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SEXUALITY AND CYST FORMATION IN THE DINOFLAGELLATE *GONYAULAX TAMARENSIS*: CYST YIELD IN BATCH CULTURES[1]

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ABSTRACT

Encystment of the toxic dinoflagellate Gonyaulax tamarensis *Lebour (var.* excavata*) was monitored in batch cultures exposed to a variety of nutritional and environmental treatments. Limitation by nitrogen (as ammonium or nitrate) or phosphorus (as phosphate) resulted in cyst formation. When the initial concentration of limiting nutrient was varied, total cyst yield (mL^{-1}) was directly proportional to the cell yield at all but the highest nutrient concentrations (where encystment was minimal). Encystment efficiency was relatively constant (0.1–0.2 cysts·$cell^{-1}$) over a 5-fold range of cell densities, indicating that 20 to 40% of the vegetative populations successfully encysted. Cyst formation was negligible in nutrient-replete medium, even with a significant reduction in growth rate due to non-optimal light, temperature, or to high batch culture cell densities. Low light levels did decrease cyst yield once encystment was initiated by nutrient limitation, but this was probably linked to smaller motile cell yield and not to a specific inhibition of encystment. In contrast, encystment was more sensitive to temperature than was growth rate: optimal cyst production occurred over a relatively narrow temperature range and no cysts were formed at*

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some temperatures that permitted growth. Cyst yield could also be reduced by chemical contaminants introduced during culture medium preparation. There is no evidence for a density-dependent induction mechanism, nor is there a link between sexuality and reduced growth rate in nutrient-replete medium.

Key index words: cysts; dinoflagellates; Gonyaulax tamarensis; *hypnozygote; resting spore,* Gonyaulax; *sexual reproduction,* Gonyaulax; *toxic blooms; paralytic shellfish poison; temperature; light*

In recent years it has become evident that many dinoflagellates are capable of both sexual and asexual reproduction (reviewed in Beam and Himes 1980, Pfiester and Anderson, 1984). Sexuality typically begins with the formation and fusion of gametes, leading to planozygote (swimming zygote) and, in many species, hypnozygote (resting cyst) formation. Observations of sexuality and encystment in freshwater species have generally been from laboratory cultures (e.g. von Stosch 1973, Pfiester 1975, 1976, 1977). Marine species have been more difficult to manipulate, and, therefore, with a few exceptions (Braarud 1945, Dale 1977a, Morey-Gaines and Ruse 1980, Walker and Steidinger 1979), most cyst/motile cell relationships were established by germinating cysts from natural sediments (Wall and Dale 1968, Dale 1977b, Anderson and Wall 1978). In many freshwater and marine studies where sexuality was induced in culture, the overall objective was often to demonstrate sexuality for a particular species, with relatively little concern for physiological induction mechanisms (reviewed in Pfiester and Anderson, in press). Our knowledge of the factors that regulate sexuality in dinoflagellates is thus quite limited due to inadequate culture technique and to the higher priority given to life history descriptions.

In most studies, sexuality has been induced by nutrient starvation, often by resuspending actively growing cells in culture medium lacking one essential nutrient, usually nitrogen (e.g. Pfiester 1975, Turpin et al. 1978, Walker and Steidinger 1979). In one instance, decreasing light intensity, photoperiod and temperature also enhanced cyst production (von Stosch and Drebes 1964). These laboratory results tend to support the intuitively appealing notion that encystment is a response to stress. There are, however, reports of encystment under seemingly favorable culture or field conditions (Zingmark 1970, Beam and Himes 1974, Morey-Gaines and Ruse 1980, Wall et al. 1970, Anderson et al. 1983).

In light of these conflicting reports and the general lack of attention given to the details of sexual induction in the past, experiments were initiated to study the encystment process in the toxic marine dinoflagellate *Gonyaulax tamarensis.* We examined the numerical yield of cysts in batch cultures under a variety of nutritional and environmental conditions. The results reported here constitute an examination of these effects using "end point" determinations of cyst abundance.

MATERIALS AND METHODS

The organism. All experiments were conducted with isolate GTMP of *Gonyaulax tamarensis* (var. *excavata*). The original culture was established by D. M. Anderson in 1978 using one cyst isolated from sediments of Mill Pond, Orleans, MA. The culture was unialgal but not axenic.

Culture medium. All experients were conducted in modifications of f/2 or h/2 media (Guillard and Ryther 1962). Maintenance and inoculum cultures were generally grown in f/2 with no added silicate (f/2-Si) using 10^{-5}M ferric sequestrene as iron source and chelator (NaFe Sequestrene, 13% Fe; CIBA-GEIGY). "Encystment" medium consisted of f/2 levels of trace metals, chelator, vitamins, and one nutrient, with the remaining major nutrient (N or P) reduced in varying amounts. Our designation "nitrate-encystment medium" thus describes media with nitrate reduced to 10% of f/2 levels (88 μM); phosphate and other enrichments were at f/2 levels, and no silicate was added. Similarly, phosphate-encystment medium had f/20 levels of phosphate (3.6 μM), and ammonium-encystment medium has h/20 levels of ammonium (50 μM). Ammonium-encystment medium was used routinely unless nutrient composition was a variable.

Unless otherwise indicated, media preparation included extensive precautions to avoid precipitation, adsorption, desorption, or chemical contamination. General procedures are those of Brand et al. (1981) with some modifications. Local seawater (ca. 31‰) filtered through glass fiber filters was autoclaved in Teflon bottles which were capped tightly immediately upon depressurization. The bottles were then cooled rapidly in a water bath, allowed to equilibrate for at least 24 h, and used within one week. Nutrient and vitamin stocks were autoclaved in individual Teflon bottles, as were trace metals, except that the iron/chelator mix was stored separately from the mixed trace metals. These stock solutions were used for several months before replacement. At the beginning of each experiment, the enrichments were added aseptically to the seawater and the medium dispensed into sterile culture vessels (tubes or flasks) that had been autoclaved containing distilled water (which was discarded before use). Pyrex culture tubes (25 × 150 mm) were used, each containing 25 mL of medium.

All plastics and glassware were routinely washed with Micro detergent, rinsed extensively, soaked several days in 2 N HCl and rinsed again with dionized, distilled, UV-irradiated water. As a further precaution, all glassware was initially coated with Surfasil (Pierce Chemical Co.) and re-coated after three or four washings.

Culturing procedures. Inoculum cultures were grown in f/2-Si and transferred during mid-exponential growth to yield an initial cell concentration of 160 mL^{-1}. The *G. tamarensis* cell concentration was determined by counts of 200 or more cells in Sedgewick-Rafter slides. Growth rates were calculated from these cell counts or from in vivo fluorescence readings (Turner Designs, Model 10) taken during intervals when the cultures were considered to be in a physiological steady state (Brand et al. 1981).

Irradiance was provided by Cool-White fluorescent bulbs at approximately 350 $\mu E \cdot m^{-2} \cdot s^{-1}$ (measured underwater inside culture tubes with a Biospherical Instruments QSL-100P probe). A 14:10 h L:D cycle was used in all experiments except one testing the effect of continuous light.

Cyst harvesting and counting. All experiments were maintained a minimum of 30 days since this represents the interval after which total cyst counts did not increase. Each tube or flask was emptied into a plastic beaker and the residue at the bottom loosened with a rubber policeman and rinsed into the same beaker with filtered seawater. The harvested culture was then sonicated (Branson S-75 sonifer) for 1 min at 1.4 A to destroy most vegetative cells and poured into a settling chamber so that the cysts could be counted with an inverted microscope. Counts before

TABLE 1. *Culture medium*[a] *and light effects on cyst yield.*

	Cyst yield[b] mL^{-1} (SE)	Percent of control
Control: NH_4^+ encystment medium (50 μM NH_4^+)	732 (182)	100
f/2-Si (883 μM NO_3^-; 36.4 μM PO_4^{3-})	3 (0.3)	0.3
NO_3^- encystment medium (88.3 μM NO_3^-)	826 (423)	112
NH_4^+ encystment medium (no vitamins, 50 μM NH_4^+)	706 (53)	96
PO_4^{3-} encystment medium (3.6 μM PO_4^{3-})	318 (43)	43
NH_4^+ encystment medium (50 μM NH_4^+ inoculum medium autoclaved in glass, all enrichments combined)	570 (60)	78
NH_4^+ encystment medium (50 μM NH_4^+; inoculum and encystment medium autoclaved in glass, all enrichments combined)	297 (73)	41
Control: NH_4^+ encystment medium (normal light, 20°C, 0.4 div.·d^{-1})	665 (182)	100
f/2-Si (normal light, 20° C, 0.4 div.·d^{-1})	0.4 (0.1)	0
f/2-Si (light reduced by 84% on Day 2, 20° C, 0.19 div.·d^{-1})	0.1 (0.1)	0
f/2-Si (temperature reduced to 8° C on Day 2, 0.15 div.·d^{-1})	0.0 (0.0)	0

[a] Unless otherwise indicated, all media were made in Teflon with composition and precautions as described in text.
[b] Mean of three replicates.

and after sonication indicated that no cysts were destroyed by this process. The criteria used to separate viable resting cysts from temporary or pellicle cysts were size, shape, contents, and wall thickness (Anderson and Wall 1978, Anderson 1980).

Temperature and light effects. Temperature was maintained at 20° C unless it was an experimental variable. In the latter instance, an aluminum bar cooled at one end and heated at the other provided a thermal gradient (four tubes at each of 16 temperatures, range 2.5 to 28° C) with irradiance from below at 435 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Watras et al. 1982). Experimental cultures were acclimated for 10 to 15 generations at or near (in the case of extreme temperatures) the experimental temperature. Growth rates were calculated for cultures in f/2-Si medium and cysts harvested from cultures in ammonium encystment medium.

When irradiance was an experimental variable, culture tubes were wrapped with layers of grey and black window screen to reduce light levels (7 irradiances, 50 to 650 $\mu E \cdot m^{-2} \cdot s^{-1}$). Growth rates were calculated for cultures in f/2-Si medium and cysts harvested from cultures in ammonium-encystment medium.

The effects of a rapid decrease in light or temperature leading to slower growth in nutrient-replete medium were tested using three sets of triplicate tubes, each containing 25 mL of f/2-Si. These were inoculated and allowed to grow exponentially under normal culture conditions for two days. Then one set was placed under reduced light (71 $\mu E \cdot m^{-2} \cdot s^{-1}$; 16% of normal), and another was placed at 8° C. Cysts were counted after 78 days since these cultures grew so slowly.

Cyst yield was also monitored under continuous irradiance. Inoculum cultures were acclimated to continuous light (350 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 15 generations in f/2-Si medium before being added to ammonium-encystment medium and incubated at 20° C for 30 days under a 24:0 h L:D cycle.

Culture medium effects. One series of experiments tested various aspects of the encystment medium recipe (variable vitamin concentration; nitrate, ammonium, or phoshate as the limiting nutrient), while also examining the need for certain procedural constraints (Surfasil-coated glassware; autoclaving in Teflon). Another experiment monitored cyst yield and peak cell concentration in a series of nutrient dilutions with N- or P-limitation. Medium was prepared with ammonium- or phosphate at concentrations between 1.6 and 97 μM or 1.1 to 7.6 μM, respectively, placed into tubes and inoculated with exponential cultures growing in typical ammonium- or phosphate-encystment medium.

RESULTS

Culture medium effects. Cysts were present to some extent in all cultures, regardless of initial medium composition. The lowest cyst yields were in nutrient-replete (f/2-Si) cultures, with production more than two orders of magnitude lower than that in our standard ammonium-encystment medium (Table 1). Cysts were present when either ammonium or nitrate was used as the limiting nitrogen source. Reduced phosphate concentrations also yielded normal cysts, but many slightly aberrant forms with the correct cell contents and wall structure but with a round to slightly oval shape were observed as well. These were not temporary or pellicle cysts (Anderson and Wall 1978) and they may have been capable of extended dormancy, but they were sufficiently different in shape from natural cysts that they were not included in our tabulations. This type of rounded cyst was more numerous in P-limited cultures than in N-limited cultures.

Omission of the vitamin enrichment from the ammonium-encystment medium had no effect (Table 1). Similarly, cysts were produced in cultures using either ferric sequestrene or an equivalent concentration of $FeCl_3$ and EDTA as chelated iron (Guillard 1973). Attempts to optimize cyst yield through manipulations of iron and chelator type in this manner gave highly variable results and no definitive optimum mixture. For consistency, all experiments reported here used 10^{-5} M ferric sequestrene with f/2 levels of all other metals.

Two simple tests were conducted to determine the value of our precautions against chemical contamination and precipitation in culture medium preparation (Table 1). When normal ammonium-encystment medium was inoculated with cells grown in f/2-Si prepared by adding all enrichments to seawater and then autoclaving the mixture in glass (non-Surfasil coated borosilicate), cyst yield was lower but not significantly different from the control method ($P > 0.05$). However, when both inoculum and encystment media were prepared as above in glass, the cyst yield dropped to 41% of the control (significantly different, $P < 0.05$).

When actively growing cells were inoculated into medium containing reduced N or P concentrations, total cyst yield was directly proportional to the initial concentration of limiting nutrient up to an optimum concentration, decreasing thereafter with higher nutrient availability. With phosphate in short supply, optimum cyst production occurred near 3 μM,

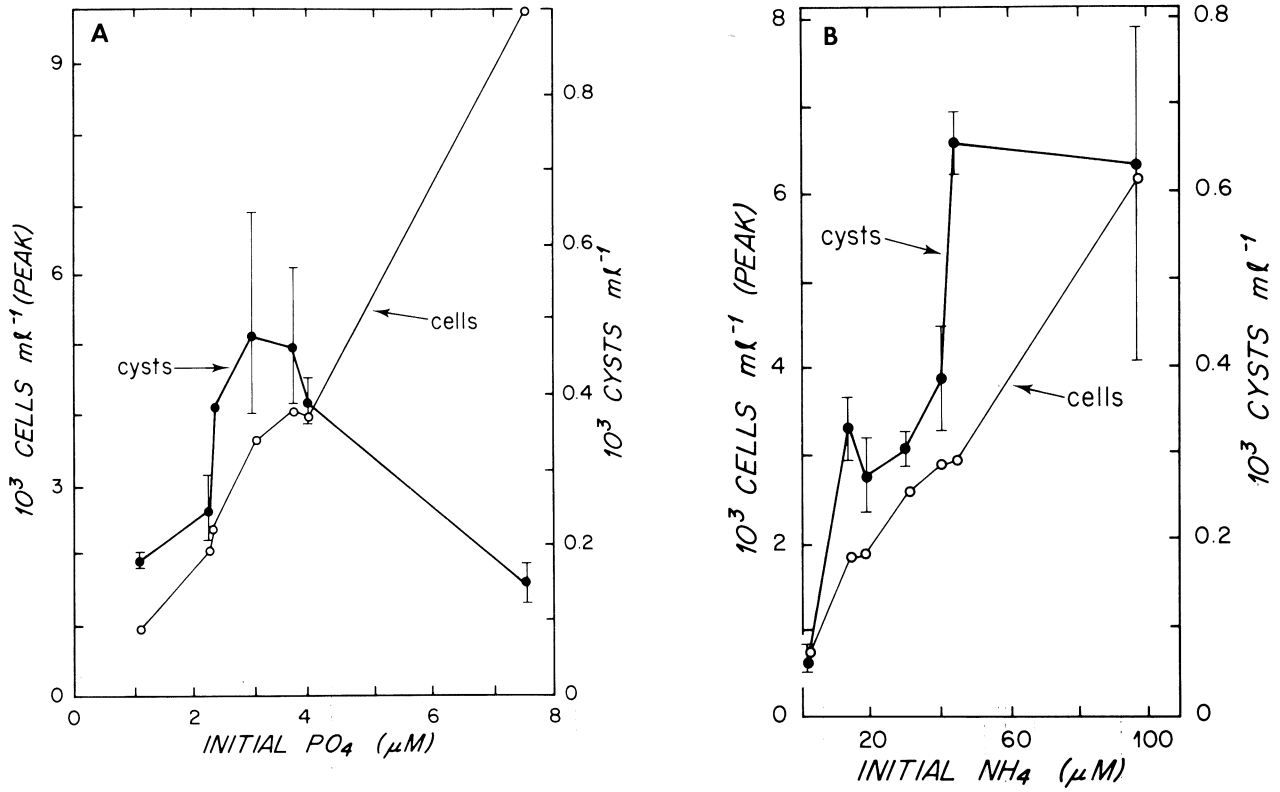

FIG. 1A, B. Cyst yield (●) and peak motile cell concentration (○) versus initial concentration of limiting nutrient. A. Phosphate limitation; B. Ammonium limitation. Vertical error bars show the range of triplicate counts.

yielding three times as many cysts as the lowest (1.1) and highest (7.6 μM) initial concentrations (Fig. 1A). The motile cell yield increased linearly with a maximum at the highest phosphate concentration. Except at the highest phosphate concentration, increases in cell density were matched by increases in cyst yield such that encystment efficiency (the cyst : cell ratio) remained fairly constant between 0.09 and 0.15. At 7.6 μM phosphate, the efficiency dropped to 0.02 cysts·cell^{-1}.

With ammonium as the limiting nutrient, the maximum cyst production occurred in the 45 and 97 μM cultures (Fig. 1B). At higher concentrations (not shown), *G. tamarensis* growth was highly erratic, with long lag phases and poor replication between cultures. This clearly reflects inhibition by ammonium since this organism grows well in f/2 where nitrate is 883 μM (with negligible cyst production; Table 1). Cell yield increased linearly with ammonium additions, as was generally the case with cyst yield (Fig. 1B) which was highest at the highest ammonium concentration (nearly twice the production at lower concentrations). Encystment efficiency varied between 0.06 and 0.20 cysts·cell^{-1}, with no apparent trend.

Temperature and light. The *G. tamarensis* growth rate varied significantly with temperature in f/2-Si medium, with no growth below 7° or above 26° C and an optimum range between 11° and 22° C (Fig. 2A). It should be noted that inoculated cells did not die at 2.5° and 5° C, but neither did they grow. Cell mortality occurred above 26° C. Between these inhibitory extremes, peak cell density remained relatively constant (Fig. 2A). Few, if any, cysts were observed in these f/2 cultures. In encystment medium, peak cell concentration was also relatively constant beween 8 and 24° C, but total cyst production was highly variable, with a sharp optimum near 21° C (Fig. 2B). Thus, 12° C was the lowest temperature at which cysts were produced, with a yield fully two orders of magnitude lower than the optimum. At high temperatures, inhibition of encystment paralleled the sharp decrease in growth rate. The large range in cyst yield at different temperatures and the relatively constant motile cell yield reflect marked changes in encystment efficiency (varying from 0.01 to 0.13 cysts·cell^{-1}).

Growth rate in f/2-Si increased as irradiance increased, reaching optimum levels at approximately 150–200 $\mu E \cdot m^{-2} \cdot s^{-1}$ with no indication of photoinhibition up to 650 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Fig. 3A). A similar trend was observed in both cell yield and cyst yield in encystment medium (Fig. 3B), with saturation at 150–200 $\mu E \cdot m^{-2} \cdot s^{-1}$ and no inhibition at higher irradiances. The tight coupling between these two parameters reflects a relatively constant cyst : cell ratio that fluctuated between 0.11 and 0.17.

When cultures were adapted to continuous light

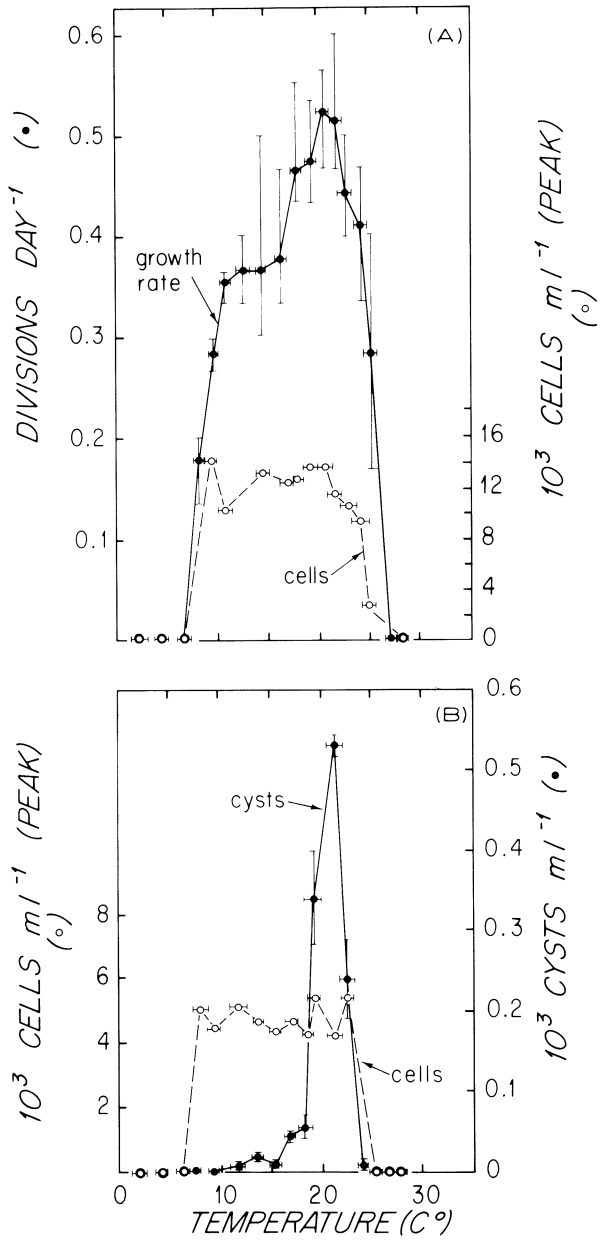

FIG. 2A, B. The effect of temperature on *G. tamarensis* growth rate, peak motile cell concentration, and cyst production in batch cultures. A. Growth rate (●) and peak cell concentration (○) versus temperature in nutrient-replete f/2-Si medium; B. Cyst yield (●) and peak motile cell concentration (○) versus temperature in ammonium-encystment medium. Vertical error bars show the range of triplicate counts; horizontal error bars indicate the range of temperature fluctuations.

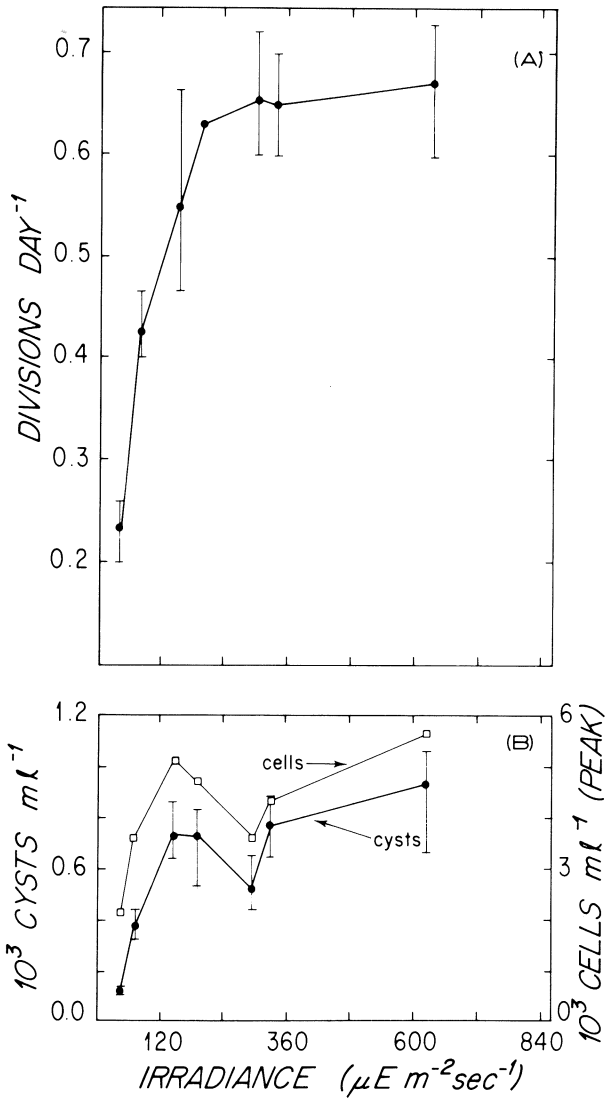

FIG. 3A, B. The effect of irradiance on *G. tamarensis* growth rate, peak motile cell concentration, and cyst production. A. Growth rate versus irradiance in nutrient-replete f/2-Si medium; B. Cyst yield (●) and peak motile cell concentration (○) versus irradiance in ammonium-encystment medium. Vertical error bars show the range of triplicate counts.

for 15 generations and inoculated into encystment medium, cysts were produced in quantities similar to those obtained under 14:10 h LD irradiance. In another experiment, a 50% reduction in growth rate in nutrient-replete medium in response to reduced light did not increase cyst yield beyond the normally low f/2-Si levels (Table 1). Similarly, a 60% reduction in growth rate due to suboptimal temperature (8° C) did not produce cysts in nutrient-replete f/2-Si medium.

DISCUSSION

As has been reported in other studies on dinoflagellate sexuality (reviewed by Pfiester and Anderson, in press), a reduction in nutrient concentrations resulted in the formation of hypnozygotes or resting cysts in *G. tamarensis* cultures. In nutrient-replete cultures, essentially no cysts were formed despite reductions in growth rate due to non-optimal light, temperature, or to high batch culture density. Once initiated by nutrient depletion, however, total cyst yield was influenced by temperature, light, and culture medium purity.

Nutrient effects. Looking first to the role of nutrients in the encystment process, it is evident that actively growing cells inoculated into a range of N and P concentrations produced relatively few cysts at the highest and lowest nutrient concentrations (Fig. 1, Table 1). In phosphate-limited cultures, cyst yield was optimal with the initial concentration near 3 μM. This finding is similar to that of Trainor (1958) who demonstrated an optimum concentration of limiting nitrogen for sexuality in batch cultures of the green alga *Chlamydomonas chlamydogama.*

Although there is likely to be an optimum initial concentration of nitrogen for *G. tamarensis* cyst production as well, we were unable to grow this species reliably at the high ammonium concentrations (>100 μM) where encystment would presumably have been low. The highest ammonium concentrations where growth was reproducible (45 and 97 μM) yielded the most cysts, but 250 μM was lethal to motile cells. Very few cysts were produced at f/2 levels of nitrate (883 μM). Toxicity at high ammonium concentrations was the only observed difference between ammonium- and nitrate-limited cyst production (Table 1; unpublished data).

It should be stressed that the high cyst yield at certain nutrient concentrations is only an indication of optimal initial conditions and should not be directly extrapolated to natural waters. The batch culture populations responded to a rapidly decreasing limiting nutrient without additional input. The dynamics of nutrient depletion and regeneration in natural waters are far more complex, and thus the timing, synchrony, and magnitude of sexual induction would presumably be different.

When initial nutrient concentrations were at or below optimal levels, there was a direct correlation between cyst yield and cell yield (Fig. 1), with encystment efficiencies fluctuating narrowly between 0.1 and 0.2 cysts·cell^{-1}. Differences between these efficiencies are not considered significant, in part because of errors associated with cyst harvesting and counting and, in part because of the lack of a systematic trend in efficiencies. Note that these efficiencies are necessarily approximate since they are calculated using peak cell concentrations and final cyst yield, two measurements taken at different times during the experiment. More accurate estimates require continuous monitoring of cell and cyst abundances throughout each experiment so that those cells that successfully fused to form cysts can be included in the motile cell totals.

Since two cells fuse to produce each cyst (Anderson and Wall 1978, Turpin et al. 1978, Anderson and Lindquist, unpublished data), the efficiency data indicate that approximately 20–40% of the motile population successfully encysted across a wide range of nutrient concentrations. This is noteworthy because it indicates that a relatively constant fraction of the cells from motile cell populations of different size successfully encysted. Intuitively one might expect encystment efficiency to be optimal at or above a specific cell density, with the lowest values at reduced cell densities where gamete interactions would be less frequent. Perhaps some characteristic of our batch culture conditions (e.g. the rapid depletion of external nutrients or the high biomass) caused this relative constancy.

Above the optimum nutrient concentrations, cyst production was low despite maximum motile cell numbers. At the extreme of nutrient excess, cyst production in f/2 was essentially zero (Table 1). Nutrient analyses indicated that both N and P were present in substantial quantities when the f/2 cultures stopped dividing. Thus, density-dependent stress factors such as self-shading, pH, carbon limitation or excretion limited growth without inducing sexuality.

Induction mechanisms. A variety of factors, alone or in combination, have been reported to induce sexuality in algae (Coleman 1962). Many of these involve nutritional or environmental stress, but induction by pheromones has also been described (Darden 1966, Starr 1970). In the latter instance, gametogenesis would occur in response to a pheromone-type substance that is either produced or is effective when cell density reaches a threshold value. If such a process were both density-dependent and independent of the nutrient status of a *G. tamarensis* population, we would not expect to see cyst yield decrease sharply with increased nutrient availability as was observed in our study (Fig. 1). Furthermore, this mechanism is not consistent with our observations of a relatively constant encystment efficiency despite 4–5 fold variations in cell density. We conclude that a density-dependent induction mechanism either does not apply to *G. tarmarensis* or that it is moderated by nutrient pools. We are now attempting to separate nutrient effects from those due to cell density through continuous culture techniques in which cell concentration can be varied without changing intracellular or extracellular pools of the limiting nutrient.

In batch culture, cysts were consistently produced under conditions where the nutrient in shortest relative supply was depleted. Since cysts were counted only at the end of our experiments, the nutrient dynamics associated with encystment remain obscure. Detailed monitoring of nutrient pools and life cycle stages is required to resolve the timing of sexual induction in this species.

It is noteworthy that sexuality occurred under both N and P limitation, as was observed by Pfiester (1976) and Watanabe et al. (1982) for *Peridinium willei* and *Scrippsiella trochoidea,* respectively. Ellis and Machlis (1968) also observed zygospore formation following phosphate limitation in the green alga *Golenkinia* sp. This suggests that metabolic pathways for these two nutrients may each include a mechanism for initiating the shift to sexual reproduction. Alternatively, since nutrient limitation can affect growth rate, con-

trol of sexuality might be closely associated with cell division. However, the latter possibility seems unlikely for two reasons. First, cysts were rare in nutrient-replete cultures that stopped growing because of density-dependent factors. Second, a 50% reduction in growth rate due to sub-optimal light or temperature did not produce cysts in nutrient-replete medium (Table 1). The clear inference is that sexuality cannot occur under nutrient-replete conditions, despite the growth-limiting effects of a variety of factors. Such inhibition is presumably common to many dinoflagellates as well, since induction of sexuality has often required a transfer of cells from normal growth medium to one lacking major nutrients (von Stosch 1973, Pfiester 1975, Turpin et al. 1978, Walker and Steidinger 1979). High nutrient concentrations have also inhibited sexuality in the green alga *Scenedesmus obliquus* (Cain and Trainor 1976).

It is not known whether non-nutritional stresses such as light or temperature would induce sexuality at lower but non-limiting nutrient concentrations in *G. tamarensis*. Our batch culture results indicate that nutrient depletion is one factor that regulates sexuality, but it is too soon to say whether this is the only induction mechanism.

Temperature and light effects. The effect of temperature on encystment is significantly different from its effect on growth rate or peak cell concentration (Fig. 2). Cells could survive at 2.5° C, but needed temperatures between 7° and 26° C to divide. This general response is similar to that reported in other studies with *G. tamarensis* (Prakash 1967, Yentsch et al. 1975, Watras et al. 1982). Between 7° and 26° C cell yield remained relatively constant in both nutrient-replete and nutrient-limited medium (Fig. 2). It is thus of note that no cysts were produced below 12° C and that total cyst yield varied by two orders of magnitude—all with the same initial nutrient availability. Apparently some metabolic process unique to gamete formation, fusion, or encystment requires higher temperatures than those that support cell division.

In the only field study to date of *G. tamarensis* sexuality, Anderson et al. (1983) observed motile cells in waters as low as 4–6° C but the first sexual stages were not seen until water temperatures reached 10–12° C. The correspondence between field and laboratory data is not surprising given that our GTMP strain was isolated from the region where that field study took place. It would be interesting to determine the temperature threshold for encystment in *G. tamarensis* isolates from colder regions.

Studies of temperature effects on sexuality in other phytoplankton species suggest that the response of *G. tamarensis* is neither unique nor universal. For example, Watanabe et al. (1982) reported that *Scrippsiella trochoidea* cyst production was directly proportional to cell yield, even at temperatures suboptimal for growth (only five temperatures were examined). On the other hand, Necas (1982) and O'Kelley (1983) found that sexuality in the green algae *Chlamydomonas geitleri* and *Chlorococcum echinozygotum* occurred over a much narrower temperature range than that permitting cell division.

In contrast to temperature, irradiance had similar effects on both growth rate and encystment (Fig. 3), with saturation at 150–200 $\mu E \cdot m^{-2} \cdot s^{-1}$ and no inhibition up to 650 $\mu E \cdot m^{-2} \cdot s^{-1}$. Reduced cyst production at low light levels is probably due to lower motile cell concentrations since the cyst : cell ratio was relatively constant at all light levels. Thus, light affects encystment at low (and presumably very high) intensities, but it is unlikely that this inhibition is unique to sexual processes.

Optimizing cyst yield. For many years, cyst-forming marine dinoflagellates have responded poorly to laboratory culture conditions, often necessitating the use of cysts from sediment samples for the description of theca/cyst cycles (Wall and Dale 1968, Dale 1977b, Anderson and Wall 1978). It now appears that some of the problems may have been due to inhibition of growth and/or sexuality by artifacts introduced by common methods of medium preparation (Table 1). Of the variations in medium composition that we tested, perhaps the most important was the avoidance of precipitates and chemical contaminants during autoclaving (Brand et al. 1981). When inoculum and encystment media were autoclaved in borosilicate glass with all enrichments combined, cyst yield was significantly lower than that in control media prepared using our "clean" protocol ($P < 0.05$). Seawater sterilized in borosilicate glass contained as much as 300 μM silicate following autoclaving, whereas Surfasil-coated glassware released less than 20 μM. It may be the silicate, the materials released with it, and/or the precipitation and scavenging of necessary elements when the medium is autoclaved as one mixture that inhibits the cells. Although tedious and somewhat expensive, the precautions described for glassware cleaning and medium preparation are recommended for optimal growth and cyst production.

It mattered little whether nitrate or ammonium was used as the limiting nitrogen source (Table 1; unpublished data). Low levels of phosphate also induced sexuality, but many of the cysts produced were not the correct shape. This morphological effect has not been observed for cysts in our laboratory cultures of *S. trochoidea* and *G. uncatenum* (unpublished data). Omission of the vitamin enrichment had no effect, perhaps because the cultures were not axenic or were prepared with natural seawater. In general, approximately f/20 concentrations of nitrate, ammonium, or phosphate yielded the most cysts. Note that this protocol differs significantly from that used on many dinoflagellates (e.g. Pfiester 1975, Turpin et al. 1978, Walker and Steidinger 1979), in that actively-growing cells are inoculated into medium containing nutrients that are depleted by sub-

sequent growth, and not into medium already lacking one element. Our approach permits an increase in biomass through growth, resulting in a higher yield of cysts. The reduced cyst abundance in low nutrient medium (i.e. 1.6 μM ammonium; Fig. 1B) is indicative of low biomass.

Culture temperatures should be maintained near the optimum for growth, and irradiance above saturation (20° C and 200 $\mu E \cdot m^{-2} \cdot s^{-1}$, respectively, for *G. tamarensis*). Our results also indicate that encystment occurred under a 14:10 h light : dark regime and under continuous light. These techniques have been used, with essentially no modification, to study encystment in two other marine dinoflagellates, *S. trochoidea* (Binder, unpublished data) and *Gyrodinium uncatenum* (Anderson, unpublished data).

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