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ENCYSTMENT OF THE DINOFLAGELLATE *GYRODINIUM UNCATENUM*: TEMPERATURE AND NUTRIENT EFFECTS[1]

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ABSTRACT

Sexual reproduction and encystment of the marine dinoflagellate Gyrodinium uncatenum *Hulburt were induced in nitrogen and phosphorus-limited batch cultures. Sexuality did not occur under nutrient-replete conditions even when growth rate was reduced by non-optimal temperatures. Growth was optimal over a broader temperature range than encystment and virtually no cysts were produced at some low and high temperatures where growth occurred.*

Most cells initiated sexuality as intracellular pools of each limiting nutrient reached minimum or subsistence levels as much as four days after extracellular nutrients were exhausted. High nitrogen cell quotas during the phosphorus experiment indicate that sexuality was induced by a shortage of phosphorus and not by an indirect effect on nitrogen uptake.

Total cyst yield corresponded to successful encystment of 9–13% of the motile populations, yet 60–85% of the plateau-phase motile cells were planozygotes (swimming zygotes formed from fusing gametes). Batch culture studies monitoring total cyst yield may thus seriously underestimate the extent of sexuality. More importantly, the number of cysts produced in a dinoflagellate population may be significantly reduced by environmental factors acting on the cells after sexual induction and fusion.

Key index words: cyst; encystment; gamete; Gyrodinium uncatenum; *hypnozygote; nutrient limitation; planozygote; temperature*

The marine dinoflagellate *Gyrodinium uncatenum* Hulburt blooms regularly in the tributary estuaries of the Chesapeake Bay, sometimes with cell densities sufficient to discolor the water. The temporal variation of these blooms can be explained in part by the characteristics of a dormant life cycle stage (resting cyst) produced during the blooms which settles to the sediments as a potential inoculum for future outbreaks. Our recent work has demonstrated that: a) normal asexual division of *G. uncatenum* can switch to sexual reproduction whereby gametes fuse to form a swimming zygote (planozygote) which subsequently becomes a non-motile dormant cyst (Coats et al. 1984); b) the location of blooms of motile cells in the water and cyst accumulations in the sediments can be determined in part by concentration and recirculation of *G. uncatenum* cells in frontal convergence zones (Tyler et al. 1982); and c) the germination of resting cysts from the "seeds beds" as temperatures reach 15° C in the spring provides an innoculum that can restore the vegetative population to overlying waters (unpublished data).

We thus have some understanding of *G. uncatenum* bloom initiation and development but know relatively little of the factors regulating sexuality, encystment, and bloom decline. Published accounts of sexual induction in dinoflagellate cultures describe a variety of causative conditions, ranging from nutrient starvation to no stress whatever (reviewed in Pfiester and Anderson 1985). Most of these efforts have been descriptive in nature, however, and provide little systematic information on growth or nutritional dynamics prior to encystment (e.g. von Stosch 1973, Pfiester 1975, Walker and Steidinger 1979). More recent studies have tested the factors that maximize total cyst yield in culture (Watanabe et al. 1982, Anderson et al. 1984). Such results are useful in guiding biochemical or physiological studies, but there is a clear need for more detailed examinations of dinoflagellate physiology during the transition from asexual to sexual reproduction.

One step in this direction was a study that monitored life cycle stages and the sizes of intracellular and extracellular pools of limiting nutrient (phosphorus) during *Gonyaulax tamarensis* encystment in batch culture (Anderson and Lindquist 1985). The experiment provided useful information on the duration of the swimming zygote stage, the fraction of the cumulative motile population that successfully encysted, and the phosphorus uptake rates as sexuality was induced. Due to the lack of suitably precise methods to distinguish gametes and early planozy-

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gotes from vegetative cells, however, no definitive conclusions could be drawn about sexual induction mechanisms and planozygote development.

In the study reported here, we used cytological markers revealed by protargol silver staining (Coats et al. 1984) to pinpoint the onset of sexuality in a *G. uncatenum* culture. Combined with measurements of limiting and non-limiting nutrient pools and studies of the effects of temperature on growth and encystment, the results add much to our understanding of sexual reproduction in this dinoflagellate.

MATERIALS AND METHODS

All experiments were conducted with *Gyrodinium uncatenum*, an estuarine organism that grows optimally in salinities between 8 and 30‰ (Tyler et al. 1982). The original isolation (strain GYRO) was by D. W. Coast from the Potomac estuary at 10‰ and 20° C. The culture was unialgal, but not axenic or clonal. Culture medium was f/2 minus Si (Guillard and Ryther 1962) in a 25‰ natural seawater base when nutrient-replete conditions were required. Nutrient limitation was imposed by transferring exponentially growing cells from f/2 minus Si into medium with f/30 concentrations of either NH_4^+, NO_3^-, or PO_4^{3-} and all other components at f/2 levels (hereafter termed encystment medium). Details of this medium and the extensive precautions taken to avoid precipitation or chemical contamination are given in Anderson et al. (1984). Cultures were grown at 20° C under 350 $\mu E \cdot m^{-2} \cdot sec^{-1}$ irradiance on a 14:10 h L:D cycle with cool white fluorescent lights.

Temperature effects on growth rate, cell yield, and cyst yield were determined using culture tubes in an aluminum temperature gradient bar similar to that described by Watras et al. (1982). Heating one end and cooling the other resulted in a temperature range from 8 to 35° C (four tubes at each of 20 temperatures). Experimental cultures were acclimated for at least 10 generations at or near (in the case of extreme temperatures) the experimental temperature. Growth rates were calculated for cultures in nutrient replete medium and cysts harvested from encystment medium limited by NH_4^+. *Gyrodinium uncatenum* cell concentration was monitored by counting at least 200 cells in Sedgewick-Rafter or Palmer-Maloney slides. Cysts were harvested by emptying each tube into a plastic beaker and loosening the remaining residue with a rubber scraper. After a thorough rinsing with filtered seawater, the harvested culture was sonicated for 1 min at 2.4 amps (Branson S-75 Sonifier) to destroy most vegetative cells. Counts with and without sonication indicated that no cysts were destroyed by this process. The sonicated liquid was then settled and the cysts counted with an inverted microscope. For a description of *G. uncatenum* cysts, see Tyler et al. (1982) and Coats et al. (1984). In the temperature experiment, cultures were incubated 45 days before cyst harvesting. In the time-course experiment, cysts were harvested daily.

A multi-tube experiment was used to obtain more detailed information on the time-course of encystment. More than 200 25 × 150 mm culture tubes were filled with 25 mL of encystment medium (with either NO_3^- or PO_4^{3-} at limiting concentrations) and then inoculated with exponentially growing cells from nutrient replete medium. Every day thereafter the number of tubes necessary to provide sufficient biomass for particulate CHN or phosphorus analysis were combined, subsampled for cell and planozygote counts, and filtered through glass fiber filters (Whatman GFF). Twenty-five mL aliquots of the filtrate were frozen and later analyzed for NO_3^- and PO_4^{3-} using the methods of Strickland and Parsons (1972). Also on a daily basis, one tube was harvested for total cyst yield as described previously. The number of planozygotes present each day was determined using the Bodian protargol silver stain technique (Tuffrau 1967) to accentuate basal bodies and flagella as described by Coats et al. (1984).

Total cellular phosphorus was assayed using a modification of the method of Menzel and Corwin (1965). Cells were filtered onto 25 mm diameter 5 μm pore size membrane filters (Gelman Metricel GA), rinsed thoroughly with filtered Sargasso seawater, and dried along with appropriate blank filters at 60° C. Dried filters were subsequently autoclaved for 20 minutes (121° C) in 15 mL of 0.17 M potassium persulfate. After cooling, sample volumes were measured and 10 mL aliquots analyzed for soluble reactive phosphorus colorimetrically (Strickland and Parsons 1972). A mean recovery of 98% of the total phosphorus present was obtained in mass balance experiments with other dinoflagellates using this method (Anderson, unpublished). Cell quotas of carbon, nitrogen and phosphorus were obtained by dividing the measured particulate C, N, or P by the number of cells filtered for analysis. In addition, the disappearance of NO_3^- or PO_4^{3-} from the medium was used with daily cell counts to give another estimate of pool sizes.

RESULTS

Gyrodinium uncatenum requires relatively warm temperatures for growth, with optimum rates between 25 and 30° C and no growth below 10° C or above 35° C in nutrient-replete medium (Fig. 1A). Over the 11.3 to 34° C range where growth rate was positive but varying six-fold, peak cell concentrations remained relatively constant (4500 cells $\cdot mL^{-1}$) with only a slight decrease at extreme temperatures. No cyst production was observed in any of the nutrient-replete cultures. Peak cell concentrations were generally lower in encystment medium (3000 mL^{-1}), but otherwise followed the same general trend observed in nutrient-replete medium (Fig. 1B). There was no growth in encystment medium at extreme temperatures (i.e. 11.3, 33, 34° C) where positive, but low growth rates were recorded in f/2 minus Si. In contrast to the relatively constant peak cell concentrations at different temperatures, total cyst yield varied from 4 to 268 cysts $\cdot mL^{-1}$, with the highest production between 23 and 27° C (Fig. 1B). This temperature effect is clearly seen in the encystment efficiency (defined as the ratio of total cyst yield to peak cell concentration [Anderson et al. 1984]) which reached a maximum level of 0.16 cysts $\cdot cell^{-1}$ at 23° C and decreased at lower and higher temperatures (Fig. 1C). It should be emphasized that these ratios only approximate the fraction of the populations that successfully encysted since some cysts are typically formed before the motile cell maximum occurs in batch cultures. A more accurate estimate of encystment efficiency can be obtained from time-course experiments (see below) that monitored motile cell, cyst, and planozygote concentrations as well as intracellular and extracellular nutrient pools on a daily basis.

During the time-course, cysts were formed in response to both nitrogen and phosphorus limitation. With NO_3^- in shortest relative supply, motile cell growth following inoculation was generally exponential through Day 8, after which the population

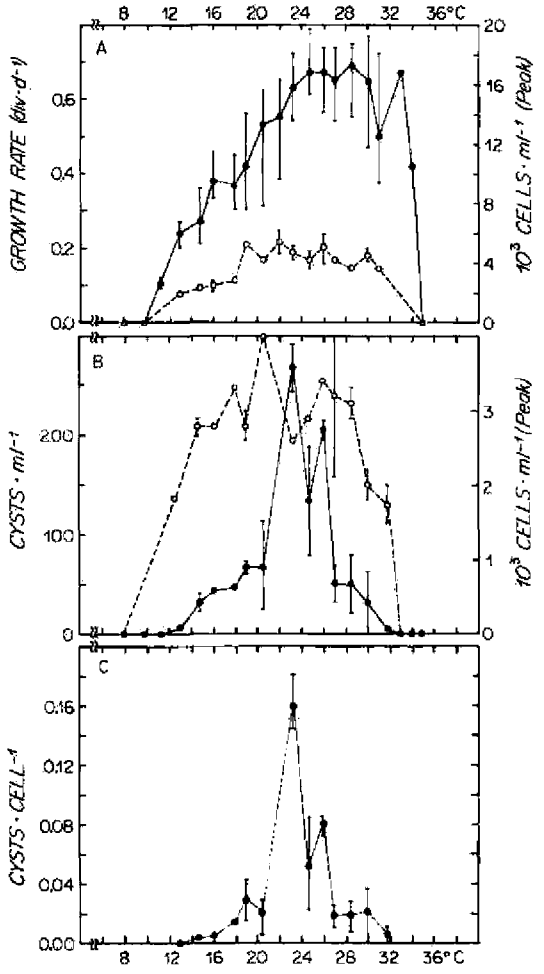

FIG. 1A–C. The effect of temperature on *Gyrodinium uncatenum* growth rate, peak motile cell concentration, cyst production, and encystment efficiency in batch cultures. A) Growth rate (●) and peak cell concentrations (○) in nutrient-replete f/2 minus Si medium versus temperature; B) Cyst yield (●) and peak motile cell concentration (○) versus temperature in NH_4^+-limited encystment medium; C) Encystment efficiency (cysts·cell^{-1}) versus temperature. Error bars show the range of replicate counts.

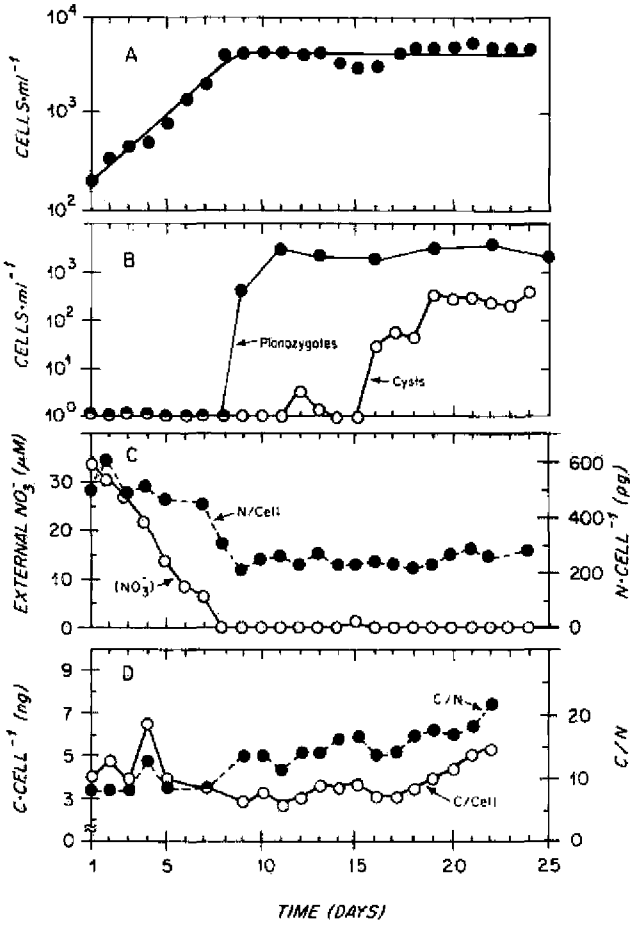

FIG. 2A–D. Time course of *G. uncatenum* encystment under NO_3^--limitation. A) Motile cell concentration (●) and log-linear and linear regressions. B) Planozygote (●) and cyst (○) concentrations. C) NO_3^- in the culture medium (○) and measured N·cell^{-1} (●); D) Carbon·cell^{-1} C/N ratio (by weight).

remained in stationary phase for two weeks (Fig. 2A). Planozygotes (the product of fusing gametes) increased rapidly in number beginning on Day 9, eventually totalling 85% of the motile population. Cysts appeared in significant numbers on Day 16, increasing to a plateau abundance of 400 mL^{-1} on Day 19 (Fig. 2C). Planozygote numbers did not decrease as cysts appeared, but instead remained constant nearly an order of magnitude higher than the final cyst abundance.

Nitrate in the culture medium steadily decreased following inoculation, reaching undetectable levels on Day 8 (Fig. 2D). Following an initial increase, cellular nitrogen remained near 450 pg N·cell^{-1} through Day 7, decreasing thereafter to an apparent subsistence quota of 230 pg N·cell^{-1}. (The subsistence quota is defined here as the quantity of cellular nitrogen that permits survival but not division.) Although not as dramatic as the decline in N pools, carbon·cell^{-1} appeared to decrease between Days 5 and 9 and may have increased slightly thereafter. The C/N ratio (by weight) was approximately 8 for the first 3 days, gradually increasing to a value near 20 at the end of the experiment. The first planozygotes appeared as this ratio reached 14.

These data make it possible to calculate uptake rates based on the disappearance of NO_3^- from the medium (Table 1). Following an initial increase after inoculation, the specific uptake rate for nitrogen was 0.3 d^{-1} during the first week of the experiment, a value close to the specific growth rate (0.4 d^{-1}). This approximate balance between cell division and uptake resulted in a nitrogen cell quota that decreased only slightly through Day 7 (Fig. 2C). As NO_3^- dropped from 6.5 μM to undetectable between Days 7 and 8, however, the uptake rate decreased to 0.16 d^{-1} while growth rate remained constant. The net effect was a rapid decrease in the nitrogen quota as the cells divided at the expense of stored reserves.

In the phosphate-limited time-course experiment,

TABLE 1. *Calculated nutrient pools and uptake rates during exponential growth.*

Daily interval	Avg (NO_3^-) (μM)	Cellular N (pg N·cell^{-1})	Uptake rate (pg N·cell^{-1}·d^{-1})	Specific uptake rate (d^{-1})	Avg (PO_4^{3-}) (μM)	Cellular P (pg P·cell^{-1})	Uptake rate (pg P·cell^{-1}·d^{-1})	Specific uptake rate (d^{-1})
1–2	32.5	519	232	0.45	2.4	239	30.2	0.13
2–3	28.8	474	155	0.30	2.1	198	29.7	0.15
3–4	24.5	426	134	0.31	1.7	138	15.1	0.11
4–5	18.0	410	153	0.37	1.5	133	6.0	0.05
5–6	11.3	321	61	0.19	0.81	117	29.5	0.25
6–7	7.7	229	19	0.08	0.16	85.3	2.9	0.03
7–8	3.3	181	29	0.16	0.07	60.4	0.1	0.0

exponential growth ceased on Day 10 (Fig. 3A). Planozygotes appeared first on Day 7, but the major increase occurred on Day 10 to approximately 2000 mL^{-1}. Cyst concentrations increased on Day 16, eventually totalling 500 mL^{-1}. Once again, the increase in cyst abundance was not associated with a detectable decrease in planozygote numbers.

Phosphate disappeared rapidly from the medium, reaching undetectable concentrations on Day 7. Cellular phosphorus steadily decreased from Day 1 to Day 10, eventually reaching an apparent subsistence quota of 29 pg P·cell^{-1} (Fig. 3D). The C/P ratio was 9 at the beginning of the experiment, increasing gradually through time to approximately 200 as the cells depleted phosphorus reserves. This ratio was about 110 when the major increase in planozygote abundance occurred between Days 9 and 10. Pools of the non-limiting nutrients nitrogen and carbon increased through time (Fig. 3E), with the C/N ratio fluctuating narrowly between 7.3 and 10. Calculated PO_4^{3-} specific uptake rates (Table 1) were always less than the specific growth rate, resulting in a steady decrease in P·cell^{-1} through time. Particulate phosphorus measurements of the 0.2 to 5 μm size fraction showed that bacterial uptake removed at most 10% of the available PO_4^{3-} through Day 10. Since a negligible number of bacteria were retained on the 5 μm filters after rinsing, particulate phosphorus measurements represent only *G. uncatenum* biomass.

Estimates of the fraction of each *G. uncatenum* population that successfully encysted under nitrogen or phosphorus limitation require a knowledge of the cumulative number of motile cells produced in each experiment. To compute motile cell equivalents, daily motile cell counts were added to the planozygote concentration plus two times the cyst concentration (the motile cell counts already include planozygotes and both cysts and planozygotes are the product of the fusion of two gametes). This is equivalent to tabulating the number of genomes (Coats et al. 1984). These calculations indicate that cumulative motile cell concentrations of 9800 and 7000 cells·mL^{-1} were achieved under nitrogen and phosphorus limitation respectively. Since cyst yield was approximately 400–500 mL^{-1} in both experiments (Figs. 2B, 3B), this corresponds to successful encystment of between 9 and 13% of the motile population. Furthermore, the large number of planozygotes remaining at the end of the experiments indicates that 55 to 85% of the motile cells fused as gametes, but most were unable to complete the encystment process.

DISCUSSION

A technique that permits identification of cells in the early stages of fusion and zygote development (Coats et al. 1984) was used to study the detailed dynamics of nutrient depletion, sexuality, and encystment in the marine dinoflagellate *Gyrodinium uncatenum*. In conjunction with batch culture studies of the *G. uncatenum* growth rate and cyst yield in response to temperature variations, the factors regulating the transition from asexual to sexual reproduction can now be examined in detail.

Induction Mechanisms and Temperature Effects

Under the experimental conditions used, encystment of *G. uncatenum* was mediated by temperature but required some degree of nutrient limitation for induction. A reduction in growth rate due to non-optimal temperatures did not produce cysts in nutrient-replete medium. The absence of sexuality in nutrient-replete cultures with high cell densities also argues that there is not a density-dependent induction through pheromone or gamone-type substances as observed for other algae by Darden (1966) and Starr (1970).

Temperature did alter both cyst yield and encystment efficiency once sexuality was initiated in nutrient limited cultures, however. Figure 1B demonstrates the magnitude of this temperature effect and Fig. 1C emphasizes the existence of a narrow temperature optimum for encystment near 23° C for this species. This is noteworthy since the relationship between growth rate and temperature has a much broader optimum range (between 19 and 31° C). Our data also demonstrate a low temperature threshold for *G. uncatenum* encystment near 15° C, four degrees warmer than the lowest temperature with positive growth (Fig. 1B). We conclude that either the temperature requirements for *G. uncatenum* encystment differ from those for growth or that temperature-induced variations in nutrient uptake and assimilation directly affect encystment in batch cultures.

Two recent studies that examined temperature

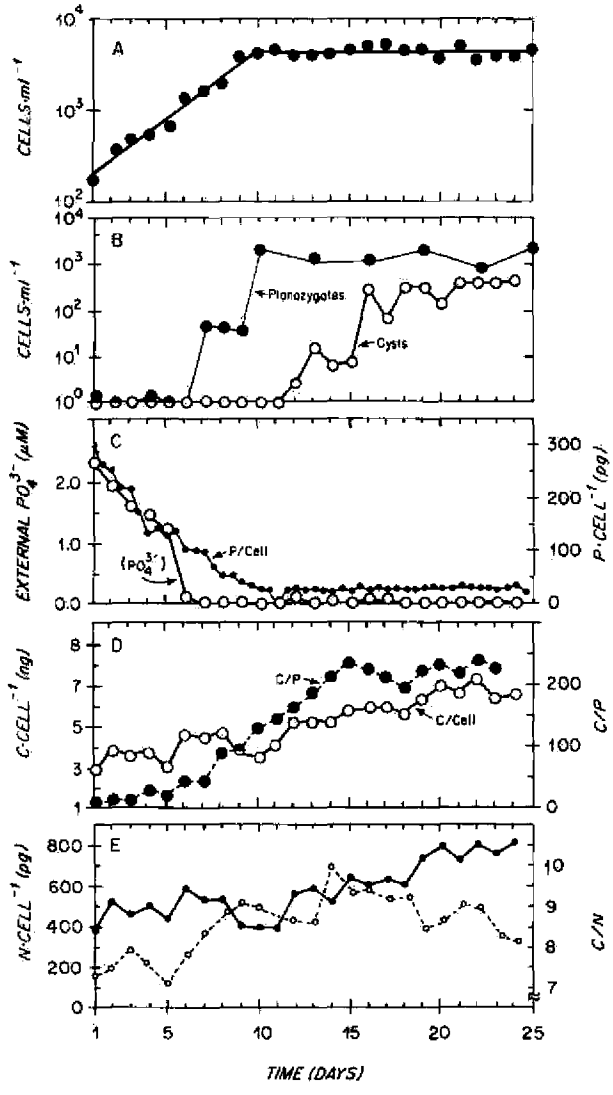

FIG. 3A–E. Time course of *G. uncatenum* encystment under PO_4^{3-}-limitation. A) Motile cell concentration (●) and log-linear and linear regressions. B) Planozygote (●) and cyst (○) concentrations. C) PO_4^{3-} in the culture medium (○) and measured P/cell (●); D) Carbon·cell^{-1} and C/P ratio (by weight); E) Measured N·cell (●) and C/N ratio (○).

and nutrient effects on dinoflagellate cyst yield (Watanabe et al. 1982, Anderson et al. 1984) are in general agreement with our *G. uncatenum* data. In both *Scrippsiella trochoidea* and *Gonyaulax tamarensis* cultures, nitrogen or phosphorus limitation produced large numbers of cysts, whereas nutrient-replete cultures exposed to non-optimal light or temperature produced few, if any. Temperature only altered cyst yield if nutrients were in short supply, as was observed with *G. uncatenum* in this study. *Gonyaulax tamarensis* had a temperature threshold for cyst formation at 12° C, five degrees warmer than the lowest temperature permitting growth (Anderson et al. 1984), whereas *S. trochoidea* produced cysts at all temperatures where growth was possible (only 5 were tested, Watanabe et al. 1982).

Nutrient Dynamics

Gymnodinium uncatenum responded to a decrease in nutrients by encysting, but the details of that transformation are not easily resolved even with daily or twice daily time-course measurements. This stems from the rapid changes in intracellular and extracellular nutrient pools in batch culture and from the difficulty in establishing the onset of gametogenesis. Our observations of fusing cells suggest that there are no obvious morphological features that distinguish *G. uncatenum* gametes from other cells in batch cultures (Coats et al. 1984). We thus relied on the protargol silver stain technique to accentuate flagella and basal bodies and to provide a clear indicator of successful gamete fusion at all stages of development. It was then possible to use the rapid increase in planozygotes as a marker for the onset of sexuality. The utility of this approach depends in part on the duration of the interval between gamete formation and fusion. Fortunately, our experience with *G. uncatenum* indicates that this process can be very rapid (a matter of hours, Coats et al. 1984), so we have good confidence that the surge in planozygote production we observed was immediately preceded by gametogenesis.

Sexuality commenced just before the population maximum was reached in both N and P-limited cultures (Figs. 2, 3). Under phosphorus limitation, the first planozygotes were seen when cellular phosphorus was still a factor of four above the subsistence level of of 29 pg·cell^{-1} (Fig. 3C), implying that sexuality was induced well before actual nutrient starvation. This was not the case in the nitrogen experiment where planozygotes first appeared as cellular nitrogen reached subsistence levels of 250 pg·cell^{-1} (Fig. 2C). Closer examination of the phosphorus data reveals that the early increase in planozygote abundance was relatively small in magnitude (30–50 mL^{-1}) on Days 6–9 when motile cell concentrations were nearly two orders of magnitude higher (Fig. 3B). The major increase in planozygote abundance to 2100 mL^{-1} occurred on Day 10, just as cellular phosphorus reached the subsistence quota. We acknowledge that sexuality may have occurred to some extent before nutrients were completely exhausted but emphasize that sexuality became the dominant reproductive mode only when extracellular nutrients were depleted and intracellular pools of the limiting nutrient had either reached, or were close to, subsistence levels. Since extracellular PO_4^{3-} was undetectable for four days prior to the large surge in sexual activity, it seems clear that sexual induction was coupled to the depletion of intracellular rather than extracellular nutrient supplies.

The importance of the limiting nutrient is emphasized by comparing nitrogen cell quotas on the day planozygotes appeared under either phosphorus or nitrogen limitation. Since these values are 388

and 208 pg N·cell^{-1} respectively, it is evident that the depletion of cellular phosphorus triggered sexual induction when nitrogen was in excess and that the observed shift in reproductive mode was not due to an indirect effect on nitrogen uptake.

In both time-course experiments, the ratio of carbon to the limiting nutrient increased through time from initial nutrient-replete values (7 and 15 for C/N and C/P by weight respectively) to higher ratios denoting severe nutrient limitation several weeks later (C/N = 22; C/P = 235). Planozygotes appeared at intermediate levels (C/N = 14; C/P = 110) that are also indicative of nutrient limitation.

The calculated nutrient uptake rates in Table 1 provide an interesting insight into the relationship between external nutrients and uptake for *G. uncatenum*. In the NO_3^- time-course, growth rate and uptake were well-balanced until the extracellular NO_3^- concentration dropped from 6.5 μM to undetectable in one day, after which the cells rapidly depleted their internal pools of nitrogen and formed gametes. In the PO_4^{3-} experiment, however, specific uptake rates were always less than the growth rate, resulting in a steadily decreasing P cell quota throughout the experiment. Thus phosphorus could not be taken up as fast as it was being utilized for growth at 20° C with 1–2 μM PO_4^{3-} concentrations. This imbalance would vary with temperature and other environmental conditions, but it does suggest that *G. uncatenum* encystment in natural waters may occur before nutrient concentrations drop to extremely low or undetectable levels.

Planozygote Development

The use of the protargol technique allows us to unequivocally distinguish planozygotes from vegetative cells. We can thus say with certainty that the high planozygote abundance at the end of our experiments (60 and 85% of all motile cells in the N and P-limited time-courses respectively) indicates that many gametes fused but were unable to complete encystment. A related observation is that the increases in planozygotes and cysts occurred 5–7 days apart—a much longer interval than the 2–3 day period observed in a natural population of the same species (Coats et al. 1984).

Several factors may have inhibited cyst formation after sexual induction, and all are associated with batch culture conditions. For example, pH, excretion products or even the absence of nutrients may have prevented encystment. Nothing is known of the nutritional requirements of planozygotes, but presumably the demands of prolonged dormancy require substantial reserves. A planozygote would have two sources of nutrients—one supplied by its gametes and the other through normal uptake of extracellular nitrogen or phosphorus. Since the depletion of extracellular nutrients in our cultures occurred very rapidly, the nitrogen or phosphorus content of individual gametes (and thus the planozygotes) probably varied considerably within the population. This is consistent with the wide variation in cell size that we observed at that stage in the cultures. Perhaps the only planozygotes that formed cysts were those that either had sufficient reserves following gamete fusion or that alternatively were able to slowly augment their nutrient pools using excreted or regenerated nutrients.

Although admittedly speculative, the hypothesized nutritional requirement for planozygotes is intuitively appealing. It is consistent with recent observations that *G. tamarensis* planozygotes fix carbon at a rate approximating that of normal vegetative cells (Anderson, unpublished data). It may also help to explain the relatively long 5–7 day interval between the increase in planozygotes and the subsequent increase in cyst abundance in our culture. In another study, a natural bloom sample of *G. uncatenum* was placed in a shipboard container and essentially the entire population encysted in less than 4 days (Coats et al. 1984). That population was confined to a small volume with no external nutrient input, but the unavoidable presence of other autotrophs and heterotrophs in the field population probably duplicated the continuous nutrient recycling that occurs in natural waters. The shorter duration of the planozygote stage in that population may reflect the dynamic nature of natural nutrient regeneration.

One implication of the residual planozygote population in our time-course experiments is that batch cultures may seriously underestimate the extent of sexual induction and pairing if total cyst yield is used as a measure (e.g., Watanabe et al. 1982, Anderson et al. 1984, Anderson and Lindquist 1985). More importantly, as we endeavor to understand the factors regulating sexual induction in dinoflagellates, we must now recognize that the number of cysts produced by a population may be significantly reduced by environmental factors acting on the cells after induction and fusion.

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STUDIES IN PENNATE DIATOMS: VALVE MORPHOLOGIES OF *LICMOPHORA* AND *CAMPYLOSTYLUS*[1]

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ABSTRACT

Using scanning electron and light microscopy, several hundred specimens of Campylostylus normanianus *(Grev.) Gerloff,* Licmophora abbreviata *Agardh,* L. gracilis *var.* anglica *(Kütz) Per. et Per., and* L. flabellata *(Carm.) Agardh were examined to elucidate their valve morphology. These species were found to be heterovalvar with respect to the presence of the labiate process in the basal apices of the cell, although one was always present at the head pole of each valve. This form of heterovalvy may be one basis for partitioning the large and variable family Diatomaceae. Because of the similar valve morphologies exhibited by* C. normanianus *and* Licmophora *species examined, it is recommended that* Campylostylus normanianus *be placed in the older genus* Licmophora, *as* L. normaniana *(Grev.) Wahrer.*

Key index words: araphid diatoms; Diatomaceae; Campylostylus; Licmophora; *scanning electron microscopy; heterovalvy; labiate process; diatom valve morphology*

The Diatomaceae is a large group of pennate diatoms comprising over 35 genera found in fresh, brackish, and even marine habitats. Many form ribbon or stellate and zig-zag colonies. They are characterized by a pronounced axial area and transapical striae, although there is much variation in fine structure. The position of the labiate process is quite variable between, and even within, genera, as they are now circumscribed (Simonsen 1979). The position and orientation of the labiate process can be used as a reliable diagnostic character as in many centric genera.

Campylostylus normanianus (Grev.) Gerloff is a distinctive species in the family Diatomaceae with greatly elongated arcuate cells and heteropolar valves. It has been found in stellate and fan-shaped colonies (Ehrlich 1975, Gerloff et al. 1978) in brackish waters. Van Heurck (1896) reported that Shadbolt found it on mahogany logs from Honduras. This species has also been collected recently in the inland United States from southeastern New Mexico (Rushforth and Johansen 1979), from a saline lagoon in the Northern Sinai, and from the Dead Sea

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