DIEL VERTICAL MIGRATION BY DINOFLAGELLATES: ROLES OF CARBOHYDRATE METABOLISM AND BEHAVIORAL FLEXIBILITY

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ABSTRACT

Some dinoflagellates are capable of sustained directed swimming, traversing about 10 m in the course of diel vertical migration. In coastal regions of the sea with stratified water columns, this behavior probably leads to enhanced growth. During the day, migrating dinoflagellates exploit high levels of irradiance near the surface, where low concentrations of inorganic nitrogen would normally limit growth; at night they descend to a layer where readily available nitrate is utilized to fulfill metabolic requirements. This process involves physiological as well as behavioral adaptations, because the uptake and assimilation of nitrate represents an unusually large metabolic demand during the dark period. An adaptation to diel irradiance, generally distributed among plants, is apparently capable of managing the special requirements of some migrating dinoflagellates: photosynthate is stored as carbohydrate during the day to be utilized at night to support the uptake and reduction of nitrate. A simple biochemical mechanism of source-sink regulation may have a central role in allocating photosynthate such that supply and demand are balanced over a diel cycle. Behavior of vertical migrators is flexible and tied to physiology so that behavior acts to regulate metabolism. The net result is optimization of growth rate within the constraints of an ecological strategy.

INTRODUCTION

In the context of migration, let us consider the dinoflagellates: the nature of their migration, the adaptive significance, and the physiological and behavioral mechanisms involved. Dinoflagellates are unicellular planktonic organisms capable of directed swimming by means of two flagella. A dinoflagellate, 50 μm in length, can sustain swimming velocities of 1 $m \cdot h^{-1}$ or more (e.g., Eppley, Holm-Hansen and Strickland 1968). In several dinoflagellate species, this swimming is directed into diel vertical migration (DVM), whereby a population will move vertically, phased to the diel cycle of solar irradiance (Forward 1976, Weiler and Karl 1979). This behavior commonly entails aggregation near the surface during the day and descent at night, with a vertical range on the order of 10 m. Vertically migrating populations of

dinoflagellates sometimes form red tides, the dense aggregations of algae that discolor coastal waters (Holmes, Williams and Eppley 1967). Although dinoflagelates undertake DVM in both the sea and freshwater under similar constraints, the work herein will mainly be concerned with events in coastal marine environments.

Several approaches have been employed to study the DVM of dinoflagellates. Observational studies (e.g., Hasle 1950, 1954 and references therein; Eppley et al. 1968; Blasco 1978) have provided the data from which hypotheses have been developed. As a result much has been learned of how the rates and timing of vertical migration are affected by environmental influences (cf. Eppley et al. 1968, Kamykowski and Zentara 1977, Heaney and Eppley 1981) and also how water movements such as internal waves or two-layer flows interact with vertical migration to promote the development of red tides or other ecologically important aggregations of dinoflagellates (Seliger, Carpenter, Loftus, Biggley and McElroy 1970; Kamykowski 1974; Seliger 1981; Brink, Jones, Van Leer, Mooers, Stuart, Stevenson, Dugdale and Heburn 1981).

Dinoflagellates are known to thrive where vertical gradients of light, temperature and nutrients are pronounced (Holmes et al. 1967, Margalef 1978). As a result, studies conducted in the laboratory have considered the biological responses of dinoflagellates to time-varying external conditions as determined by the migration of phytoplankters through vertical gradients. These studies have involved examining the physiology or behavior of dinoflagellates in response to light, temperature, salinity, or nutrients, varied individually (Hand, Collard and Davenport 1965; Harrison 1976; Forward 1976 and references therein; Kamykowski 1981a; Harding, Meeson and Tyler 1983). Results of such studies can be crucial in describing ecologically important phenomena. For instance, the avoidance of low salinity water by the red-tide dinoflagellate *Prorocentrum mariae-lebouriae* (Parke and Ballentine) Loeblich leads to confinement of a population in a subsurface layer that is transported from the southern Chesapeake Bay to the northern bay, where a bloom occurs annually (Tyler and Seliger 1978, 1981).

Recent work has demonstrated that in some ecologically relevant situations, the effects on DVM of any one environmental factor cannot be viewed in isolation: for example, several factors can strongly influence DVM simultaneously, as when nitrogenous nutrition substantially modifies the light- and temperature-dependent vertical movements of dinoflagellates grown in stratified water columns (Heaney and Eppley 1981; Cullen and Horrigan 1981). Progress in understanding the DVM of dinoflagellates thus depends on better appreciation of the action of all factors, internal and external, that influence the behavior and growth of migrating phytoplankton (Kamykowski 1979). Ideally, the functional relationships between interacting factors can be,described so the behavior of dinoflagellates can be represented by simple but realistic models.

In this paper, I focus attention on the physiological correlates of DVM and postulate an underlying regulatory mechanism that may operate in a simple fashion to optimize the growth rate of vertically migrating phytoplankton within the constraints of the ecological strategies of different species. The interaction of light and nutrients

in affecting the behavior and growth of migrating phytoplankton can thereby be considered within a conceptual framework that encompasses both the holistic concept of "optimization" and a mechanistic description of biochemical regulation. It is hoped that this proposition will incite a fresh look at the interaction of light and nutrients in the context of migration of phytoplankton in natural waters.

THE ADAPTIVE ADVANTAGES OF DVM

It might be expected that DVM of dinoflagellates confers upon them some benefit in terms of net growth rate in the natural environment, this benefit being the "adaptive advantage" of DVM. The potential adaptive advantages of DVM can be divided into those that enhance the specific growth rate of the organisms and those that reduce losses due to predation, sinking or physical transport to regions unfavorable for growth. Indeed, it has been shown that the DVM of dinoflagellates, as it interacts with hydrographic motions, can produce a concentration mechanism that helps to maintain populations in specific environments (Seliger et al. 1970, Tyler and Seliger 1978, 1981; Kamykowski 1974, 1981b). Also, resistance to predation (Wyatt and Horwood 1973; Fiedler 1982; Huntley 1982) is clearly an adaptation that would enhance persistence of a population. Without detracting from the potential importance of these types of adaptations to the ecological success of dinoflagellates, I would like to focus on physiological mechanisms that might, in conjunction with DVM, enhance specific growth rates of phytoplankton. These mechanisms should be important because regardless of why dinoflagellates migrate, natural selection fosters physiological adaptations that maximize specific growth rates within the constraints of ecological strategies. Further, I choose to concentrate on the migration of dinoflagellates in stratified coastal waters nearshore, where red tides and other ecologically important aggregations of dinoflagellates can occur (e.g., Holmes et al. 1967, Tyler and Seliger 1981, Lasker 1975, Huntley 1982). An important characteristic of these coastal systems is a sharp gradient of nitrate concentration in the euphotic zone, within the range of vertical migration (e.g., Cullen, Stewart, Renger, Eppley and Winant 1983).

The Physiological Benefit of DVM

Although the potential influence of DVM on the growth of dinoflagellates has been studied, the subject has not been recently reviewed and a strong consensus on the physiological advantages of DVM has yet to be developed. Accordingly, a brief critique is in order.

Gran (1929) recognized that the motility of dinoflagellates enabled them to exploit the dissolved nutrients over a relatively large part of the water column, attaining in the process higher densities than nonmotile diatoms under conditions of stratification and nutrient depletion. Holmes et al. (1967) presented calculations which indicated that the concentrations of particulate nitrogen which were associated with red-tide blooms of dinoflagellates could develop only if dissolved nitrogen were taken

up through much of the water column and concentrated as cellular material by phototactic phytoplankton. A hypothesis was presented to explain how some dinoflagellates attain high concentrations in surface waters even though nitrate is depleted and the water is thus unable to support net growth of phytoplankton. At night, dinoflagellates descend to a layer where readily available nitrate is utilized to fulfill growth requirements: migration to the surface during the day produces visible blooms that are commonly observed (Holmes et al. 1967, Eppley et al. 1968). The optimization of growth rate is illustrated by considering the limitations which affect a nonmotile phytoplankter. In the surface layer, nutrients limit growth; if the organism grows where nutrients are adequate, light limits growth (Dugdale 1967). Nocturnal uptake of limiting nutrient is thus hypothesized as an important adaptive advantage of DVM (Eppley and Harrison 1975, Harrison 1976).

Tests of the hypothesis that nocturnal uptake of nitrate is important to migrating dinoflagellates have involved demonstration of the physiological capacity to assimilate nitrate in the dark and attempts to quantify the relative importance of light versus dark uptake of nitrate by populations of migrating dinoflagellates. Given the complexity of interacting factors that affect migratory phytoplankton in the sea, one would hope that experiments on populations in nature could resolve the validity of the hypothesis. Results of experiments in the field are the subject of some contention, however. Packard and Blasco (1974) and MacIsaac (1978) performed studies on a "natural population dominated by *Gonyaulax polyedra*." Packard and Blasco's measurement of nitrate reductase activity suggested that *Gonyaulax polyedra* (Stein) has a special ability to assimilate nitrate in the dark: little diel variation in nitrate reductase activity was observed. In contrast, similar measurements on diatoms show a pronounced drop in nitrate reductase activity at night (Packard and Blasco 1974). It was also observed that the reduction of nitrite to ammonia could proceed with NADH as a cofactor rather than NADPH, as is common for algae and higher plants. Packard and Blasco pointed out that reduction of nitrate to ammonium was thus not tied to photosynthesis and could proceed at night. MacIsaac (1978) measured rates of nutrient uptake using the stable isotope ^{15}N as a tracer. She found that rates in the dark were clearly insufficient to support active growth of a population. In reference to the hypothesis that dinoflagellates have a special capacity for uptake of nutrients in the dark, she states that her results "do not support this hypothesis, nor does it appear that any special cellular characteristics beyond migration are required to explain occasions of dinoflagellate dominance." Recently, Dortch and Maske (1982) studied a bloom dominated by *Gymnodinium sanguineum (splendens)* K. Hirasaka, a motile, nonthecate dinoflagellate. They measured enzyme activities and rates of nitrate uptake and concluded that rates in the dark were significant and that the development of the population could probably be attributed to vertical migration and nocturnal uptake of nutrients.

The apparent conflict between MacIsaac's findings and those of others can probably be resolved by considering the types of measurement made and the environmental context. First, the coastal upwelling system off Baja California, site of the studies by Packard, Blasco, and MacIsaac (cf. Walsh, Kelley, Whitledge,

MacIsaac and Huntsman 1974), was much more energetic and less vertically stratified than the red-tide environments described by Holmes et al. (1967) and thus may represent a different but no less interesting situation in which dinoflagellates can out-compete diatoms (cf. Blasco 1977). It should be noted in this regard that despite the characterization of the population as dominated by *Gonyaulax*, the organism constituted, on the average, much less than half the biomass of the phytoplankton, not the type of domination characteristic of red tides (Blasco 1977). Second, field measurements of nitrogen uptake utilized relatively large inoculations of tracer (10 μg-at N·l^{-1}) so that in situ rates of uptake were not directly measured. Because a primary reason for the importance of nocturnal nutrition is the absence of external nutrients in surface waters during the day, in situ rates rather than maximal rates, are preferable data. The hypothesis concerning the relative importance of nocturnal nitrate uptake was therefore not tested in an entirely appropriate fashion.

Unlike experiments on natural populations, laboratory studies on cultures of dinoflagellates have produced clear-cut results. The red-tide dinoflagellate *Gonyaulax polyedra*, grown in a light-dark cycle, could indeed satisfy its requirements for growth with nitrate taken up during the dark period (Harrison 1976). Experiments performed on cultures grown in 2-m water columns confirm that migrating populations of dinoflagellates can grow on inorganic nitrogen obtained during nocturnal descent. Evidence included disappearance of nitrate from the medium during the dark period (Cullen and Horrigan 1981) and a rapid increase of cellular N and subsequent continuation of growth after nocturnal exposure to nitrate (Heaney and Eppley 1981).

It should be noted that the perceived ability of an alga to take up and assimilate nitrate nocturnally can be in large part determined by the conditions under which the experiments are performed. For example, the migratory dinoflagellate *Heterocapsa (Cachonina) niei* (Loeblich III) Morill and Loeblich has been characterized as having a limited ability to take up and reduce nitrate at night (Hersey and Swift 1976). Consistent with this pattern, a synchronous culture of *Heterocapsa* exhibited no net synthesis of protein during the dark period of a diel cycle (Loeblich 1977). An experiment performed in our laboratory demonstrates that a modification of culture conditions can elicit a pronounced change in the magnitude of nocturnal nitrate assimilation. This is shown in Figure 1 where a culture of *H. niei*, grown with adequate nitrate in the media, takes up about 60% less nitrate nocturnally as compared to during the day. After a short period of nitrate depletion, the culture readily takes up and reduces added nitrate either during the light or dark period. Vertical migration of *H. niei* into a nutrient depleted surface layer during the day may be analogous to our experimental manipulation in that it may induce sufficient internal nitrogen depletion to stimulate effective nitrate uptake at night (e.g., Harrison 1976). It is interesting that this response is not restricted to vertical migrators. Our results for a similar experiment on the diatom *Skeletonema costatum* (Greve.) Cleve were nearly identical (results not shown), and both sets of results were generally consistent with the observed responses of other types of microalgae such as *Chlamydomonas* and *Dunaliella* (Syrett 1981).
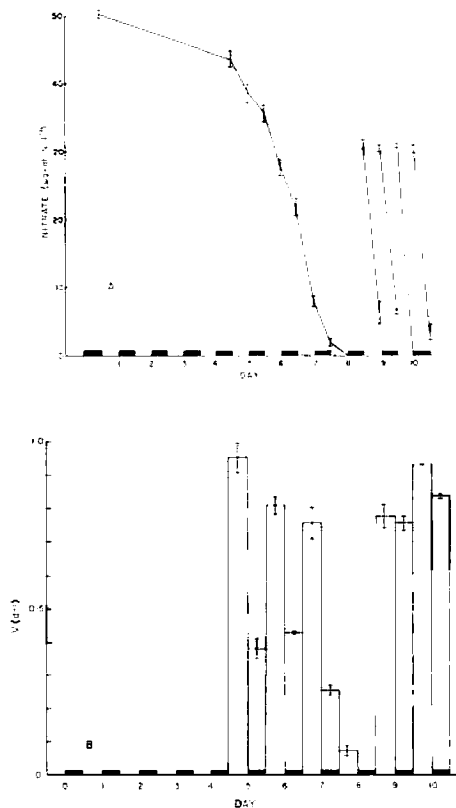
Figure 1

Diel pattern of nitrate uptake as affected by nitrogen depletion: *Heterocapsa niei*. Two cultures were grown at 20°C on a 12:12 light-dark cycle with photon flux density of 125 $\mu E \cdot m^{-2} \cdot s^{-1}$. All nutrients were present in excess except nitrate, which was initially 50 μg−at $N \cdot l^{-1}$.

A. Pattern of nitrate depletion: nitrate was depleted by day 8 and remained undetectable thereafter. On days 9 and 10, at the beginning of the light and dark periods, subsamples from the nitrate depleted cultures were inoculated with nitrate at 30 μg−at $N \cdot l^{-1}$. Concentrations at the beginning and end of each 12 h incubation period are plotted.

B. Rate of uptake: the specific rate of uptake of nitrate (v: μg-at nitrate-N taken up per μg-at cellular N per day) was calculated using an exponential model, $N_t = N_0 e^{vt}$ where N is cellular nitrogen (sum of the initial inoculum and the cumulative nitrate-N taken up), and t is 0.5 days. The dimension of v is d^{-1}. Measurements of intracellular nitrate + nitrite indicated that concentrations were sufficiently low to be ignored in these calculations. Error bars connect determinations for the two independent cultures, grown in parallel. Unpublished data of J.J. Cullen and Zhu Mingyuan.

Due to the severe limitations on experimentation on migrating populations at sea (Dortch and Maske 1982), an accurate description of the nutritional patterns of natural populations of migrating dinoflagellates will be difficult to obtain. Meanwhile, the hypothesis relating the success of some migrating dinoflagellates to the nocturnal uptake of nutrients should be retained.

PHYSIOLOGICAL CORRELATES OF DVM

As described above, diel vertical migration exposes the cell to an unusual type of environmental variability: a large diel variation of external nutrient concentration with a maximum at night. Such variation is exceptional if not unique with respect to phytoplankton. The diel pattern of nutrition, with nitrate uptake predominantly in the dark, is equally unusual. From an evolutionary perspective, one might suggest the DVM has been selected for in these dinoflagellates because they can take up nitrate in the dark. Considering experimental results demonstrating the inducibility of nocturnal nitrate uptake (Fig 1 and Syrett 1981), one could propose the alternate hypothesis that the nocturnal uptake of nitrate by dinoflagellates can be attributed primarily to their ability to migrate vertically. These hypotheses can be addressed by identifying the physiological correlates of DVM and considering the degree to which observed patterns represent specialized physiological adaptations to DVM rather than normal responses to an unusual regime of environmental variability imposed by DVM. An examination of the responses of plants to a diel-varying environment is illuminating in this context.

Unicellular algae and higher plants have adapted to spending about half their lives in the dark by maintaining metabolic processes in the absence of light at the expense of photosynthetic products stored during the day. A manifestation of this adaptation is a diel cycle in the concentration of starch or some other carbohydrate (Preiss and Levi 1979; Cook 1966; Eppley, Holmes and Paasche 1967; Loeblich 1977; Foy and Smith 1980; Fig 2). The regulation of starch synthesis is thus a subject of special interest because it is clear that regulatory processes are effective in matching the diurnal accumulation of starch to the nocturnal demand. Geiger and Giaquinta (1982) and Challa (1976) have shown that in the leaves of some higher plants the rate of accumulation of carbohydrate is inversely related to the length of the photosynthetic period such that relatively greater quantities of carbohydrate are available for longer nights. Experiments conducted on blue-green algae yield similar results (Foy and Smith 1980). These observations support a hypothesis that the accumulation of carbohydrate results from a "programmed synthesis that is influenced by the energy demand of the diurnal dark period" (Chatteron and Silvius 1979). This hypothesis is relevant to the study of dinoflagllate migration because assimilation of nitrate represents a large demand for metabolites (reductant, carbon skeletons and ATP; Syrett 1981, p. 201): according to the hypothesis, the diurnal accumulation of carbohydrate should be dependent to some extent on the amount of nitrate assimilated at night; in turn, nocturnal nitrate assimilation depends on storage products such as carbohydrate.

It is instructive to think in terms of a source-sink balance, well recognized in the plant literature but incompletely understood (Wareing and Patrick 1975). The source is photosynthesis and the sink, broadly speaking, is metabolism and growth. When considering the leaves of higher plants, a major sink is sucrose for export through the phloem (Geiger and Giaquinta 1982), whereas in unicellular algae, the synthesis of protein is typically the important end product (Myers 1951).
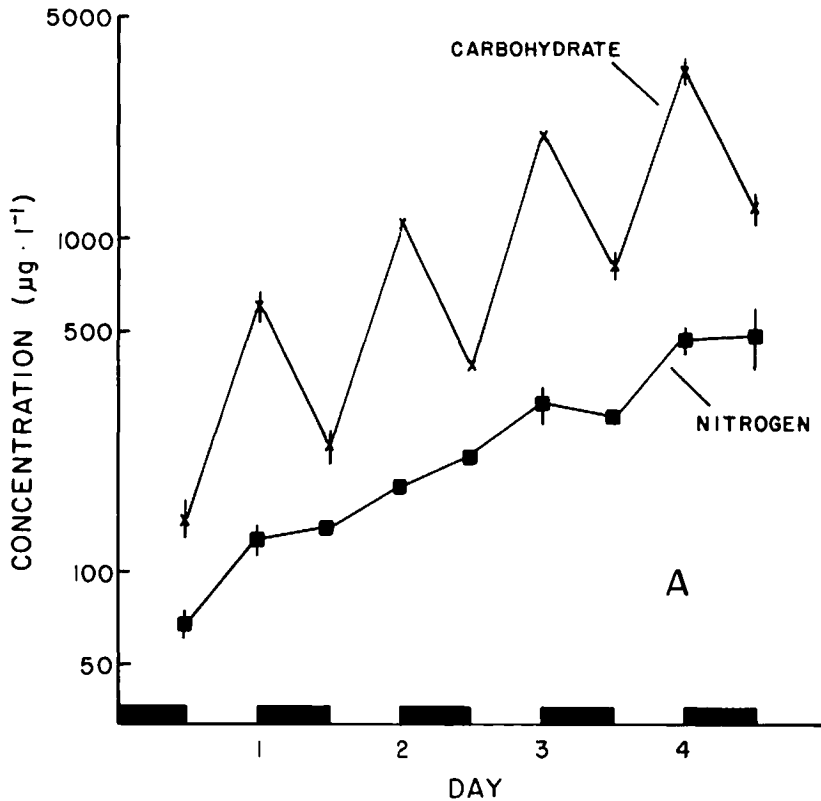
Figure 2
   Diel variation in carbohydrate content of plants grown on light : dark cycles. *Heterocapsa (Cachonina) niei*, a dinoflagellate which is known to migrate but is considered to have a limited ability to assimilate nitrate at night (Hersey and Swift 1976, Loeblich 1977): ×, carbohydrate (dextrose equivalents, phenol-sulphuric acid method); filled boxes, particulate nitrogen (micro-Kjeldahl method). Logarithmic transform: error bars represent the 95% confidence limits for carbohydrate, and the range of duplicates for nitrogen (when the range exceeds the dimension of the symbol). Culture conditions as in Fig 1, except nitrate was present in excess. Unpublished data of Zhu Mingyuan and J.J. Cullen.

Empirical evidence consistent with source-sink regulation in the allocation of photosynthate is plentiful. The C/N ratio in algae is responsive to the ratio of source (photosynthesis) to sink (nitrogen assimilation and concurrent protein synthesis) in a pattern that of course reflects the relative rates of carbon assimilation and nitrogen assimilation but which also facilitates restoration of a typical C/N balance upon return to optimal conditions after a period of deprivation (cf. Myers 1951; Eppley and Renger 1974; Lehman and Wober 1976; Morris 1981; Syrett 1981; Rivkin, Voytek and Seliger 1982). The plasticity of C/N is attributable in large part to accumulation and mobilization of carbohydrate, although Fogg (1959) implicates lipid. Carbohydrate is accumulated in response to nutrient depletion or to increments of supersaturating light intensity (source exceeds sink), and it is degraded in darkness, in the absence of CO_2, or when adequate nutrients are restored after nitrogen depletion (sink exceeds source). Similarly, in the leaves of higher plants, starch levels also respond to

experimentally imposed changes in the source-sink balance. After other leaves in the plant are shaded to increase demand for sucrose, starch levels in the lighted leaf decline in a reversible response (Thorne and Koller 1974), whereas when a leaf is removed to minimize sinks for photosynthesis, starch is accumulated in the leaf at a greater rate than when attached (Heldt, Chon, Maronde, Herold, Stankovic, Walker, Kraminer, Kirk and Heber 1977).

Biochemical studies of the regulation of starch formation are consistent with the notion that the accumulation of carbohydrate is governed by a source-sink balance. Central to the regulatory scheme is the ratio of 3-phosphoglycerate (PGA) and other photosynthetic intermediates to inorganic phosphate (P_i) in the chloroplast (Preiss and Levi 1979, Heldt et al. 1977). If the utilization of photosynthetic intermediates for metabolic processes is less than the production by photosynthesis, PGA accumulates and the production and storage of starch is stimulated; if the demand for intermediates exceeds the supply, starch will be degraded and utilized. As far as the primary products of photosynthesis are concerned, drains to sucrose or protein synthesis may act equivalently, given that an efficient shuttle (Heldt and Rapley 1970; Kelley, Latzko and Gibbs 1976; Heldt et al. 1977) promotes transfer of PGA, triosephosphates and P_i across the chloroplast envelope, thus assuring unobstructed communication between source and either sink (cf. Bassham and Buchanan 1982). It is thus reasonable to assume as a working hypothesis that source-sink regulation of carbohydrate metabolism, essentially as described for higher plants (and cyanobacteria: cf. Lehman and Wober 1976, Foy and Smith 1980), applies to microalgae in general.

Specifically, I would like to suggest that this nonspecialized biochemical mechanism is sufficiently flexible to support the physiological correlates of DVM, enhanced storage of carbohydrate during the day and significant uptake and assimilation of nitrate at night at the expense of carbohydrate. This mechanism of source-sink regulation bridges the temporal gap between dark and light metabolism such that photosynthesis during the day can affect nitrate assimilation at night and nocturnal nutrition can determine the allocation of photosynthate during the day. According to the source-sink hypothesis, when migration through a subsurface nutricline forces a diel cycle on the availability of nitrate, nitrogen deficiency during the day should stimulate the accumulation of carbohydrate (Mykelstad 1974; Haug, Myklestad and Sakshaug 1979), concurrently enhancing the capacity for sustained uptake and reduction of nitrate in the dark. If inorganic nitrogen is depleted throughout the water column, accumulated carbohydrate is not mobilized at night and the carbohydrate content and C/N of the population will rise dramatically in a well-recognized response to depletion of nitrogen (Strickland, Holm-Hansen, Eppley and Linn 1969; Heaney and Eppley 1981; Cullen and Horrigan 1981). As discussed later, there are behavioral as well as physiological responses to sustained nutrient limitation.

Few data are available to examine directly the regulation of metabolism during DVM. However, some preliminary observations (J.J. Cullen and Zhu Mingyuan, unpublished) are consistent with the principles of source-sink regulation as outlined above. A culture of *Heterocapsa niei*, grown at 20°C, L:D 12:12, with nitrate as a nitrogen source (Fig 3A), displays a regular diel pattern of carbohydrate : nitrogen

content indicative of balanced growth over 24 h, which was accomplished by matching the diurnal accumulation of carbohydrate to the nocturnal demands. Immediately after nitrogen is depleted from the media, relative synthesis of carbohydrate during the day is enhanced (as might be expected, given that N is unavailable) and nocturnal utilization of carbohydrate is reduced, due presumably to the restriction of catabolic activity by N-depletion. The net result is a rapid, substantial increase in carbohydrate : nitrogen comparable to that observed in numerous studies on the effects of nitrogen depletion (e.g., Haug et al. 1979). Exposure of subsamples from the nitrogen-depleted culture to nitrate at 30 μg-at·l^{-1} resulted in vigorous uptake and reduction (Fig 1) and concomitant changes in carbohydrate metabolism (Fig 3B and C). Net synthesis of carbohydrate was reduced if nitrate was added at the beginning of the light period and consumption of carbohydrate in the dark was greatly enhanced by the addition of nitrate at the beginning of the dark period.

By comparing the magnitude of nocturnal nitrate uptake with the amount of excess carbohydrate utilization associated with the uptake (Fig 3B and C), a rough stoichiometry can be calculated (Table 1): for each nitrate ion taken up and reduced, about 6 CH_2O units of starch are utilized, theoretically enough to supply the necessary electrons, ATP, and carbon skeletons for assimilation of nitrate to cell proteins (cf. Syrett 1955, Raven and Beardall 1981). Some aspects of source-sink regulation are thus illustrated. The presence of a nitrogen source during the day affects the degree to which carbohydrate accumulates, and the nocturnal utilization of carbohydrate is to a large extent dependent on the uptake of nitrate. These data are not sufficient to demonstrate that enhanced diurnal accumulation of carbohydrate associated with nitrogen depletion is necessary to fuel nocturnal nitrate assimilation associated with vertical migration through a nitrate gradient. Experiments to test this hypothesis are underway.

It is noteworthy that the behavioral patterns of some vertical migrators seem to be closely linked to physiological responses to nutrition. Laboratory experiments have shown that when nutrients are replete throughout the water column, vertical migration is such that the maximum time is spent near the surface, within the constraints of completing nocturnal descent. When nitrate is depleted in the surface layer, behavior is modified to provide more exposure to nutrients at depth while still maintaining a high rate of photosynthesis during the day. *Gonyaulax polyedra* increases its exposure to nutrients by beginning its nocturnal descent early (Heaney and Eppley 1981), whereas *Gymnodinium sanguineum (splendens)* restricts its diurnal ascent to a subsurface layer, often close to the nitracline, in which light intensity is approximately saturating for photosynthesis (Cullen and Horrigan 1981). When nitrogenous nutrients are not available within the range of vertical migration, the pattern of migration in *Gonyaulax polyedra* is modified further such that it no longer migrates to the surface during the day. These behavioral changes occur when excess carbohydrate accumulation as depicted in Figure 3A would be expected. It is possible then, that changes in behavioral patterns are directly associated with changes in chemical composition or physiological state. Better studied are the responses of cyanobacteria
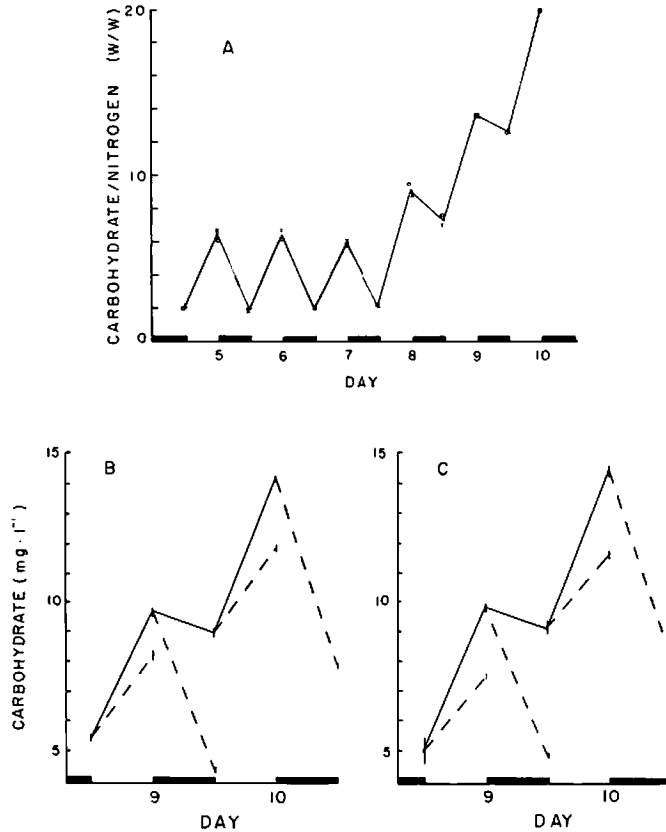
Figure 3

Diel variation of carbohydrate content of *Heterocapsa niei* as affected by nitrogenous nutrition. Same experiment as described in Fig 1.

A. Carbohydrate/nitrogen (ratio by weight): o and x are independent replicate cultures. Carbohydrate measured by the phenol-sulphuric acid method. Nitrogen, as nitrate, was depleted from the media on day 8. Cellular nitrogen was determined from the disappearance of nitrate, by difference.

B,C. Carbohydrate concentration after nitrogen depletion on independent replicate cultures. Solid line represents nitrogen-depleted culture as in A; dashed line represents subsample exposed to 30 μg-at·l^{-1} nitrate. Vertical bars indicate standard error of the mean. Unpublished data of J.J. Cullen and Zhu Mingyuan.

(see Klemer 1985 for a detailed discussion). When excessive accumulation of carbohydrate is stimulated, downward movement results (Oliver, Utkilen and Walsby 1983; Klemer 1985), which decreases irradiance and increases the likelihood of encountering nutrients. When conditions encourage the depletion of stored carbon (adequate nutrients, subsaturating irradiance), upward movement is observed. The response of some cyanobacteria to CO_2 depletion might represent an unfortunate consequence of source-sink regulation. Accumulation at the surface in nuisance blooms occurs, perhaps because the biochemical manifestation of CO_2 limitation is similar to that of insufficient light, in that the supply of photosynthetic intermediates does not meet the metabolic demand.

Table 1

Utilization of carbohydrate during the dark period in the presence
and absence of nitrate. The experiment was performed on nitrogen-
depleted cultures of *Heterocapsa niei*, day 9 (see Figs 1,3). Pairs of
numbers are determinations for independent replicate cultures.

		1200 h	2400 h	Decrease
CONTROL	Carbohydrate ($\mu g \cdot l^{-1}$)	9760, 9908	9024, 9166	736, 732
	Nitrate (μg-at $N \cdot l^{-1}$)	0, 0	0, 0	0, 0
EXPERIMENTAL	Carbohydrate ($\mu g \cdot l^{-1}$)	9760, 9908	4417, 4868	5343, 5040
	Nitrate (μg-at $N \cdot l^{-1}$)	30.61, 30.07	6.13, 6.50	24.48, 23.57

DEFINITION OF "EXCESS" CARBOHYDRATE UTILIZATION:
(decrease in experimental) − (decrease in control) = 4607, 4298 $\mu g \cdot l^{-1}$

MOLAR RATIO OF EXCESS CARBOHYDRATE UTILIZED TO
NITRATE TAKEN UP: 6.27, 6.08

The intraspecific patterns in migratory behavior can thus be linked to relative fluxes of light and nutrients. How can interspecific patterns be explained? One possibility is that the thresholds for behavioral response to source-sink imbalance differ between species according to ecological strategy. (1) On one extreme might be *Gonyaulax polyedra*, which can tolerate very high irradiance and low nitrate during the day so long as a source of adequate nutrients is encountered at night to support growth and to utilize stores of carbohydrate (e.g., Heaney and Eppley 1981). It appears that photosynthate can be stored to a considerable extent before behavior is substantially affected. (2) *Gymnodinium* may represent an intermediate case. When nutrients are not available in the surface layer, the organism avoids high light intensities (which would stimulate excessive storage of carbohydrate, cf. Cook 1966) and aggregates at a lower light level that is still nearly saturating for photosynthesis (Cullen and Horrigan 1981). Nonetheless, nitrate is taken up to a significant extent at night, so a good deal of source-sink imbalance is accommodated. (3) Phytoplankters that aggregate in well-defined strata but do not undergo significant diel migration (e.g., in some instances, *Ceratium tripos* (O.F. Muller) Nitzsch; Eppley, Reid, Cullen, Winant and Stewart 1984) would, according to the source-sink hypothesis of behavioral regulation, tolerate relatively little imbalance in the fluxes of photosynthate and nutrients before adjusting vertical position. Diurnal variation of chemical composition would thus be minimal and accordingly, assimilation of nutrients would be in large part light-driven, i.e., nocturnal assimilation of nitrate would be relatively unimportant. Each of these strategies represents an ecological trade-off that would be effective in a restricted range of environmental conditions.

Although one is limited to speculation in discussions of mechanisms by which behavior interacts with physiology to regulate the growth rate of vertically migrating dinoflagellates, it is possible to define clearly the adaptive significance of behavioral flexibility in the natural environment. By linking behavioral plasticity to physiological state, some environmental stresses are avoided. In stratified coastal waters (cf. Eppley and Harrison 1975, Cullen and Eppley 1981) a source of adequate nutrients will be found within the relatively shallow euphotic zone; by shifting migrational patterns downward in response to nutrient depletion, migrating dinoflagellates in coastal waters need not deal with high irradiance *and* nutrient starvation (cf. Prézelin 1982). Likewise, because adequate nitrate is available within the range of migration of populations in the lower euphotic zone, vertically migrating phytoplankton need not adapt to low irradiance without a source of nutrients available (cf. Prézelin and Matlick 1983).

SUMMARY AND CONCLUSIONS

It has been demonstrated that plants have developed simple regulatory mechanisms that tend to optimize the allocation of photosynthate for maximal growth; as a result, enough carbohydrate is synthesized and stored during the day to satisfy nocturnal metabolic needs. In stratified waters characteristic of many coastal environments, vertically migrating phytoplankton stand to benefit greatly from such a strategy. During the day, they can migrate into well-lighted but nutrient-poor strata where the storage of carbohydrate would be stimulated by both high irradiance and low nutrient concentration. At night, they can descend to water rich in nutrients where the processes of nutrient uptake and assimilation depend on storage products. Because dinoflagellates differ in their abilities to take up and assimilate nutrients in the dark (Hersey and Swift 1976) and also differ in their patterns of vertical migration (Eppley et al. 1984 and references therein), it is likely that physiological and behavioral patterns are coordinated, making behavior an important aspect of metabolic regulation. Indeed, available data indicate that nutrient-dependent modification of vertical migration acts to enhance growth of some dinoflagelates; that is, the nutritional status of the phytoplankters profoundly influences their vertical migration such that their overall pattern of vertical displacement supports balanced supply rates of photosynthate and nutrients. Behavioral repertoires differ between species, apparently reflecting ecological trade-offs, but in each case demonstrating that in dinoflagellates migrational patterns are altered to avoid the stresses of limiting nutrients or overly high irradiance.

Note added in proof: Interspecific variation in dark nitrogen uptake by dinoflagellates is discussed in a recent paper by Paasche, Bryceson, and Tangen (Journal of Phycology 20: 394-401, 1984).

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