stein) Loeblich are examined. Cysts undergo a mandatory period of dormancy lasting approximately 25 days, during which germination does not occur. The duration of this period is not affected by temperature. Once the dormancy period is completed, germination is regulated by external factors. Cysts germinate optimally in nutrient replete medium at temperatures greater than approximately 14°C. At lower temperatures or in nutrient-depleted media germination rate is dramatically slowed, although the final germination frequency appears unchanged. The large Q_{10} of this temperature effect (ca. 11) suggests that the reduction in germination rate at lower temperatures is not merely the reflection of generally reduced metabolic rates, but rather the result of a temperature response specific to germination. At the highest temperatures tested (22–25°C), germination rate remains maximal although vegetative growth is greatly reduced. A shift in temperature or nutrient conditions, per se, is not necessary for germination. The relatively short dormancy period combined with the absence of a requirement for a dramatic shift in environmental conditions could facilitate rapid cycling between resting and vegetative stages in natural* S. trochoidea *populations. At the same time, the dramatic reduction in germination rate at low temperatures would permit cysts of this species to serve as overwintering cells as well.*

Key index words: cyst; dinoflagellate; dormancy; germination; Scrippsiella

Many dinoflagellates produce resting cysts during their life history (Wall 1971, Dale 1983). The roles suggested for these cysts (and for algal resting stages in general), include short- and long-term survival under unfavorable conditions, bloom initiation, species dispersal, reproduction, and preservation of genetic variation (Wall 1971, Anderson and Wall 1978, Coleman 1983, Dale 1983, Anderson 1984). Despite their intuitive appeal, the importance of these roles to dinoflagellates in nature remains largely hypothetical. The extent to which a cyst could fulfill any particular one of these functions will depend, in part, upon the physiological and environmental regulation of its germination.

Most data concerning the regulation of germination in dinoflagellate cysts are derived from studies which have had as their major goal the elucidation of dinoflagellate life histories, rather than the investigation of factors affecting the observed transformations (Dale 1983, Pfiester and Anderson 1986). Furthermore, the majority of those studies which have systematically addressed the latter concern have employed cysts recovered from the field (Huber and Nipkow 1923, Anderson and Wall 1978, Anderson and Morel 1979, Anderson 1980, Endo and Nagata 1984). Such cysts are necessarily formed under, and have been exposed to, variable and undefined environmental conditions.

One generalization that has emerged from these studies is the existence of "dormancy periods" lasting weeks to months, during which cyst germination apparently cannot occur (Huber and Nipkow 1923, Wall and Dale 1968, von Stosch 1973, Pfiester 1975, Anderson 1980, Endo and Nagata 1984). Reports of this type of endogenous control of germination have primarily consisted of casual observations of reduced germination in young cysts under conditions conducive to germination in more mature cells. In some cases the duration of dormancy is affected by storage temperature: cold storage increases the dormancy period in *G. tamarensis* cysts (Anderson 1980), but decreases it in *Peridinium cinctum* (Dürr 1979).

Once the dormancy period has been completed, the extent to which cyst germination occurs depends largely on environmental conditions. To date, temperature has been widely implicated as the primary environmental factor regulating germination in such non-dormant (or "quiescent") cysts. In general, low temperatures inhibit germination, while shifts upward promote it (Wall et al. 1967, Wall and Dale 1968, 1969, von Stosch 1973, Pfiester 1975, Anderson and Morel 1979, Chapman et al. 1981, Endo and Nagata 1984). Cysts of *Ceratium hirundinella* stored at constant low temperature remained quiescent, but viable, for years (Huber and Nipkow 1923).

Other environmental parameters have received much less attention with respect to cyst germination. Factors such as light and nutrients are generally believed not to exert a strong influence on germination, though they obviously would greatly affect the subsequent survival and growth of excysted cells (Huber and Nipkow 1923, Anderson and Wall 1978, Dale 1983).

In the present study we have undertaken a detailed investigation of the environmental and en-

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dogenous control of cyst germination in the marine dinoflagellate *Scrippsiella trochoidea*. As culture-produced cysts were used throughout, we can provide documentation of the effects of temperature, nutrients, and cyst age on cells whose prior growth and storage conditions were carefully controlled. The effects of light on germination in cysts of this species are reported elsewhere (Binder and Anderson 1986).

MATERIALS AND METHODS

The organism. Initial experiments employed a non-axenic multiclonal culture of *Scrippsiella trochoidea* (Stein) Loeblich, designated ScrpMxB, originally established from Perch Pond (Falmouth, Massachusetts) by D. M. Anderson. A clonal axenic culture (designated SA10) was subsequently established from ScrpMxB by repeatedly rinsing an individually isolated motile cell in drops of sterile medium. SA10 was routinely tested for contamination using various marine bacteria growth media (Hoshaw and Rosowski 1973); occasional direct inspection of old cultures under phase contrast illumination at ×400 confirmed the absence of bacterial contaminants. Species identification of this clone was confirmed by K. A. Steidinger (pers. comm.).

Throughout this paper we use the terms "resting cyst" and "cyst" to refer to thick-walled, non-motile, presumably physiologically inactive, dinoflagellate life cycle stages. In *S. trochoidea* these cells are the product of sexual fusion, and may therefore also be refered to as "hypnozygotes" (Watanabe et al. 1982, Dale 1983).

Culturing. General procedures were as outlined in Anderson et al. (1984). *Scrippsiella trochoidea* cultures were routinely grown in f/2-enriched Vineyard Sound seawater (31 ppt), minus silicate, in 25 × 150 mm borosilicate culture tubes (Guillard and Ryther 1962). For cyst production, cultures were grown in reduced-nutrient media. The standard encystment medium was $\frac{1}{10}$ × h/2-enriched seawater (Guillard 1975), minus nitrate and silicate (50 μM NH_4, no added NO_3). Media were autoclaved in culture tubes complete with nutrients; in contrast to the case for *Gonyaulax tamarensis* (Anderson et al. 1984), growth and encystment in *S. trochoidea* were not adversely affected by media thus prepared. Cultures were maintained at 18° C under a 14:10 h LD cycle, with cool white fluorescent bulbs providing illumination at approximately 450 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR.

Experiments addressing the effects of temperature on growth rate and germination were performed using a temperature gradient bar, as described in Anderson et al. (1984). Vegetative growth rate was calculated from in vivo fluorescence (Turner Designs Model 10) of well acclimated cultures considered to be in balanced growth (Brand et al. 1981).

Cyst storage and germination. After cysts appeared in culture, the tubes were generally stored without further manipulation. Since storage and germination conditions were varied experimentally, each particular treatment employed will be noted in the Results section.

For any single culture, we define cyst age = 0 when cyst abundance is 50% of the maximum abundance achieved in that culture. Since cysts are generally produced over a period of several days (Fig. 1), cyst ages specified in this study have a standard deviation of approximately ±1 day.

Germination experiments were conducted in two ways. In early experiments, individual cysts were isolated by micropipette into 130 μL of medium in the wells of 96-well tissue culture plates (Costar, Cambridge, Massachusetts). These plates were then placed under the specified germination conditions and inspected every few days at ×100 on an inverted microscope. The appearance of swimming *S. trochoidea* cells indicated that germination had occurred. At least 50 cysts were isolated and scored for each experimental treatment.

In the course of these initial experiments, we found that the calcitic cyst wall which was left after germination remained intact and was easily recognized. Thus the appearance of empty cysts, rather than swimming cells, could be used as an indicator of germination. Periodically scoring the proportion of empty cysts in a suspension (usually 0.1 mL aliquots in a Palmer-Maloney slide) is far more convenient and allows greater experimental flexibility than searching for the appearance of swimming cells in individual tissue culture plate wells; this method was used exclusively in subsequent experiments.

In some cases, cysts were separated from other cells and resuspended in the specified medium for storage or germination. Following concentration by centrifugation (typically 500 *g* for 20 min in a horizontal rotor), cysts and vegetative cells were separated using a Percoll-sorbitol density step-gradient. "Percoll-SSW" (Price et al. 1978) without Tris was employed. Solutions of Percoll (Pharmacia Fine Chemicals) + sorbitol and $MgCl_2$ + seawater were autoclaved separately, and combined aseptically just prior to use; the pH of the combined solution was 7.9–8.1, its density was approximately 1.15 g mL^{-1} (Price et al. 1978). After centrifugation as above, cysts formed a pellet at the bottom of the tube, while vegetative cells accumulated at the Percoll-seawater interface. Cysts were rinsed free of Percoll by resuspending the pellet in sterile seawater and centrifuging again.

RESULTS

Encystment. In encystment-medium batch cultures, *S. trochoidea* grows exponentially at a rate of approximately 1 doubling per day (Fig. 1). A day or two after stationary phase is reached, the first cysts appear; cyst abundance then increases over the next 3 or 4 days. Final cyst yield is typically 10% of the maximum vegetative cell number.

Age effects. The pattern of germination for cysts of different ages is shown in Figure 2A, B. These cysts were all produced at the same time and stored at 18° C under a 14:10 h LD cycle. After 4, 13, 20, and 29 days of storage, cysts were isolated into tissue culture plates and incubated at 15° C in f/2 medium (light regime unchanged). A negative relationship between the time necessary for germination and cyst age at the start of the incubation is obvious for cysts between 4 and 20 days old (Fig. 2A). Ultimate germination frequency was high in all four series (>90%), and was independent of cyst age at the start of incubation on the time scale of this experiment. When the same germination data is plotted against an X-axis of absolute cyst age (rather than incubation time), the cumulative percent germination is a clear function of cyst age alone (for cysts younger than 20 days old), and is independent of the age at which these cysts were first placed under germinating conditions (Fig. 2B).

In the same experiment, cysts stored at 3° C prior to incubation showed much the same pattern of germination as those stored at 18° C. In fact, the median germination times (by definition, the time required for 50% germination) for cysts of the same age, stored at either temperature, were essentially identical (Fig. 3).

The assessment of the effects of aging on germination can be extended by comparing both the median germination times and the ultimate germination frequencies observed among cysts of various

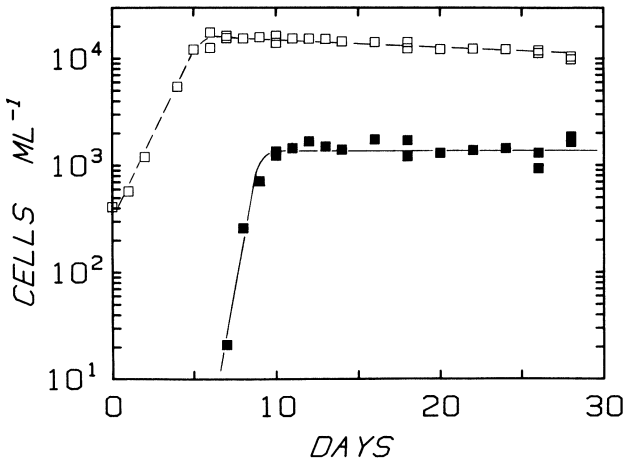

FIG. 1. Vegetative growth and cyst production by ScrpMxB in encystment-medium batch culture. Vegetative cells (□) and cysts (■) per milliliter; lines drawn by eye.

ages, in all the sundry *S. trochoidea* germination experiments we have run to date (Figs. 3, 4). Included here are data from the treatments considered to be optimal for germination (i.e. nutrient replete, exposed to light, under permissive temperatures) within all the experiments from which the relevant parameters can be extracted.

Again, the negative relationship between germination time and cyst age results in a minimum germination age of approximately 25 days (Fig. 3). For cysts older than 25 days, germination time appears to slowly decrease with increasing cyst age; a minimum germination time of 2 to 3 days is reached among cysts stored at 3° C for 75 days or longer (Fig. 3).

The ultimate germination frequency achieved in these experiments varied from 60 to 100%, with no clear pattern regarding cyst age or storage temperature (Fig. 4). The considerable variability in these data could obscure a subtle downward trend in germination frequency with increasing age; in any case, a substantial proportion of cysts retain the ability to germinate after a year of dark storage at 3° C. No data are available for cysts stored at 18° C beyond 100 days.

Temperature effects. Cysts stored at 3° C in the dark for more than 3 months were incubated at various temperatures in f/2 in the light (14:10 h LD), and scored for excystment over time (Fig. 5A, B). For temperatures above approximately 14° C (up to at least 25° C) median germination time was 5 days or less, but it increased sharply at incubation temperatures below this range (Fig. 5A). Median germination time at 3° C was greater than 75 days (the final time point of this experiment); no germination had been detected in the 1° C treatment at that time. Except for these two lowest temperatures, ultimate germination frequency appeared to be independent of incubation temperature (Fig. 5A). Thus, while

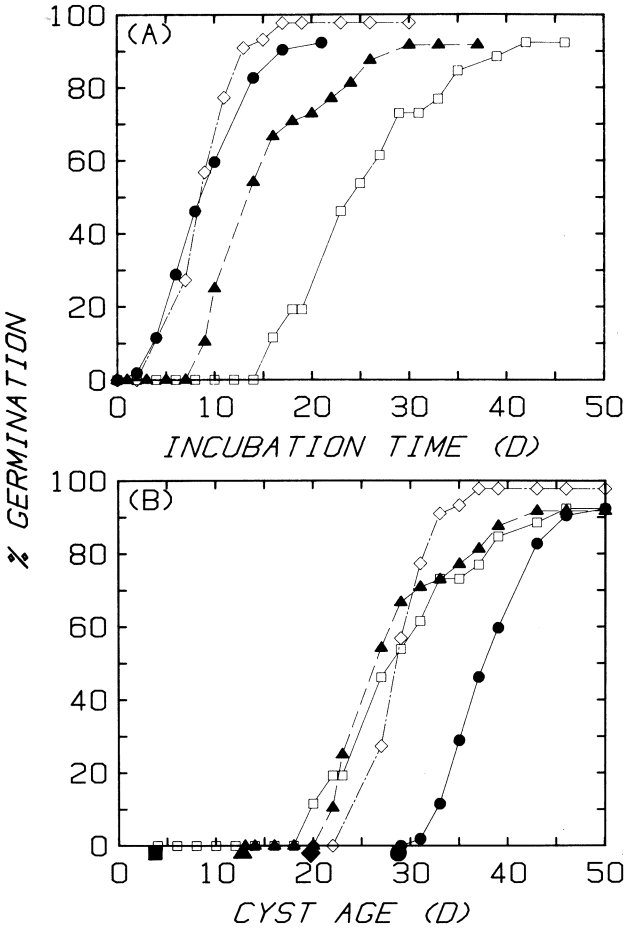

FIG. 2A, B. Germination kinetics for cysts stored in light at 18° C for 4 (□), 13 (▲), 20 (◇), and 29 (●) days prior to nutrient enrichment and shift to 15° C. (A): Data plotted against incubation time at 15° C. (B): Same data plotted against cyst age; enlarged closed symbols indicate the start of the 15° C incubation for each of the corresponding series.

germination rate at 6° C was an order of magnitude slower than the rate observed at higher temperatures, the germination frequency finally achieved was the same.

The optimal temperature range for vegetative growth (in acclimated cultures) was different than that for germination (Fig. 5B). Maximum growth rate was achieved between 10° C and 20° C. Above this range growth rate fell off rapidly; no growth occurred above 23° C. Growth rate decreased more gradually at temperatures below the optimal range, and at 2° C was still 50% of the maximum.

Medium effects. Cysts stored undisturbed in their original cultures, under unchanged light and temperature conditions, do eventually germinate (Fig. 6). The final proportion of cysts germinating under these circumstances appears comparable to that achieved in parallel cultures spiked with nutrients, but the time required for germination is greatly increased when no nutrients are added. The pH of

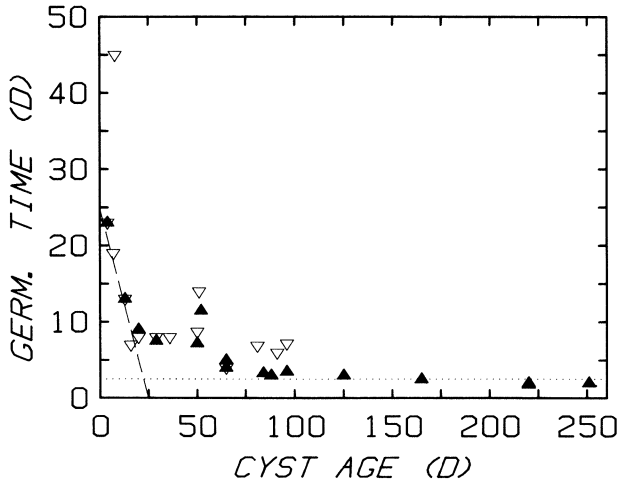

FIG. 3. Median germination time versus cyst age at start of incubation. Data derived from all applicable *S. trochoidea* germination experiments run to date (see text). Closed symbols: cysts stored at 3° C prior to germination; Open symbols: cysts stored at 18° C. Broken line represents germination at a constant *age* of 25 days; dotted line represents a constant germination time of 2.5 days.

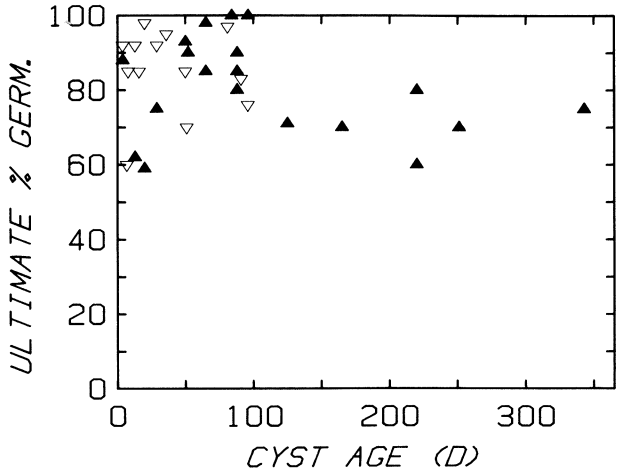

FIG. 4. Ultimate germination frequency versus cyst age. Data derived from all applicable *S. trochoidea* germination experiments run to date (see text). Closed symbols: cysts stored at 3° C prior to germination; Open symbols: cysts stored at 18° C.

undisturbed cultures was not different from those enriched with nutrients.

Cysts harvested from culture and separated from vegetative cells prior to resuspension in unenriched Sargasso Seawater likewise showed a dramatic reduction in the rate of germination relative to f/2-enriched controls (Fig. 7). These cysts had reached 50% germination at 65 days (the last time point of that series), while the median germination age was 22 days in the enriched controls.

DISCUSSION

Germination in *Scrippsiella trochoidea* cysts is regulated by a variety of environmental and physiological factors. The effects of these parameters can be manifested either as total suppression of germination, as was the case during the dormancy period and under light deprivation (Binder and Anderson 1986), or as changes in the kinetics of germination, as occurred with nutrient and temperature variations. This study (together with that described in Binder and Anderson 1986) is one of the first detailed investigations of the environmental regulation of germination in marine dinoflagellate resting cysts formed in culture and manipulated under controlled conditions. It is thus free from potential artifacts associated with sonication, isolation, and other techniques necessary for work with cysts recovered from natural sediments, as well as from complications arising from the uncertain environmental histories of such cysts. The results provide valuable insights into the potential role of cysts in the ecology of this common marine dinoflagellate.

Encystment. Cyst formation in *Scrippsiella trochoidea* has been described by a number of authors (Braarud 1958, Wall et al. 1970, Watanabe et al. 1982). Watanabe et al. (1982) reported that these cysts are the product of sexual fusion, an observation we did not attempt to confirm. Both Braarud (1958) and Wall et al. (1970) noted that cysts appeared in batch cultures without specific manipulations or dramatic shifts in light or temperature. While the present data confirm this observation, the fact that no cysts were observed in culture until after stationary phase had begun is inconsistent with the contention that cyst formation in *S. trochoidea* is favored by conditions optimal for vegetative growth (Wall et al. 1970). Instead, it is likely that the onset of nutrient limitation is responsible for sexual induction and encystment in this species, as has been reported for a number of other dinoflagellates (Pfiester and Anderson 1987).

The number of cysts produced in our *S. trochoidea* cultures was approximately 10% of the maximum vegetative cell number, corresponding to a 20% rate of participation in sexual fusion (assuming that each cyst is a zygote, produced by the fusion of two gametic cells; Watanabe et al. 1982). This rate of gametic fusion is considerably less than the 60% reported by Watanabe et al. (1982) for the same species, but is similar to that observed in *Gonyaulax tamarensis* and *Gyrodinium uncatenum* cultures (Anderson et al. 1984, Anderson et al. 1985). The disparity between the present results and those of Watanabe et al. (1982) is probably related to the different induction methods employed, although inter-clonal variability cannot be discounted (Pfiester 1975). The two *S. trochoidea* clones are obviously different in other respects, particularly in their growth rate response to temperature (cf. Watanabe et al. 1982, our Fig. 5B).

As demonstrated by Anderson et al. (1985) for *G. uncatenum,* cyst production may not fully reflect the extent of gametic fusion in culture. In their cultures,

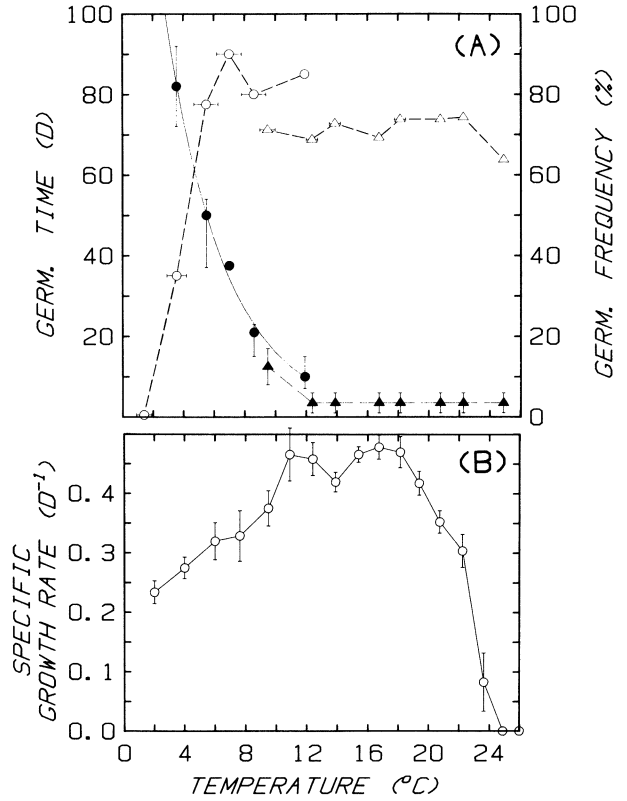

FIG. 5A, B. Effect of temperature on *S. trochoidea* germination (A) and growth rate (B). (A): Median germination time (▲, ●) and ultimate germination frequency (△, ○) for cysts stored at 3° C (in the dark) prior to experiment. Two symbol shapes correspond to two separate experiments. Median germination time estimated by linear interpolation of germination data at each temperature; vertical bars represent the resolution of these estimates (as determined by sampling schedule); horizontal bars show the daily range in temperature. Solid line is the least squares fit of the germination times from the second experiment (●) to an exponential model ($Y = 205e^{-0.255(T)}$; $r^2 = 0.994$). (B): Mean specific growth rate ± one SE, derived from 3–10 independent estimates at each temperature.

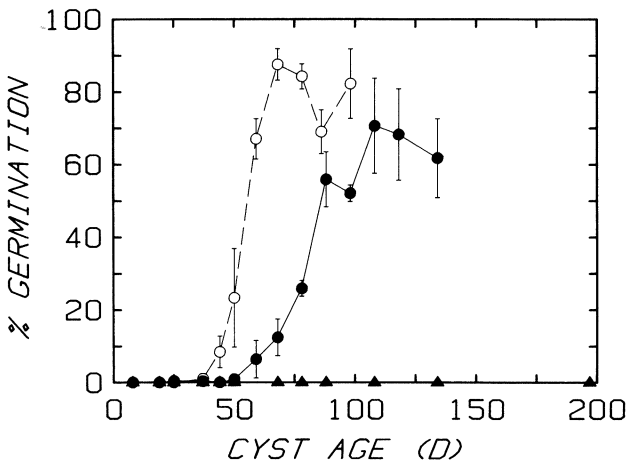

FIG. 6. *S. trochoidea* germination at 18° C, 14:10 h LD, in the absence of environmental changes (●), with nutrient enrichment (○); and at 3° C in the dark (▲). Means ± SE (n = 3).

a large proportion of the cells formed planozygotes but failed to encyst. Thus the relatively low final cyst yield in their study, and perhaps in the present one, reflects the inadequacy of culture conditions for the transformation from planozygote to cyst, rather than for the transformation from vegetative to sexual reproduction.

Dormancy and quiescence. Reference to dormancy is often made in discussions about dinoflagellate cysts, but the term is rarely defined and the concepts associated with it remain vague. We will adopt the terminology used by Pfiester and Anderson (1987), wherein "dormancy" refers to a curtailment of germination as the result of an endogenous condition (i.e. as the result of some property of the cyst itself). The term "quiescence," on the other hand, will be applied when germination is inhibited by an exogenous, or environmental, factor. Thus dormant cysts cannot germinate, even under optimal environmental conditions, while quiescent cysts are competent to germinate but are inhibited from doing so by some environmental factor. For the purposes of this discussion, the terms "germination" and "excystment" are used synonymously.

Scrippsiella trochoidea cysts undergo a requisite period of dormancy lasting approximately 25 days (Fig. 3). During this period, cysts placed under normally optimal germination conditions fail to germinate. Once the dormancy period is completed, cysts incubated under the same conditions germinate readily, while those placed under non-permissive conditions (see below) remain quiescent. *Scrippsiella trochoidea* cysts have remained quiescent, but viable, for 340 days (at 3° C in the dark) as of this writing (Fig. 4), and they are fully expected to survive considerably longer.

Analogous periods of dormancy have been observed in other dinoflagellate species (Dale 1983, Pfiester and Anderson 1987). Although the cysts of one species (*Peridinium gatunense*) can germinate within 12 h of their formation (Pfiester 1977), most species undergo dormancy periods lasting from 7 to 20 weeks. Therefore the 3.5 week dormancy period observed for *S. trochoidea* is short relative to most dinoflagellates studied to date.

The duration of dormancy in a number of species can be affected by the conditions under which cysts are stored. A low temperature treatment shortens the dormancy period in *Peridinium cinctum* (Dürr 1979) and results in more complete germination (once permissive conditions are restored) in *Gymnodinium pseudopalustre* and *Woloszynskia apiculata* (von Stosch 1973). On the other hand, low storage temperature lengthens the dormancy period in cysts of *G. tamarensis* and a *Peridinium* sp. (Anderson 1980, Endo and Nagata 1984). In contrast to these observations, temperature has no effect on the duration of dormancy in *S. trochoidea.* New cysts stored at

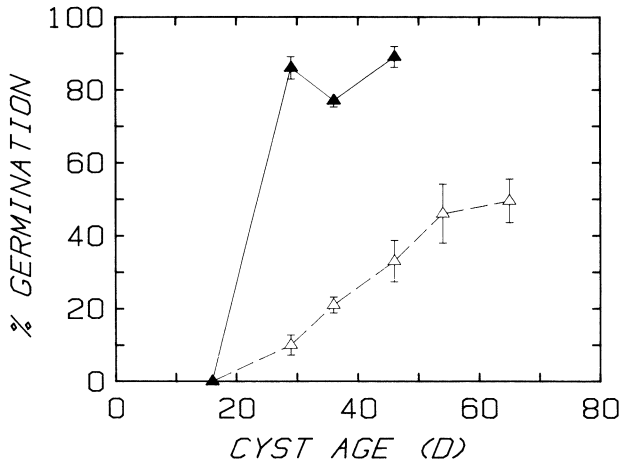

FIG. 7. Germination of *S. trochoidea* cysts separated from vegetative cells at age 17 d and incubated at 18° C in f/2, 14:10 h LD (▲), and in unenriched Sargasso seawater (△). Means ± one SE (n = 3).

3° C or 18° C displayed the same 25 day delay in germination (Fig. 3).

The extent to which the dormancy period in *S. trochoidea* or other dinoflagellate species reflects a period of development (analogous to "morphological dormancy" in plant seeds; Nikolaeva 1977), or instead, an endogenous inhibition of an otherwise competent cell (analogous to "physiological dormancy"), is not known at present. A negative relationship between the length of the dormancy period and temperature, as reported for *G. tamarensis*, could be indicative of a developmental requirement which takes longer to complete at lower temperatures (Anderson 1980, Dale 1983). By implication, those cases in which cold treatment improves germination should not involve such development, but rather some sort of endogenous inhibition, which is broken by cold storage. Our observation that the duration of dormancy in *S. trochoidea* is independent of temperature obviously fits into neither of these categories, and may well foreshadow the diversity of behaviors which might be observed as more dinoflagellate cysts are examined.

Excystment. The relationship between median germination time and cyst age can be separated into three hypothetical developmental phases (Fig. 3). For cysts within the first phase, from age 0 to approximately 25 days, the median germination time appears to be controlled strictly by cyst dormancy. The observed linear decrease in germination time within this interval is the mathematical consequence of a minimum germination *age* of approximately 25 days.

At the other extreme, cysts more than 75 days old appear to approach a minimum germination time of approximately 2.5 days. This asymptotic value may be set by the absolute minimum time required to complete the physiological or developmental changes which underlie excystment.

The intermediate phase, between 25 to 75 days, appears to represent a transition from the dormancy period, when germination is impossible, to the final phase, when cysts are prepared to germinate optimally. Although variability in the data obscures the details of this phase, some physiological changes apparently occur in cysts that remain quiescent, resulting in increased germination rate in these cysts once permissive conditions are established.

Spontaneous excystment. The requirement for a shift in environmental conditions to trigger germination has been discussed in connection with a number of dinoflagellate species. However, the distinction between a requirement for an actual environmental change versus a requirement simply for a given set of permissive conditions has not yet been carefully examined (Dale 1983). Anderson (1980) showed that an upshift in temperature from 4° C to 15° C or a downshift from 22° C to 15° C resulted in germination in *G. tamarensis* cysts, but he pointed out that since no treatment involving both storage and germination at 15° C had been performed, he could not establish a need for a temperature shift, per se, as opposed to a simple need for incubation at 15° C. In fact, a number of dinoflagellates have been reported to germinate in the absence of obvious environmental changes, although quantitative data have not been presented (von Stosch 1973, Pfiester 1975, 1976, 1977, Pfiester and Skvarla 1979).

Scrippsiella trochoidea cysts do not require environmental shifts to initiate germination. Rather, our data are consistent with the concept of a permissive "window" of environmental conditions, within which non-dormant cysts will germinate, but outside of which such cysts remain quiescent (Dale 1983, Pfiester and Anderson 1987). Thus, cysts left undisturbed in their parent cultures, under unchanged light and temperature conditions (14:10 h LD, 18° C), did germinate (Fig. 6). Germination was significantly delayed relative to nutrient-enriched treatments, but the final proportion of cysts germinating in the two treatments was comparable. This experiment utilized separate culture tubes at each time point in order to avoid potential artifacts associated with repeated handling of the same tube over time. However, the possibility of other artifacts resulting from the presence of vegetative cells in the suspension (e.g. organic or inorganic nutrient release, oxygen concentration changes, etc.) cannot be discounted. These effects could be particularly pronounced at the bottom of culture tubes where dead and senescent vegetative cells as well as all the cysts accumulate. Therefore, while there is no doubt that temperature and light shifts are unnecessary as triggers of germination in *S. trochoidea* cysts, the conclusion that shifts in water chemistry are likewise not required must remain tentative.

Temperature effects. Temperature is the environmental variable most often cited as controlling germination in dinoflagellate cysts (Dale 1983, Pfiester

and Anderson 1987). Low temperature maintains quiescence in cysts of many species; a return to higher temperatures appears sufficient to initiate germination. Few studies have addressed the effects of incubation temperature on germination in any detail.

Huber and Nipkow (1923) reported that germination rate in *Ceratium hirundinella* varied with temperature in much the same way as it does in *S. trochoidea* (compare their fig. 11 with our Fig. 5A). Within a certain temperature range, the time required for germination is at its minimum; beyond that range, germination time increases sharply. For *C. hirundinella,* this increase in germination time was observed above the optimum temperature range as well as below it; *Scrippsiella trochoidea* might show a similar increase above its optimal range, but no such increase was detected at the highest temperature tested (25° C).

It appears likely that the apparent decrease in "ultimate" germination frequency in *S. trochoidea* cysts at temperatures below 5° C is an artifact of the finite duration of the experimental incubations (see below). Similar decreases in germination frequency at non-optimal temperatures have been reported in *Peridinium cunningtonii* and a *Peridinium* sp. (Kadota et al. 1984, Endo and Nagata 1984). However, the lack of kinetic data in the first example, and the relatively short incubation time in the second, make it impossible to be sure that the observed relationships between temperature and germination frequency are not in fact reflections of the effect of temperature on germination *rate.* In the present study, no significant germination was detected at 5–6° C until 25 days after the start of the experiment, yet germination at this temperature did finally reach a frequency of 75%.

The relationship between germination time and temperature in *S. trochoidea* and *C. hirundinella* suggests that for these species at least, the permissive temperature "window" for germination does not have clearly defined boundaries outside of which germination is impossible. Instead, beyond the optimal temperature range, germination occurs at progressively slower rates. This reduction in the rate of germination, as reflected by an increase in median germination time, is well described in *S. trochoidea* by an exponential function, the slope of which corresponds to a Q_{10} of approximately 11 (Fig. 5A; $r^2 = 0.995$, $P < 0.001$). Using this relationship, the extrapolated germination time for *S. trochoidea* cysts at 1–2° C is approximately 150 days, or twice as long as the duration of our temperature experiment. Thus, the possibility of germination at such low temperatures cannot be excluded. In this connection, note that in the present study the cysts reported to have remained quiescent for 340 days at 3° C were stored in the dark.

Although these data argue against a simple on/off response by cysts to temperature, the graded response observed is quite precipitous. Thus, although the germination rate at 3–4° C was approximately 6% of the optimal rate, vegetative growth rate was reduced by only 50% at these low temperatures (Fig. 5B). Furthermore, the Q_{10} values of 11 and 9 for germination rate in *S. trochoidea* and *C. hirundinella,* respectively, are far above the values reported for various metabolic processes in other algae [Soeder and Stengel 1974; Q_{10} for *C. hirundinella* germination calculated from data in Huber and Nipkow (1923) between 5° C and 20° C]. Therefore, while the response of cysts to temperature is not strictly an on/off one, neither does it appear to be a simple reflection of a general metabolic slowdown with decreasing temperature. Rather, the steep slope of germination time versus temperature suggests the operation of a specialized control mechanism.

The optimal temperature range for germination in *S. trochoidea* does not coincide exactly with the optimal range for growth (Fig. 5). Aside from the drastic reduction in germination rate at temperatures causing only moderate reduction in growth rate at the low end of the temperature range, large reductions in growth rate are apparent at the high end, above 23° C, where germination still occurs. Huber and Nipkow (1923) noted similarly that the optimal temperatures for excystment in *C. hirundinella* were higher than the optimal range for morphological development of the germling (21–24° C vs. 15–20° C, respectively).

Medium effects. Nutrient concentrations and other water chemistry variables are not generally considered to exert significant influence on germination in dinoflagellate cysts. In fact, those few studies which have addressed the question have concluded that germination is insensitive to these variables, although subsequent survival and development of the germling may obviously be affected (Huber and Nipkow 1923, Anderson and Wall 1978, Anderson and Morel 1979). In contrast, the present study establishes that water chemistry can indeed influence germination in *S. trochoidea* cysts. In two experiments involving nutrient-enriched and unenriched media, germination was significantly retarded in the unenriched treatment. However, final germination frequencies appeared comparable, or only slightly reduced in the unenriched treatments (Figs. 6, 7).

Other factors can apparently modify these nutrient effects. The germination rate in cysts stored at 3° C in the dark for 3 months and then returned to 18° C (14:10 h LD) was comparable in enriched and unenriched treatments (data not shown). Furthermore, we consistently found that when cysts were individually isolated into tissue culture plates, germination in unenriched media was equivalent to that in enriched treatments. Perhaps the environmental perturbations which are necessarily associated with such manipulations were responsible for stimulating germination in these treatments.

The inability of other studies to demonstrate nutrient effects may reflect real differences in germination behavior among dinoflagellate species. However, considering that the response reported here involved the kinetics of germination rather than ultimate germination frequency, and that this response could be overridden by other factors, the possibility of similar subtle effects of nutrient conditions on germination in the other dinoflagellate species examined cannot be excluded. We note that most previous studies of nutrient effects have involved individually isolated cysts.

Ecological implications. It is apparent that the germination behavior of *S. trochoidea* cysts is determined by no single factor, but rather is the result of the interaction of several. Temperature, nutrient conditions, light regime, and cyst age all may affect germination. Caution is advised in using our laboratory results to predict cyst behavior in the natural environment where other variables may further complicate the picture. With this caveat in mind, some general implications of the present results to the ecology of *Scrippsiella trochoidea* can be discussed.

The relatively short dormancy period in *S. trochoidea* cysts, the lack of a requirement for dramatic environmental shifts, and a rather broad optimal temperature range for germination and growth could all facilitate a rapid cycling of the *S. trochoidea* population between its vegetative and dormant phases repeatedly during the year. Cysts of this species could thus be acting as mechanisms for survival during short term environmental adversity. Such a role in short term survival would be less appropriately applied to cysts of dinoflagellate species with longer dormancy periods which might only seed one or two discrete blooms in a year (Anderson and Morel 1979).

Despite the ability of *S. trochoidea* cysts to support rapid population turnover, the quiescence of these cysts under cold, dark conditions is consistent with the longer term, over-wintering role often assumed for dinoflagellate cysts. Although our data suggest that germination might be possible even at very low temperatures, the rate of such germination would be so low as to render it ecologically insignificant in many cases. For example, the extrapolated median germination time of 5 months at 1–2° C is far longer than the 2 to 3 months during which such temperatures are actually experienced in local, well mixed waters. On the other hand, in deeper areas where low temperatures occur throughout the year, low temperature germination, no matter how slow, could be of great significance. Note, however, that an unsatisfied requirement for light could well limit germination in these areas (Binder and Anderson 1986).

The pronounced retardation of germination in *S. trochoidea* cysts at lower temperatures (<10° C) which still support good vegetative growth could result in an apparently unnecessary delay (at least in terms of temperature tolerance) in seeding spring blooms of this species. Conversely, germination at high temperatures (>20° C) could release germlings into conditions which are unfavorable for growth. Thus, *S. trochoidea* cysts appear not to be finely tuned as "timing mechanisms" for bloom initiation.

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