Observations on the Growth and Motile Behavior of the Colonial Diatom *Bacillaria paradoxa* in Culture.

by

© Margaret R.M. Kapinga

A Thesis Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Botany
University of Manitoba
Winnipeg, Manitoba

ISBN 0-315-51607-0

OBSERVATIONS ON THE GROWTH AND MOTILE BEHAVIOR OF THE COLONIAL DIATOM *Bacillaria Paradoxa* IN CULTURE

BY

MARGARET R.M. KAPINGA

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

We often think that when we have completed our study on one, we know all about two, because "two is one and one". We forget that we have still to make a study of "and". ~ *Sir Arthur Eddington*

ABSTRACT

The colonial motile diatom, *Bacillaria paradoxa* Gmelin, is known to exhibit a remarkable form of gliding motility in which entire colonies rhythmically expand and contract as single units. Cultures of *Bacillaria* grown under an alternating light/dark regime were found to exhibit rhythms in cell motility with the majority of colonies being motile during the light periods and aligned and non-motile during the dark periods. In continuous darkness this rhythm was lost, with the majority of colonies remaining non-motile and aligned. Cultures also showed rhythms in cell division with peaks generally occurring 6 hours after the onset of the dark period. This rhythm persisted for 2 cycles in continuous darkness although at lower amplitudes.

Light intensities providing photon density fluxes greater than 17μmole/m^2/second were found to reduce the percentage of motile cells per colony by 50% after one hour of exposure. At light intensities providing 150μmole/m^2/second colonies became aligned and non-motile within minutes and chloroplasts moved from normally peripheral positions to more central ones. Maximum cell velocities were reached at 25^{o}C. At temperatures near 10^{o}C and above 30^{o}C colonies were found to become reversibly non-motile and aligned.

Examination of cleaned frustules with SEM revealed no silica structures by which cells can attach to one another. It is speculated that the mucilage

excreted through raphes may provide the means for cell attachment and that the silica raphe protrusions of adjacent cells fit closely together preventing this mucilage from rapidly dissolving. It is speculated that the raphe mucilage may have an anomalous viscosity and that this may be responsible for the alignment of non-motile colonies. An evaluation of the motile behavior of *Bacillaria* colonies in terms of recent models of gliding motility in diatoms suggests that the apparent co-ordination between cells within a colony is controlled both by mucilage secretion and microfilament activity.

ACKNOWLEDGEMENTS

In all of life nothing is ever accomplished alone and so too, with the completion of this thesis I find myself reflecting on the different ways in which so many people have helped me and to whom I owe my sincere thanks. Thanks are extended to the Botany Department, including support staff, professors and fellow students for their interest, helpful advice and encouraging words. The time, facility use and expert advice generously given by Hedy Kling of the Freshwater Institute for SEM work is greatly appreciated. Likewise, the donation of cultures and willingness to offer help expressed by David Czarnecki of Loras College is also greatly appreciated. Thanks are extended to each member of my examining committee for their commitment of time and valuable insights regarding this thesis. Among these I especially want to thank Dr. Gordon Robinson for his generous loan of equipment and advice regarding the collection and culturing of *Bacillaria*. The helpfulness and friendship extended to me by all those who have been a part of the wide variety of scientists in the lab of Dr. Richard Gordon has been a great source of encouragement. Sincere thanks is expressed to Richard Gordon for his willingness to be my advisor, his patience in the working out of this project, for his challenge to reach into many new areas of scientific endeavor and for allowing this project to be funded through an NSERC grant. Finally, to my family, I wish to express my deepest thanks for never failing to demonstrate their love and support towards me.

TABLE OF CONTENTS

Page

Page

Results

LIST OF FIGURES

LIST OF TABLES

Page

INTRODUCTION

Diatoms are single-celled algae which produce rigid cell walls composed of silica. The cell wall, or frustule, is constructed of two almost equal halves which fit together like a Petri dish. Each half of the frustule is composed of a valve, which forms the larger outer surface and a girdle, a circular band of silica attached to the edge of the valve. The larger outer half of the frustule is referred to as the epitheca and the smaller inner half as the hypotheca. The frustules are often adorned with a variety of complex ornamentations and perforations, giving diatom cells a remarkable beauty. Some diatoms have been found to exhibit a form of gliding motion. This movement is characterized by an active displacement of the organism without the involvement of a visible structure and without a change in shape of the organism (Jarosch 1962). The mechanism of movement in diatoms is not clearly understood but motility is usually associated with the possession of a raphe, a long narrow slit in the cell wall.

Motile diatoms usually glide as single cells along some type of solid substrate. *Bacillaria paradoxa* Gmelin represents a special case, in that as well as being motile, it is colonial, with the cells in a colony being attached valve to valve and normally gliding only against each other. The only other colonial diatoms that have been found to exhibit gliding motility are tube-dwelling diatoms. These diatoms form extensive colonies enclosed in tubes of mucilage but the cells do not grow attached to one another and as a result move individually within the mucilage. In active *Bacillaria* colonies,

cells glide past each other in such a way that the entire colony moves in a rhythmic and co-ordinated fashion, often appearing to expand and contract as a single unit. This movement is highly regular and can be sustained for many cycles. Many other examples of sustained rhythms of co-ordinated activity have been found in biology, ranging from slime molds (Gerisch and Wick 1975) to heart cells (Ypey *et al.* 1979). If the activity of a population is synchronized, it is generally assumed that some type of synchronizer exists for the system, either externally or intrinsically (Nanjundiah 1986). If synchronization is due to an external stimulus or Zeitgeber, the endogenous rhythm of each oscillator is entrained, resulting in the entire population oscillating in phase and at the same frequency as the Zeitgeber. A common example of a Zeitgeber is the natural alternation of day and night, which can cause the synchronization of physiological rhythms in both plants and animals. Intrinsic synchronizers are not as easily recognized. In some cases, individual cells or units have been found which drive the entire system. For example, certain slime mold amoebae periodically release cyclic AMP which acts as an intercellular signal, inducing neighboring cells to also secrete cyclic AMP (Gerish and Wick 1975). In other instances, synchronization is a result of mutual entrainment. This has been observed in populations of yeast cells (Hess and Boiteaux 1971) and embryonic heart cells (Ypey *et al.* 1979), where if two populations each oscillating in different phases or at different frequencies are brought into contact the combined population will oscillate in phase at the same frequency. A similar phenomenon has been observed among some species of fireflies which produce rhythmic flashes of light (Buck and Buck

1976).

In one complete cycle of movement, cells within a *Bacillaria* colony move from a stationary extended position, past each other to another extended stationary position, reverse direction and then glide back to their original position. Colonies in extended positions remain stationary for approximately 10 seconds. During this time, cells are arranged almost end to end forming a single straight line or psuedofilament. Cells resume gliding almost simultaneously and as they do, colonies appear to contract from both ends with their two halves passing each other as cells continue to glide towards the next extended position. This type of behavior along with the fact that isolated single cells have been shown to oscillate with a frequency similar to that of whole colonies (Drum *et al.* 1971) suggests that the co-ordinated movements of *Bacillaria* colonies may be a result of some type of entraining interaction between cells.

Although the movements of *Bacillaria* colonies have been observed for many years there is limited information available in the literature as to its behavior in culture. It is necessary to have a thorough understanding of this organism before questions regarding the nature of any possible cell-cell interactions can be answered. Therefore, the purpose of this study was to first grow *Bacillaria* in culture and describe its behavior as well as some of the characteristics of its growth. Once this was established, the influence of light and temperature on motility was investigated, paying particular attention to the conditions under which motility no longer occurred and to the conditions which brought about a reduction in the degree of co-ordination between cells.

LITERATURE REVIEW

1. Gliding Motility in Diatoms. Gliding motility occurs in a variety of organisms, including some bacteria, blue-green algae, diatoms, desmids, flagellates, protozoa and certain reproductive cells of red algae and fungi (Jarosch 1962; Doetsch and Hageage 1968; Nultsch 1974; Stebbings and Hyams 1979; Russell and Sinden 1981). This type of movement is distinct from swimming in that no visible organelles are involved, no change in shape of the organism is revealed using light microscopy, and the organism must be in contact with a solid substrate (Jarosch 1962). Gliding rates are generally slower than the speeds shown by swimming cells, with reported rates ranging from 0.03 μm/second to 29 μm/second (Edgar 1979; Castenholz 1982). Most gliding organisms have the ability to suddenly stop moving and reverse direction (Halfen 1979). Reversals often depend on environmental conditions such as temperature and light or result from physical obstructions in the path of the organism (Burkholder 1934; Jarosch 1962).

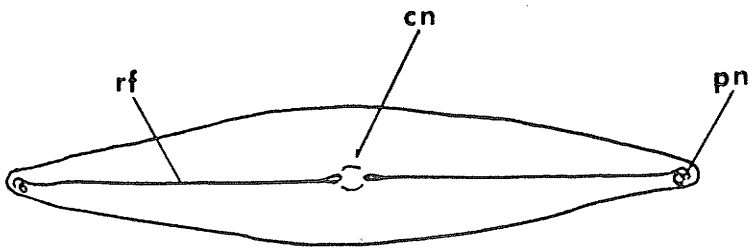
Although gliding motility has been investigated since the mid 1800's, no uniform cellular mechanism has been established for the phenomenon and it has been suggested that the structural basis may be quite diverse and polyphyletic in origin (Castenholz 1982). However, in most cell types studied, gliding movement is associated with the production of mucilage (Nultsch 1974; Mackenzie and Walker 1983; Edgar and Pickett-Heaps 1984). Many other types of cell motility have been attributed to cytoskeletal activities (Allen 1981; Williamson 1981) and recently the involvement in gliding motility of

proteins such as actin has been considered by researchers (Webster, Cooksey and Rubin 1985; Russell and Sinden 1981). Some gliding bacteria have been found to possess numerous small discs on their cell walls which resemble the basal parts of rotary flagella (Pate and Chang 1979) and it has been suggested that gliding in this case may be a result of the spinning of these discs.

Among the pennate diatoms (those with bilateral symmetry in valve view), only those which possess a raphe are capable of exhibiting gliding motility (Patrick and Reimer 1966). Although the structures and positions of raphes are diverse, they all open to both the exterior and interior of the frustule and thus allow the possibility of interaction between cytoplasm and substratum (Edgar and Pickett-Heaps 1984). Raphes can occur in one or both valves and generally run along the longitudinal axis, being divided by a hyaline area, in which may occur a central nodule, and being terminated by polar nodules (helictoglossa). In cross section, the raphe fissure (Figure 1) often appears in the shape of a tongue-and-groove structure (Edgar and Pickett-Heaps 1984). At the central nodule the raphe widens to form two central pores while at the polar nodules, the raphe forms a twisted and curved slit (terminal fissure) on the outer surface of the valve and enlarges into a funnel-shaped structure within the inner surface (Patrick and Reimer 1966). The size of the raphe tends to vary with the size of the diatom but length can range from 10-100 μm and width from 20-250 nm (Drum and Hopkins 1966).

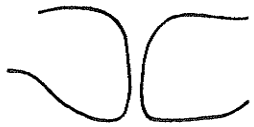
There is no evidence for the occurrence of protoplasm within the raphe (Drum and Pankratz 1964) but strands of mucilage have been observed (Drum

Figure 1. (a) Generalized view of a pennate diatom valve showing raphe fissure (rf), central nodule (cn) and polar nodules (pn). Cross-section of raphe fissure (b) between central and polar nodule, (c) at central nodule, where raphe widens to form a central pore. Not to scale. (From Edgar and Pickett-Heaps 1984.)



1(a)

1(b)



1(c)

and Hopkins 1966; Edgar and Pickett-Heaps 1982; Edgar 1983; Rosowski *et al.* 1983). These strands are approximately 1 μm in length and 96 nm in width. Individual strands are positioned perpendicular to the raphe and project through the raphe along its entire length. The strands are capable of adhering to the substratum by their distal ends (Edgar 1983; Roswoski *et al.* 1983) and provide the cell-substratum adhesion necessary for motility. If adhesion to a solid substrate is prevented in motile diatoms, locomotion is prevented (Drum and Hopkins 1966). However, only a small portion of the raphe actually needs to be in contact with the substratum in order for both adhesion and motility to occur (Drum and Hopkins 1966; Houpt 1980). As diatoms move over the substratum they leave behind deposits of adhesive mucilage, referred to as diatom trails (Drum and Hopkins 1966). This mucilage is highly soluble and quickly disperses but has been observed using small particles as markers (Drum and Hopkins 1966) or special fixation procedures (Edgar and Pickett-Heaps 1982).

It had been believed, until recently, that the only diatoms capable of gliding were pennate forms. However, there have now been reports of movement also occurring among centric diatoms, i.e., those with approximately radial symmetry in valve view (Pickett-Heaps *et al.* 1986; Medlin and Crawford 1986). Centric diatoms have been observed to shuffle back and forth as well as rotate in the plane of the valve while moving forward. This movement is thought to be due to the active secretion of mucilage through the labiate process, a tube-like structure which penetrates the valves of centric diatoms. It has been suggested that the raphe fissure of

pennate diatoms might have evolved from the labiate process (Hasle 1974) and therefore, the discovery of motile centric diatoms suggests that a functional as well as a morphological relationship may exist between the two valve structures (Pickett-Heaps *et al.* 1986).

Many motile pennate diatoms move irregularly back and forth in short jerks and travel in straight paths only for small distances (Nultsch 1974; Edgar and Pickett-Heaps 1984). Often cells move in curved paths and circles. Nultsch (1956) classified diatom movements into three groups based on raphe shapes but no clear correlation between raphe or cell shape and path pattern has actually been established (Edgar and Pickett-Heaps 1984). The velocities of diatoms often depends on the nature of the substratum (Harper and Harper 1967). However, each diatom generally has a characteristic range of speeds at which it moves. Analysis of digitized film (Edgar 1979) has shown that gliding movement in diatoms is characterized by rapidly changing velocities. Large changes in speed were found to occur within tenths of a second, giving accelerations of over 100 $\mu m/s^2$ in some species. Diatom cells were also found to be capable of coming to very rapid halts. Due to their small size, diatoms have a very low Reynolds number in water. This implies that there is little resistance to flow and that their movements are not affected by inertial forces. This accounts, in part, for their ability to change speed so rapidly.

A phenomenon that is often associated with motile diatoms is the movement of particles along the raphe systems. Diatoms moving in media containing visible particles such as organic matter, Indian ink or clay, will

have particles in the vicinity of the raphes move longitudinally along them in both directions (Drum and Hopkins 1966). Particles can travel in opposite directions along the same part of a raphe and can do so at different speeds, although their speed is not necessarily related to their size. The direction of particle movement along raphes not attached to substrata does not appear to have any relation to the direction of cell movement. Particles streaming along the raphe adjacent to the substratum almost always move in a direction opposite to that of the cell. However, commencing either just before of immediately after cell reversal, particles near the raphe at the trailing end of the cell will stream in the same direction as the cell for a short time. As the cell continues in its new direction, the particles again stream in an opposite direction to that of the cell (Drum and Hopkins 1966; Edgar and Pickett-Heaps 1984). The movement of particles along the raphe is believed to be a reflection of the force driving the cell and may provide some some clues as to the mechanics of diatom motility.

A variety of different factors have been found to influence the movements of gliding diatoms. Their behavior in the dark varies, with some diatoms reducing their speed and becoming essentially motionless after 4 to 24 hours of continuous darkness (Hopkins 1963). Others continue to remain active in the dark, utilizing ATP supplied from oxidative phosphorylation (Hopkins 1966; Nultsch 1971). Light usually has a positive photokinetic effect on diatoms, causing movement to be accelerated up to an optimum, beyond which their is no increase in acceleration (Nultsch 1970). The measured photokinetic action spectrum of the diatom *Nitzchia communis* was found to

be similar to the whole cell absorption spectrum, showing maxima between 430 and 440 nm and also at 670 nm (Nultsch 1971). Since the active light appears to be absorbed by the photosynthetic pigments, it has been suggested that photokinesis in diatoms is due to an additional ATP supply from photosynthetic phosphorylation (Nultsch 1970, 1971). Diatoms are also positively phototactic, moving towards light sources with wavelengths less than 550 nm and at intensities of up to 40,000 lux (Nultsch 1971). Above 550 nm phototactic activity is not observed. Some negative reactions have been observed at higher light intensities (Nultsch 1974) but it was found that the sensitivity of diatoms to light can also depend on their physiological state, with phototactic reactions increasing after dark treatments of 6 to 8 hours (Nultsch 1971). The lack of phototactic responses induced by red light suggests that no correlation between photosynthesis and phototaxis exists and that the phototactic and photokinetic responses may not involve the same photoreceptor. The phototactic photoreceptor is most likely a carotenoid and/or a flavoprotein, pigments which are known to absorb light between 360 nm and 500 nm (Nultsch 1971).

Temperature has been shown to affect the speed at which diatoms move (Jarosch 1962; Hopkins 1963). Hopkins (1963) found that diatoms generally move most rapidly between 10°C and 17°C, although movement does occur over the range of 0°C to 30°C. Diatoms immobilized by cold temperatures usually recover their motility when warmed but at temperatures higher than 35°C, immobilization is not always reversible (Drum and Hopkins 1966; Edgar and Pickett-Heaps 1984). Not surprisingly,

adhesion is also affected by temperature. Drum and Hopkins (1966) found that diatoms cooled to -1°C were still capable of adhering to a substrate but that at temperatures above 40°C, detached diatoms were not able to readhere.

Observations on plasmolyzed diatoms have shown that motility continues as long as the protoplast maintains some degree of contact with the valve at the raphe (Nultsch 1962). If, for diatoms moving on a glass substrate, plasmolysis occurs at just one half of the raphe in contact with the substratum, movement continues and will only cease when the protoplast has withdrawn from both of the raphe endings (Drum and Hopkins 1966). Detergents have also been reported to inhibit diatom locomotion while increasing adhesion (Drum and Hopkins 1966; Harper and Harper 1967). At concentrations of 0.0005 %, Teepol was found to cause immotility, while slightly lower concentrations only resulted in irregular movements. Similar results have been found with other detergents but some of these are lethal at the inhibitory concentrations (Edgar and Pickett-Heaps 1984).

A variety of drugs have been tested for their influence on diatom motility. Substances which disperse mucous were generally found to inhibit both adhesion and locomotion (Drum and Hopkins 1966). Actin-based microfilaments which are involved in the generation of movement in other cell types (Wessells *et al.* 1970; Yahara *et al.* 1982) are known to be disrupted by cytochalasins. Several different cytochalasins have been tested for their effect on diatom movement. It was found that cytochalasin B and dihydrocytochalasin B did not significantly inhibit motility (Spangle and Armstrong 1973; Webster *et al.* 1985). However, both cytochalasin D and E

were reported to reversibly inhibit motility, with cytochalasin E also inducing an increase in the amount of mucilage secreted (Webster *et al.* 1985).

Microtubules are also known to generate certain types of movements in cells (Cappuccinelli 1980). Colchicine, podophyllotoxin and vinblastine are all inhibitors of microtubular systems (Manso-Martinez 1982; Webster *et al.* 1985). Tests of these drugs on diatoms have shown that colchicine did not affect motility but did increase the level of mucilage secretion. Both podophyllotoxin and vinblastine were found to reversibly inhibit motility and increase secretion (Webster *et al.* 1985). These results indicate that both actin-based and tubulin-based structures may play roles in the motility of diatoms.

Studies on the marine diatom *Amphora coffeaformis* have shown that it requires extracellular, millimolar levels of calcium for motility (Cooksey and Cooksey 1980). Movement was also found to be inhibited by the drugs D-600 and ruthenium red, both of which inhibit calcium influx but not efflux from cellular compartments. When Sr^{2+} was provided as a substitute for Ca^{2+}, motility was inhibited but the adhesion of cells to glass culture tubes was not affected. This suggests that adhesion and motility may involve separate physiological processes. Other studies have considered the influence of low-level magnetic fields on the movements of diatoms (Smith *et al.* 1987). It was demonstrated that cell movement could be controlled by controlling the movement of calcium ions across cell membranes with the proper combination of DC and AC magnetic flux densities of an applied magnetic field.

Many different theories have been proposed to explain the cellular mechanism for gliding motility in diatoms. Most theories describe the raphe as the site from which the propulsive force acts. Due to the relatively high refractive index of the silica frustule, the protoplasm beneath the raphe is difficult to see in light microscopy. As a result, the theories proposed over the past one and a half centuries have incorporated a wide range of ideas. Many of the early researches attributed diatom motility to cilia or psuedopodia sticking out through the raphe fissure (see Hopkins 1967; Edgar and Pickett-Heaps 1984). However, electron microscope studies have now revealed that no protoplasm or organs of locomotion occur in the raphe of motile diatoms (Drum and Hopkins 1966; Hopkins and Drum 1966). Other early theories which are no longer seriously considered include suggestions that motion is a result of an external undulating membrane (Wenham 1856), a vibrating inner pectin membrane moving streams of water (Leibisch 1929), osmotic currents (Smith 1853; Nägeli 1860, Smith 1888) and the expulsion of gas generated in light (Jackson 1905).

One theory, which was once widely supported, attributes the movement of diatoms to the streaming of protoplasm in the raphe (Schultze 1865; Pfitzer 1871; Englemann 1879; Müller 1889; Palmer 1910; Smith 1938). Observations of particle movement in the vicinity of the raphes is the basis for this theory, since it was believed that invisible streams of protoplasm were responsible for particle movement. Müller (in Edgar and Pickett-Heaps 1984) suggested that the streaming itself was a result of turgor pressure and that the friction generated between protoplasm and substratum was sufficient to move a

diatom. This theory was criticized by Martens (1940) since he could not find a relationship between the direction and velocity of cell movement and the direction and velocity of particle movement. A correlation between particle movement along the raphe adjacent to the substratum and cell movement has since been found and now the major criticism of this theory is the lack of protoplasm occurring in the raphe. Lauterborn (1896) suggested that mucilage was excreted by the diatom and that this circulated in the raphe rather than protoplasm and was thus responsible for cell movement. Müller, however, did not believe that diatoms were capable of producing enough mucilage to account for their observed movements. (Gordon and Drum (1970) estimated that if half the volume of a diatom cell were devoted to raphe fluid it could move continuously for 20 minutes without synthesizing more fluid.) Jarosch (1962) believed that movement was a result of submicroscopic transverse waves occurring in extramembraneous protein fibrils. He suggested that the fibrils were connected through the raphe to the protoplasm and that they either caused the secreted mucilage to shift or caused the cell itself to move over the substratum. However, there is no evidence of protein fibrils occurring in the raphe. Harper and Harper (1967) proposed that diatoms secrete a mucopolysaccharide strand into the raphe. On contact with water, the strand swells into a flexible and sticky form. The strand is held in place against the raphe either by osmotic forces or by the interfacial tension between a liquid in the raphe and water. Continued secretion lengthens the strand and if it contacts a rigid object it adheres, with the pressure from further secretion pushing the diatom long the substratum.

The examination of diatoms with the electron microscope gave researchers new insights into the cellular mechanism for locomotion. In addition to discovering that the raphe contained no protoplasm, ultrastructural studies revealed features within the protoplasm that could be linked to the generation of movement. Drum and Hopkins (1966) examined longitudinal sections of motile diatoms using electron microscopy and found that one to two bundles of longitudinally oriented microfilaments occurred in the protoplasm adjacent to the raphes, paralleling the whole length of the raphe. In addition, cytoplasmic vesicles (crystalloid bodies) containing fibrous strands 0.5 to 1.0 μm in diameter were found adjacent to the raphes. Based on their observations, Drum and Hopkins suggested that external stimuli initiate contraction of the microfilaments near the raphes as well as release of the contents of the crystalloid bodies. The released material is forced out of the cell by the microfilaments and into the raphe where it expands upon hydration and streams until it strikes an object. Adhesion occurs once contact is made. If the object is relatively small, it is transported along the raphe system but if it is large enough it remains in place and acts as a substratum against which the diatom moves.

Gordon and Drum (1970) proposed a theory in which capillary pressure provides the force for diatom movement. They suggested that the fibrillar contents of the crystalloid bodies are released into the raphe, where they undergo hydration. Once the fluid is fully hydrated it no longer wets the raphe walls, but remains attached to the substratum, appearing as trail material. As hydration removes the fluid, more unreacted fluid is drawn

along the raphe from the leading end of the cell by capillary pressure, thus causing the diatom to move forward. The direction of movement can depend on either an asymmetric distribution of release of fluid from the crystalloid bodies along the raphe or on differing rates of hydration along the raphe (Gordon 1987). Based on this theory, Gordon and Drum estimated the maximum force exerted by a moving diatom to be between 1 and 50 millidynes. This is within the range found by Harper and Harper (1967), who measured the force exerted by diatoms against glass fibres.

Further ultrastructural studies have shown that microfilaments occur in two distinct bundles on either side of the internal raphe fissure (Edgar and Pickett-Heaps 1984). There has been some evidence suggesting that these microfilaments may be composed of the protein actin (Edgar and Zavortink 1983; Webster *et al.* 1985) but this has yet to be proven conclusively. The crystalloid bodies are now known to contain fibrillar mucopolysaccharide (Edgar and Pickett-Heaps 1982) and have been found in all motile diatoms studied ultrastructrally, appearing especially abundant near the cell apices. Based on their observations, Edgar and Pickett-Heaps (1984) have proposed a theory of diatom gliding in which the internal microfilament bundles provide the driving force. They suggest that the mucopolysaccharide fibrils are released into the raphe canal where they hydrate and elongate, forming mucilaginous strands which protrude from the raphe fissure. The ends of the mucilage strands inside the raphe remain attached to membrane complexes which project from the plasmalemma and which are formed from the membranes of the crystalloid bodies in which the mucilage was synthesized.

The opposite ends of the mucilage strands outside the raphe adhere to the substratum. Actin filaments move the membrane complexes and their attached mucilage strands along the raphe, displacing the whole cell. The mucilage strands move easily through the raphe due to the frustule being coated with a hydrophobic lipid layer (Edgar and Pickett-Heaps 1983) and are broken free from the plasmalemma when they reach the trailing end of the cell. In order to account for the bidirectional movement of diatoms, Edgar and Pickett-Heaps suggest that the actin filaments in each bundle are oriented in one direction and that the two bundles have opposite polarities. The direction of movement of mucilage strands could then be controlled by the alternate activation of the actin bundles. The major criticism of this model is that the mechanism responsible for movement is internally located, resulting in an inefficient loss of force exerted against the substratum (Harper and Harper 1967; Gordon 1987), though the inefficiency has yet to be estimated.

Gordon (1987) suggested that the microfilaments are only involved in controlling the direction of cell movement and that a pair of oppositely oriented microfilaments slide relative to one another, blocking access of the crystalloid bodies to the pores at the ends of the raphes. Depending on which pores were blocked, this would result in the diatom moving forward, stopping or reversing. Although the exact underlying cellular mechanism for diatom gliding has not been established, it is clear that the production of mucilage and its adhesion to a substratum is necessary for gliding to occur. The location of microfilaments near the raphe indicates that they also have a role to play in motility, either in providing the motive force or in influencing

the direction of movement.

2. Rhythmic Phenomena in Algae. Physiological rhythms have been found to occur in all groups of eukaryotic algae, with many of these rhythms following the natural alternation of light and dark in a 24 hour cycle. Rhythms are considered to be endogenous if the oscillations are found to continue under conditions of constant irradiance and temperature (Sweeney 1969). Circadian rhythms, i.e., endogenous rhythms with a period of approximately 24 hours, occur in a variety of processes. Two species that have been studied extensively are *Euglena gracilis* and *Gonyaulax polyedra*. *Euglena* a flagellated alga, exhibits circadian rhythms in phototaxis, cell division, vertical migration, cell shape and protein synthesis (Bruce and Pittendrigh 1956; Edmunds 1966; Round and Palmer 1966; Lonergan 1983; Quentin and Hardeland 1986). *Gonyaulax*, a dinoflagellate, has been found to demonstrate circadian rhythms in bioluminescence, cell division, photosynthesis and protein synthesis (Hastings and Sweeney 1958; Sweeney and Hastings 1958; Sweeney 1960; Volnandt and Hardeland 1984).

Circadian rhythms also occur in the vertical migration of diatoms living in the upper few millimeters of the sediments in both marine and freshwaters. Round and Eaton (1966) found that the upward migration of freshwater diatom populations occurred at least 2 hours before natural dawn. After remaining on the surface for approximately 9 hours, diatoms would begin to move back down into the sediments, returning to the surface at the same time the following day. The downward descent always occurred before

the onset of the dark period. This rhythm in migration was also found to occur in the laboratory under conditions of continuous light and continuous darkness. In order for the diatoms to remain on the surface of the sediment they must exhibit some motility and therefore the rhythm in vertical migration may also reflect a rhythm in motility.

Diatoms that inhabit tidal mud flats have been found to show a rhythm in vertical migration which is adapted to conform to tidal changes (Round and Palmer 1966). When the sediments are exposed by low tides, diatoms generally move to the surface during the daylight, remaining there throughout the day and migrating downwards before sunset when the light intensity falls. However, if the incoming tide covers the sediment before a decrease in light intensity occurs in the evening, the diatoms will migrate downwards, preventing the tide from washing over them. Therefore, a combination of high light intensity and exposed sediment results in the diatoms coming to the surface. Investigation of this behavior in the laboratory, away from tidal influences, has shown that the vertical migration continues under conditions of constant illumination and temperature. The rhythm of this migration shows a typical circadian periodicity which is phased by the last tide experienced in nature. However, some species studied continue to show tidal rhythms in the laboratory under constant conditions (Palmer and Round 1967; Happey-Wood and Jones 1988). *Hantzschia virgata* was found to have a migration period of approximately 24.8 hours in the laboratory, appearing on the surface of the sediment a little later each day at a time corresponding to low tide. *H. virgata* did not surface in the dark but

skipped a period of migration if it was scheduled to occur at night (Palmer and Round 1967). A similar rhythm was found for *Pleurosigma angulatum* (Happey-Wood and Jones 1988). However, after several days in constant conditions, this diatom showed a twice daily migration rhythm, with cells moving to the surface every 12.4 hours at times which correspond to both periods of tidal exposure in the field. *P. angulatum* also was found to show persistent endogenous tidal rhythms in the speed of movement of cells, with maximum rates occurring every 12.4 hours at times which corresponded to tidal exposure in the field.

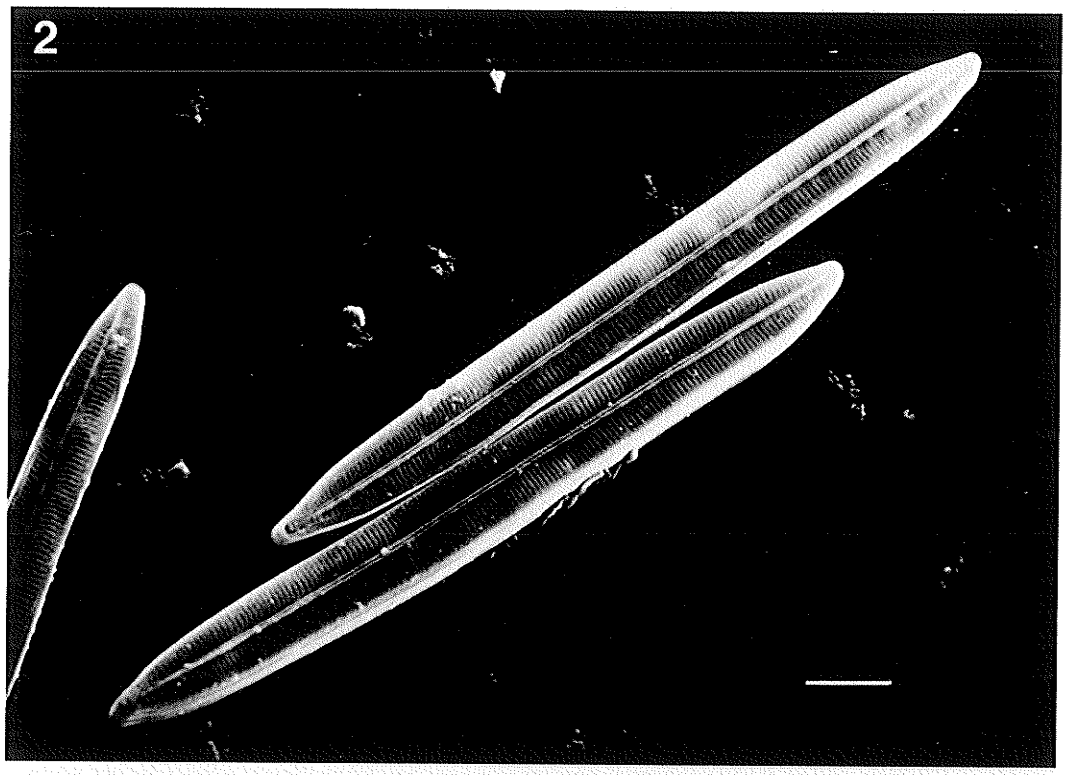
Endogenous rhythms which follow the lunar tidal cycle are not common in algae, but there have been reports of intertidal algae showing a periodicity of reproductive activity which coincides with specific periods of the lunar tidal cycle (Tanner 1981).

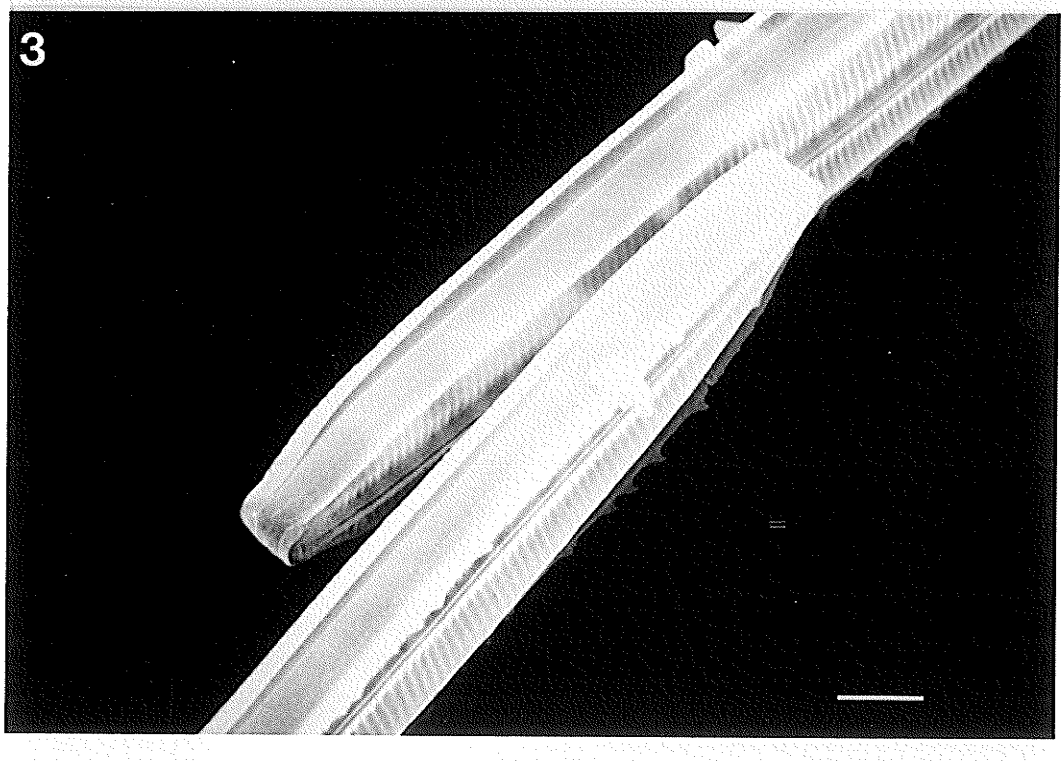
3. The Biology of *Bacillaria*. *Bacillaria paradoxa* Gmelin (= *B. paxillifer* (O.F. Müll.) Hendy) is a colonial pennate diatom belonging to the family Nitzschiaceae (Hustedt 1930). The genus *Bacillaria*, related to the genera *Hantzschia*, *Cylindrotheca* and *Nitzschia*, contains only one species, *B. paradoxa*. Individual *Bacillaria* cells are normally symmetrical in shape about the apical, transapical and pervalvar axes of the frustule (Figures 2 & 3). The cell wall is transapically striated with 20-25 striae occurring every 10 µm. These striae represent rows of small round pores having diameters of approximately 0.08 µm and lying approximately 0.16 µm apart from each other in the cell wall. In valve view the cells appear linear-spindle shaped

Figure 2. External valve view of *Bacillaria paradoxa* .

Scale bar = 7.7 μm.

Figure 3. Girdle view of two *Bacillaria paradoxa* cells positioned valve to valve. Scale bar = 3.6 μm.


2


3

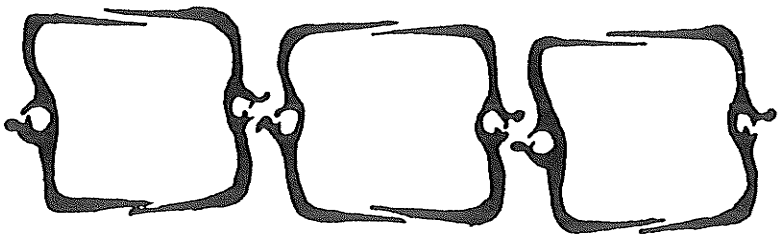
with the ends being sometimes slightly extended (Figure 2). Valves are reported to be 60-150 µm long and 4-8 µm wide. In girdle view the cells appear rectangular and are approximately 6 µm broad (Hustedt 1930; Caljon 1983).

Each valve has two raphe fissures, placed end to end, which run longitudinally along the length of the valve and which are located in the summit of a central keel. The keel is formed by silica arches and appears as a tubular canal projecting from the face of the valve (Figures 4,5 & 6). In cross-section, the raphe fissures in *Bacillaria* are in the shape of a tongue and groove structure (Figure 5) and are similar in appearance to those found in some *Nitzchia* species (Drum and Pankratz 1965). Beneath the raphe fissures, spanning transversely across the canal, are internal bars of silica which join the two parts of the valve on either side of the raphe (Figures 5 & 7). In the light microscope these bars appear as punctae. *Bacillaria* cells have 5-8 silica bars occurring every 10 µm with a somewhat irregular spacing (Figure 7). On one side of each raphe fissure is a siliceous protrusion which in cross section appears as a hook (Figure 5). The protrusions occur on opposite sides of the raphe in neighboring cells and it has been suggested that they hold the cells together in a colony either by interlocking or by each protrusion fitting into the outer raphe groove of the adjacent cell (Drum *et al* 1966). No evidence has been found for cytoplasmic connections between cells in a colony (Drum and Pankratz 1965).

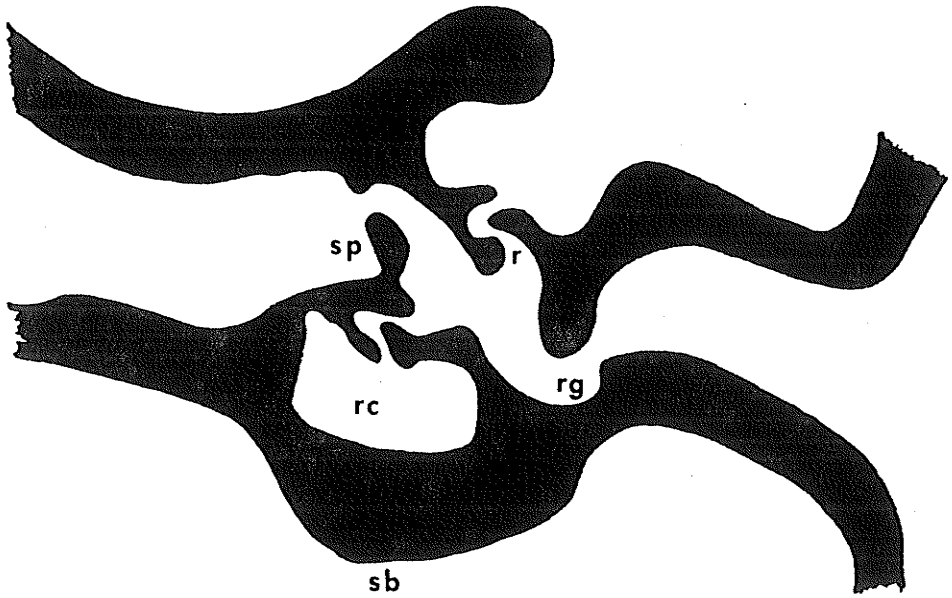
Bacillaria is an organism with a wide geographical distribution, being reported from Africa, Europe, North America (McIntire and Moore 1977) and

Figure 4. Schematic drawing of a 3-celled *Bacillaria* colony in cross-section. (After Drum & Pankratz 1966.) Scale bar = 5 μm.

Figure 5. Tracing from an electron micrograph of adjacent cell raphes of two *Bacillaria* cells in cross-section showing the raphes (r), raphe canals (rc) silica protrusions (sp), raphe grooves (rg) and internal silica bars (sb). (After Drum & Pankratz 1966). Scale bar = 0.6 μm.



4



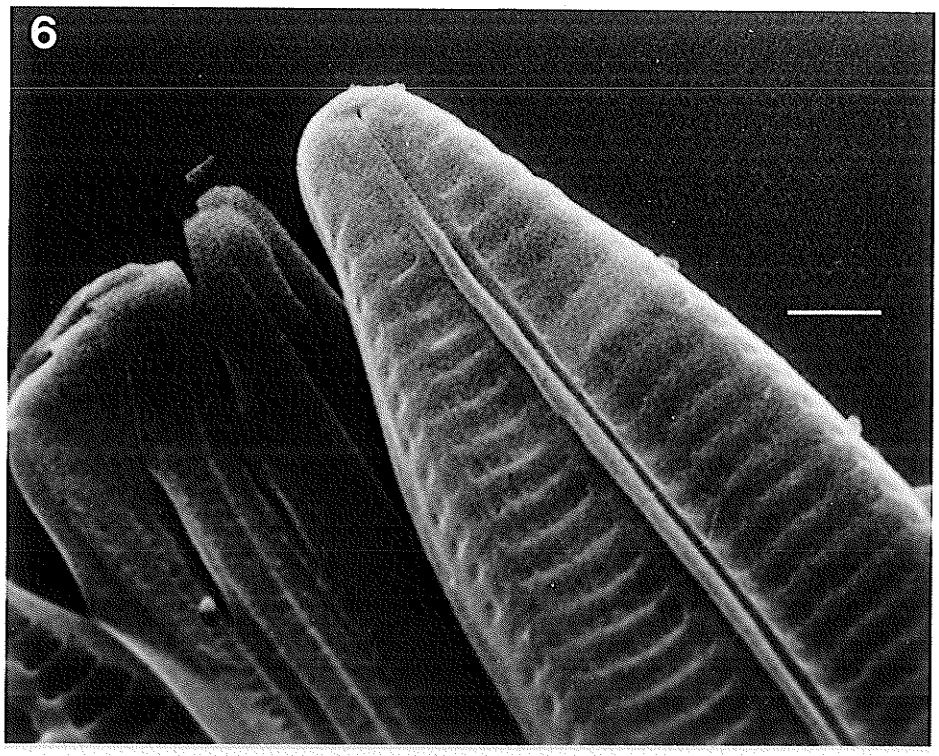
5

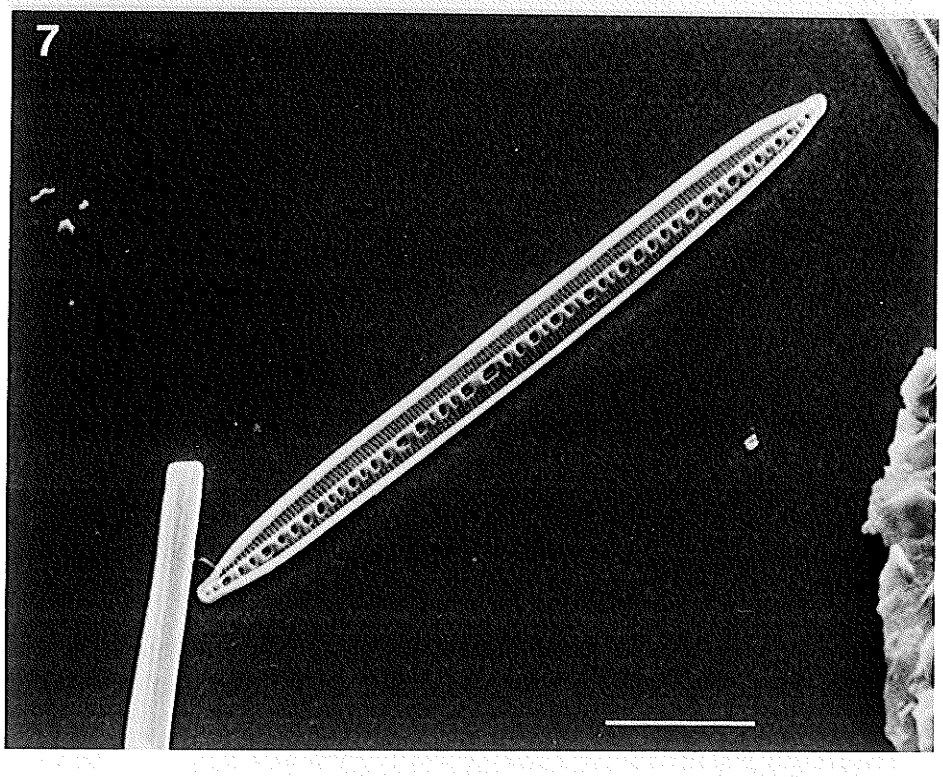
Figure 6. External valve view of *Bacillaria paradox*. raphe.

Scale bar = 1.1 μm.

Figure 7. Internal valve view of *Bacillaria paradoxa*.

Scale bar = 14.5 μm.


6


7

Asia (Guillard and Kilham 1977). It also has the ability to live in a wide range of habitats and is considered to be benthic, periphytic and planktonic (McIntire and Overton 1971; Hoek *et al.* 1979; Round 1981; Caljon 1983; Admiraal 1984). However, it is found less frequently in the plankton and therefore can be classified as tychoplanktonic (Beaver 1981). Although *Bacillaria* is common in many coastal regions, I could find no record of it occurring in the open ocean. Round (1981) suggests that *Bacillaria* lives in loose association with the surface of the sediment and that whenever the sediments are disturbed it finds its way into the inshore plankton. *Bacillaria* is classified as a holeuryhaline species (Caljon 1983; Rincé *et al.* 1981; Hoek *et al.* 1979), indicating that it can exist in sea water, brackish water and fresh water. It is pH indifferent, occurring in waters at around pH 7 (Hustedt 1957) and in areas rich in inorganic nutrients (Caljon 1983). *Bacillaria* also ranges from being oligosaprobic (Beaver 1981) to mesosaprobic (Caljon 1983), preferring waters that contain a slight to moderate amount of dissolved organic matter. Ecological studies of diatom communities in North America have shown that *Bacillaria* is often more abundant in late spring and summer when visible radiation is considerably higher than in late fall and winter (McIntire and Overton 1971).

Diatoms are a diverse group of organisms and studies of diatoms grown in culture have shown that they can vary considerably in their physiological processes (Lewin 1953; Eppley 1977; Brand *et al* 1983). The principle photosynthetic pigments are chlorophyll *a*, c_1 and c_2 , and fucoxanthin (Jörgensen 1977). Fucoxanthin is a carotinoid pigment and appears

golden-brown, giving diatoms their characteristic color. Photosynthesis in diatoms occurs mainly in the visible range of the spectrum between 400nm and 700nm but the amount and duration of light for optimum photosynthesis varies greatly with the species (Patrick and Reimer 1966). Although diatoms are considered to be primarily photoautotrophic, producing all essential metabolites, their growth is often improved by the presence of small amounts of organic substances (Lewin and Guillard 1963; Prakash *et al.* 1973). The exact nature of these organic substances as provided by soil extracts and extracts from algae and natural sea water is not clear, but it has been suggested that their stimulatory effect on diatom growth may be due to chelating activity (Prakash *et al.* 1973), allowing cells to utilize trace metals more readily. Vitamin requirements have also been found among many diatoms, especially for vitamin B_{12} and thiamine (Droop 1957; McLachlan 1959; Lewin and Lewin 1960; Guillard and Cassie 1963; Guillard 1968).

The mineral requirements of diatoms are similar to those of most plants. They require nitrogen, phosphorus, sulphur, calcium, magnesium, potassium, iron and manganese, as well as a number of trace elements. In addition, diatoms require silica for both frustule formation and cell division. Some diatoms are able to use organic sources of nitrogen such as urea and amino acids in place of inorganic nitrate or ammonia (Stephens and North 1971; Carpenter *et al.* 1972; McCarthy 1972). Certain species can use organic phosphorus compounds in place of inorganic phosphate (Chu 1946; Harvey 1953; Kuenzler 1966). There also exist some diatoms that have the ability to grow in the dark as facultative heterotrophs, using organic substrates as their

carbon and energy sources (Lewin 1953; Lewin and Lewin 1960). Soluble silica, monosilicic acid ($Si(OH)_4$), is required both for silica shell formation (Lewin 1955) and DNA synthesis (Darley and Volcani 1969; Sullivan and Volcani 1973). The amount of silica per cell varies over a wide range both between and within species and is partially dependent on the concentration of Si in the growth medium (Werner 1977). Jörgensen (1953, 1955) compared the silica content of *Bacillaria* cells from cultures containing varying silica concentrations. He found that in cultures containing 0.8 mg Si/L, *Bacillaria* had a silica content of 50pg Si/cell while cells from cultures containing 5.8 mg Si/L had a higher silica content of 250pg Si/cell. The amount of silica per cell also depends on the rate of cell division, with rapidly dividing cells depositing thinner shells than cells which divide more slowly (Lund 1950; Jörgensen 1955; Lewin 1957). The silica shells of living diatoms appear to be protected by the layer of organic material that surrounds the frustule (Lee 1980). After cell death, depending on the conditions, the silica frustules can either dissolve or remain intact and accumulate on the bottom of water bodies, forming a diatomaceous ooze.

The growth rates of diatoms in culture have been shown to be quite variable between species and even between clones (Lewin and Guillard 1963; Patrick and Reimer 1966; Medlin and Wilson 1979; Seaburg *et al.* 1981). Environmental factors such as temperature and light intensity greatly influence the growth of diatoms. The effects of these factors cannot be considered separately since, for example, diatoms can exhibit different light intensity optima for growth at different temperatures and at different

nutrient concentrations (Durbin 1974; Holt and Smayda 1974; Rhee and Gotham 1981). Generally, an increase in temperature results in an increase in diatom growth up to an optimum temperature, after which growth rates will decline. Light-dark cycles also affect the growth of diatoms (Castenholz 1964; Paasche 1968; Holt and Smayda 1974). Some diatoms are daylength dependent, showing a reduction in growth rate with shortened daylengths, while others show little difference in growth rates. Some diatoms exhibit a relative inhibition of growth under long daylength or continuous illumination. It should be noted that these results also depend on the temperature and light conditions and often are influenced by the pre-conditioning of the cultures being studied (Durbin 1974). Edenhofner and Schmid (1984) found that growth rates could be increased further if the dark cycle was combined with a lower temperature compared to that of the light cycle.

The life cycle of *Bacillaria* consists for the most part of vegetative cell division in which mitosis occurs. The division takes place longitudinally in the valvar plane. Prior to cell division there is an increase in cell volume accompanied by a sliding apart of the girdle bands. This results in the epitheca and hypotheca being pushed away from each other and the cell becoming broader in girdle view (Round 1973). This is followed by a mitotic division of the nucleus and a division of the protoplasm caused by the invagination of the plasmalemma. After the protoplasm has divided, vesicles produced by the Golgi apparatus collect beneath the plasmalemma (Stoermer *et al.* 1965). These vesicles fuse to form the silicalemma which is

the membrane of the silica deposition vesicle. Separate vesicles are formed for each new hypovalve as well as for each new girdle band and the valves of the parent cells become the epithecas of the daughter cells with each daughter cell producing a new hypotheca (Figure 8). The cycle of cell division is completed by the separation of the daughter cells, although in *Bacillaria* the daughter cells generally remain connected.

Mature valves are incapable of growth and it is believed that this is responsible for the reduction in cell size which occurs in some diatoms following vegetative division. An explanation of this was first set forth by McDonald (1869) and Pfitzer (1871) who suggested that at each cell division one of the two daughter cells is reduced in size along the apical axis by an amount equal to twice the thickness of the girdle band (Figure 8). This results in the average cell size of a population derived from a single cell gradually shifting to smaller dimensions. Although this reduction in cell size has been recorded for both natural and cultured populations it does not occur in all diatoms and when it does occur it does not always do so consistently (Crawford 1980). Cells in a reducing population of diatoms can, in some instances, return to their original size via sexual reproduction and the formation of auxospores (Fritsch 1956). Auxospores are resting cells which usually develop from zygotes formed by the fusion of two gametes. Once the zygote is formed, it escapes the parent cell, elongates and secretes an organic wall, followed by a siliceous wall inside the organic wall (Geitler *in* Patrick and Reimer 1966). The epitheca is formed first, although without a girdle band, followed by a normal hypotheca. The cells produced by subsequent

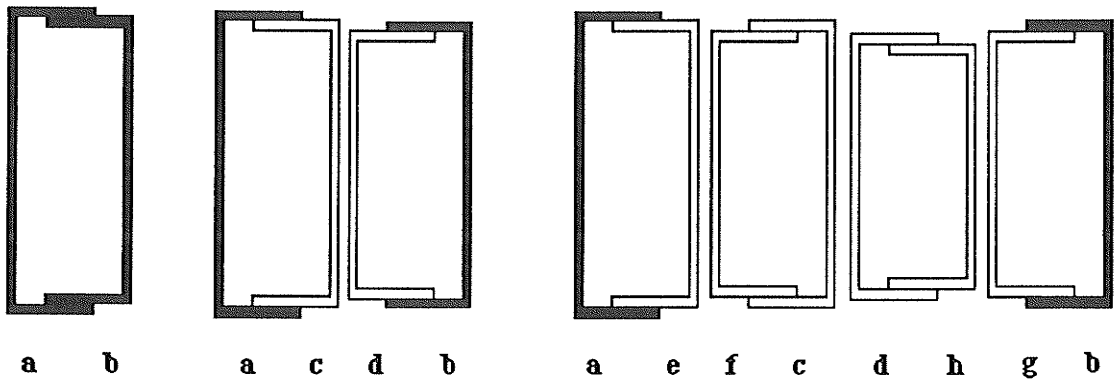


Figure 8. Schematic drawing of diatoms in girdle view indicating the reduction in size that occurs during vegetative cell division. (After Hendey 1964.)

vegetative divisions possess both valves and girdle bands.

Several different types of sexual reproduction and auxospore formation have been observed in diatoms and their forms are generally classified into a five-type system (Patrick and Reimer 1966; Drebes 1977). This system consists of the Normal Type, in which two mother cells each produce two iso-gametes which fuse in pairs to produce two auxospores; the Transitional Type, in which spermatozoa and an egg cell are formed; the Reduced Type, in which two mother cells each form one gamete, which fuse to produce a single auxospore; Automixis, in which a single auxospore arises from an unpaired mother cell; and Apomixis, which includes parthenogenetic and purely vegetative auxospore formation. Sexual reproduction is observed rarely in diatoms but it appears that there is little correlation between the type of auxospore formation and taxonomic position of the species. In fact, several different types of auxospore formation have been observed in different varieties and forms of the same species (Patrick and Reimer 1966). There is little information available on the type of auxospore formation exhibited by *Bacillaria*. According to Patrick and Reimer (1966) *Bacillaria paradoxa* can tentatively be classified as producing auxospores through automixis or through apomixis. In *Bacillaria* automixis is thought to occur autogamously, without the production of gametes; meiotic cytokinesis is suppressed and two of the four nuclei produced fuse in the undivided protoplasm. Certain diatoms have been reported to experience cell size regeneration not through sexual reproduction but rather through the extrusion of cytoplasm and the subsequent formation of new and larger valves (Gallagher 1983; South and

Whittlick 1987).

Like many other pennate diatoms possessing raphes, *Bacillaria paradoxa* exhibits a form of gliding motility which does not involve any visible structure or change in shape. However, while most motile diatoms glide against a variety of substrates, the cells within a *Bacillaria* colony normally glide only against each other. (Isolated single cells of *Bacillaria* are capable of moving on particulate substrata but not on flat surfaces (Drum *et al.* 1966)). Colony movement is highly characteristic, with the cells gliding past each other in such a way that the whole colony appears to move in a rhythmic and co-ordinated manner, often expanding and contracting as a single unit (Figure 9). In extended colonies the cells are arranged almost end to end forming a psuedo-filament. As the colony contracts, the cells reverse their direction of movement and the ends of the colony become drawn towards the centre, with the two halves passing each other as the colony extends fully again. Under certain conditions colonies are also capable of remaining contracted, with the cells positioned side by side in almost perfect alignment (Figure 10). Jarosch (1962) found the relative velocity of a living cell against a dead one to be 7-8 μm/second, while the relative velocity of two living cells was found to be 14-15 μm/second. He also reported that colonies had a constant rhythmic period of 80 seconds at 20°C and that this period remained constant in strong light, in solutions of altered osmotic concentrations and in the presence of narcotics, although the amplitude of shifting decreased, i.e., the extent of cell displacement decreased. In addition, a rise in temperature was reported to result in both a shorter period and a small decrease in amplitude.

Figure 9. Motile *Bacillaria paradoxa* colony contracting and expanding:

a-c. Extended colony moving to a contracted position;

d. Contracted colony;

e,f. Colony moving to an extended position in which the cells now become oppositely oriented.

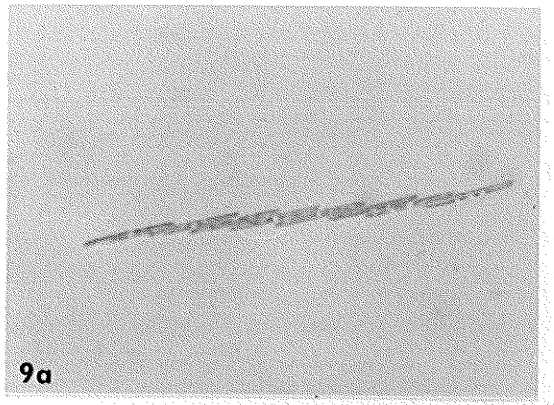
Scale bar =75 μm.

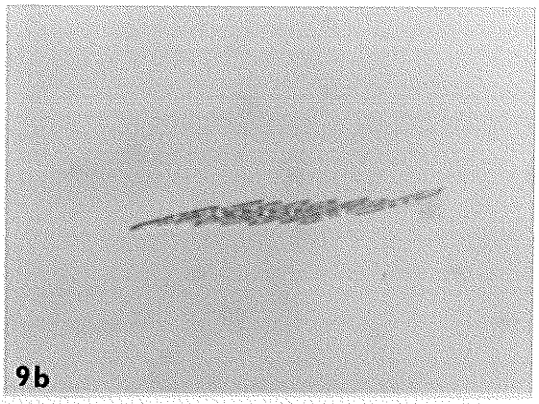
Figure 10. Non-motile *Bacillaria paradoxa* colonies in contracted and aligned positions.

a. 33-celled colony.

b. 21-celled colony.

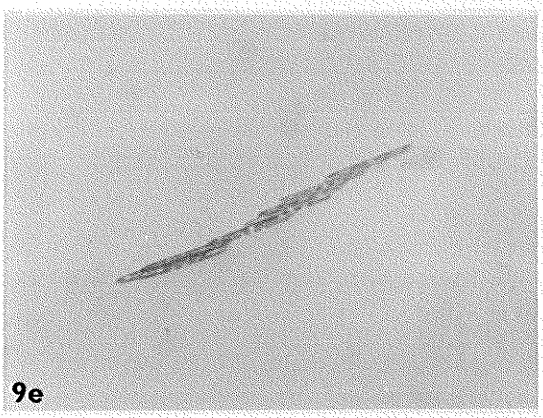
Scale bar = 42 μm.

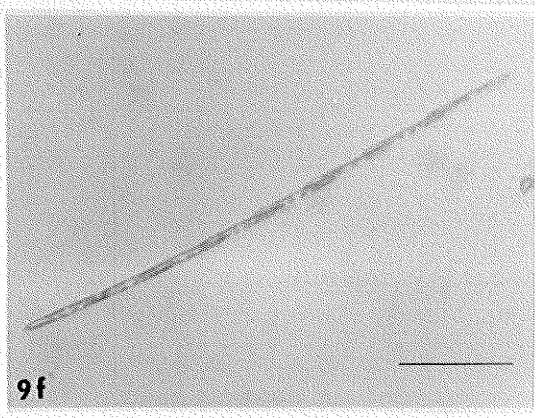

9a

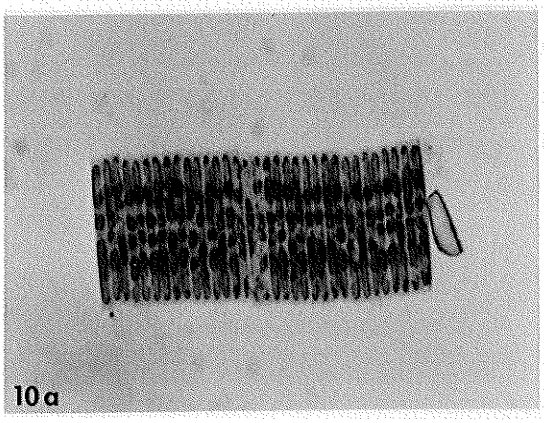

9b

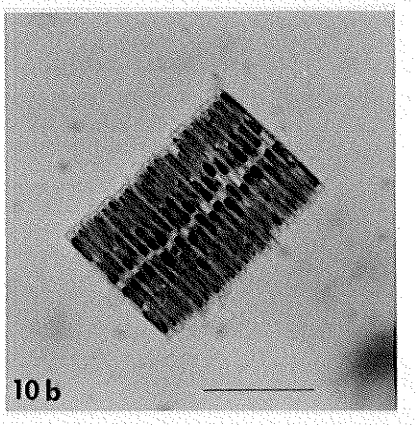

9c


9d


9e


9f


10a


10b

Funk (1914, 1919) investigated the effects of daylight and mechanical stimuli on the movements of *Bacillaria*. While observing undisturbed colonies in glass containers exposed to natural light, he found that during the early morning hours most of the colonies were in a contracted form (Figure 10). Very few colonies showed any movement and those that did moved very slowly. As daylight increased, more colonies started to move and by 10:30 a.m. many of the colonies were extended into long psuedo-filaments. Most of the colonies remained in extended, motionless positions until the daylight began to decrease. By 4:30 p.m., the colonies began to oscillate, showing their characteristic type of movement. As light decreased further, colonies gradually began to move less and to remain in contracted positions. By the time darkness arrived, the colonies were found to be in the same condition as in the early morning hours. The transition from the day to the night position was reported to have taken 1-1.5 hours. Funk performed his experiments in Naples during the month of September but he did not record the sunrise and sunset times. However, according to the Air Almanac of 1988, sunrise time can be estimated at occurring in Naples between 4:30 a.m. and 5:00 a.m. and sunset time estimated at 5:30 p.m. The behavior of *Bacillaria* was observed for an extended time and it was obviously related to certain periods in the day. However, it was not determined whether this periodicity was a direct result of the cyclic light-dark environment or whether it was an endogenous rhythm capable of being sustained under constant conditions.

In examining the effect of mechanical stimulation on *Bacillaria*, Funk regarded colonies remaining in extended, motionless positions as being in a

resting and stimuli-receptive condition. He found it was possible to induce cells in such a condition to move by disturbing them with the tip of a fine brush hair. The degree of movement and the number of cells involved depended on the strength of the stimulus. In colonies which were lightly pushed, only the cells in the area directly disturbed responded. These cells were observed to glide past one another and return with the same speed to an extended position, without causing any visible changes in the rest of the colony. Stronger stimuli induced more cells to glide, which would then also remain for a longer length of time in a contracted position. If colonies were disturbed vigorously enough, they were observed to oscillate several times before finally returning to an extended resting position. If a colony was disturbed again while still in a stimulated position and before any return movement had begun, it would remain in this position for a longer time before extending again. If the return movement had already begun, further disturbances would not induce a reversal, but after extending, the colony would continue to oscillate for some time and colony movement could therefore be sustained through persistent disturbance. Funk did not report on the effects of disturbing contracted colonies.

The rhythmic behavior of *Bacillaria* was initially attributed to the periodic activity of extra-membraneous protoplasm (Funk 1914, Kamiya 1959). However, when Drum and Pankratz (1965) examined cross sections of *Bacillaria* with an electron microscope, they found no evidence for cytoplasmic connections and they suggested that the cells are held together only by interlocking raphes. Yet, Jarosch (1958) considered the substance

holding the cells together to be very elastic, since he observed extended colonies curving and snapping back rapidly when their ends met with a resistance which was suddenly removed. Single cells of *Bacillaria,* isolated by the laser beam destruction of adjacent cells, were observed to oscillate with a frequency similar to that of the whole colony (Drum *et al.* 1971). This observation led to the suggestion that the behavior of colonies was a result of some form of interaction between individual cells which was capable of synchronizing their oscillations.

METHODS AND MATERIALS

1. Culture Techniques. Four clones of *Bacillaria paradoxa* were available for experimental use and observation. Three of the clones were isolated from Delta Marsh, Manitoba and one clone, originating from the Mississippi River in Dubuque, Iowa, was obtained from Dr. David Czarnecki of Loras College, Dubuque. The first clone from Delta Marsh was isolated in September 1986, the second in July 1987, and the third in October 1987. The clone originating from the Mississippi River was established in the summer of 1987. These clones were designated as I_2, I_3, I_5, and I_c respectively. Since all the cells within a colony of *Bacillaria* are descendants of one cell it possible to establish clonal populations in culture by isolating single colonies rather than single cells. Single colonies were isolated by successive transfers with a micropipette through a series of sterile medium washes. The cultures established were uni-algal but non-axenic (not bacteria free). The practice of using non-axenic algal cultures is recognized as an acceptable method for maintaining cultures in a morphologically healthy state and various studies have indicated that the presence of bacteria in diatom cultures often leads to improved growth (Pringsheim 1946; Kain and Fogg 1958; Round 1981; Czarnecki 1987).

Cultures were grown in Woods Hole MBL pH 7.2 medium (Nichols 1973), modified to include boron, increased silica (Goldsborough and Robinson 1984) and soil extract. The concentrations of nutrients and vitamins used in preparation of this medium are given in Table 1. Most compounds were prepared as stock solutions at 100-fold concentrations.

Table 1. Culture medium for *Bacillaria paradoxa*.

Compound	Concentration (mg per litre of medium)
$CaCl_2.2H_2O$	36.76
$MgSO_4.7H_2O$	36.97
$NaHCO_3$	12.60
K_2HPO_4	8.71
$NaNO_3$	85.01
$Na_2SiO_3.9H_2O$	227.40
Na_2.EDTA	4.36
$FeCl_3.6H_2O$	3.15
$CuSO_4.5H_2O$	0.01
$ZnSO_4.7H_20$	0.022
$CoCl_2.6H_2O$	0.01
$MnCl_2.4H_2O$	0.18
$Na_2MoO_4.2H_2O$	0.006
H_3BO_3	0.0099
Thiamine.HCL	1.0×10^{-4}
Biotin	5.0×10^{-7}
Cyanocobalamin (B_{12})	5.0×10^{-7}
Soil extract	0.25 litre/litre of medium

Silicon was added as a powder, Na_2.EDTA and $FeCl_3.6H_2O$ were combined and diluted 5-fold, and the vitamins were prepared at 10,000-fold concentrations. Soil extract was prepared by autoclaving and filtering 10 grams of soil in one litre of deionized and distilled water. The extract was added at a concentration of 250 ml per litre of medium. Soil, free of chemical fertilizers and herbicides, was obtained from Cheetam Soil Supplies Company Limited of Winnipeg. This soil had a salinity level (electrical conductivity) of 0.9 ms/cm and contained 9% organic matter (soil analysis was performed by the Manitoba Provincial Soil Testing Laboratory). All nutrients, including the soil extract, were added before autoclaving except for Na_2.EDTA, $FeCl_3.6H_2O$, and the vitamin stocks. These were filter-sterilized (using a 0.2 µm membrane filter) and added to the medium while it was still hot. The pH was adjusted to 7.8 with HCl prior to autoclaving after which it dropped to 7.2.

Cultures were contained in 16 mm x 125 mm polystyrene culture tubes with plug-seal caps. The culture tubes were held in open wire racks within a growth cabinet and illuminated from below with cool white fluorescent lamps providing an irradiance of approximately 80 µmol $m^{-2}s^{-1}$. Light periodicity was kept at a 12 hr light:12 hr dark cycle using an automatic timer and temperature was maintained at $20 \pm 2^{o}C$. In order to provide uniform material for experimental purposes new clones were re-established every few months by isolating single colonies from each culture line.

2. Growth Rates. Growth rates were estimated by measuring the increase in cell number over time. When *Bacillaria* is grown in large volumes under static conditions several problems are encountered in estimating cell number. Colonies tend to clump together, making it difficult to determine actual cell numbers and clumps of colonies often become stuck to the sides of sampling pipettes and tubes. In order to avoid these problems, cultures of *Bacillaria* were grown in test tubes which held approximately 15 mL of medium. This allowed the cultures to be vigorously shaken by hand several times daily, preventing excessive clumping of the colonies.

Cell numbers were counted from 0.5 mL samples which were removed daily from culture tubes. A drop of Lugol's solution (Lillie 1965) was added to each sample, which instantly killed the cells. Samples were placed into small petri dishes which had 2mm grids etched on their bottoms and the total number of colonies was counted using a Wild stereomicroscope. Samples were taken daily from three separate tubes for each clone. Since *Bacillaria* is colonial, it is possible to estimate cell number by determining the average number of cells per colony and multiplying this value by the number of colonies counted. The average colony size was determined separately each time cell numbers were estimated and was based on counts of 100 colonies.

The average number of divisions per day (k) were calculated, assuming exponential growth, from the following equation (Guillard 1973):

$$k = \frac{\log_2(N_1/N_0)}{t_1 - t_2},$$

where N is the concentration of cells in the culture, t is the time in days and

the subscripts denote values at two different times.

3. Cell Division Rhythms. In order to detect the presence of rhythms in cell division *Bacillaria* cultures (clone I_3) were grown in tubes containing 15 mL of media and exposed to a 12 hour light : 12 hour dark cycle under the conditions indicated above. Tubes were inoculated at a density of 500 cells/mL and allowed to reach a density of approximately 1200 cells/mL before samples were removed. In order to provide culture material from both the light and dark periods, the lighting regime was staggered so that cultures passed through dark and light periods simultaneously. Cultures were kept in darkness by wrapping individual tubes in aluminum foil, placing them in wire racks and covering the entire rack of tubes again with foil. Three tubes were removed from both the light and dark cycles every two hours between 9:00 a.m. and 9:00 p.m. for four days. Care was taken not to disturb the cultures and cells were killed by adding a drop of Lugol's solution to each tube.

In order to determine if there was a daily peak in cell division, the number of recently divided cells was counted. These cells remain attached for some time following division, therefore, the number of cell divisions occurring was determined by counting the number of paired cells. Paired daughter cells can be distinguished from an otherwise normally attached pair of cells in a colony by the fact that they are attached along the entire valve face and appear somewhat thinner in girdle view. Approximately 200 cells were counted for each sample. In order to eliminate the influence of a cyclic light

environment on cell division rhythms, the above procedure was repeated with cultures that were first exposed to a 12 hour light : 12 hour dark cycle and then kept in countinuous darkness for 60 hours.

4. Motility Rhythms. The samples removed for the above experiment were also used to determine if daily rhythms in the movement of *Bacillaria* colonies occurred. Lugol's solution was observed to kill cells almost instantly and therefore was used to preserve colony configuration at the time of sampling. The behavior of colonies is such that they appear in essentially only three different configurations: completely extended; partially extended; and aligned and contracted. It is not possible to determine from light microscope observations whether or not colonies found in completely extended or partially extended configurations had been actively motile just prior to their death (see review of Funk's observations (1914, 1919) in Literature Review, section 3 and Observations on Motility in Results, section 3). However, colonies that are in the aligned, contracted configuration are never motile. Observations on colony behavior have shown that colonies in the extended configuration can easily be induced to move through mechanical disturbance whereas colonies in the aligned, contracted configuration cannot. (Funk referred to colonies remaining extended as being in a stimuli-receptive position.) Therefore, colonies found in the fully extended and partially extended configurations were scored as being motile or potentially motile and aligned and contracted colonies were regarded as being non-motile. The degree of motility at each sampling time was determined by

counting the number of extended and partially extended colonies found among 100 colonies from each sample. This was done for cultures grown under a lighting regime of 12 hours light : 12 hours darkness as well as for cultures kept in continuous darkness for 60 hours.

5. Influence of Light on Motility. In order to observe the behavior of *Bacillaria* with a minimum of disturbance, colonies were grown in small, petri dishes sealed with parafilm which were allowed to sit on a lab bench for several days. Room temperature was maintained at 24 ^{o}C and light was provided during the day by fluorescent lamps at a photon flux density of 30 μmole m^{-2} s^{-1}. Observations of colonies were made periodically throughout the day for several days using a Wild M 400 dissecting microscope.

The effect of light intensity on the movement of *Bacillaria* was investigated by exposing individual colonies (clone I_3) to a range of light intensities at a constant temperature. During the experiments, temperature was maintained at 20.9 ^{o} $\pm$ 0.8 ^{o} C. Light was provided by a 60W 12V illuminator from a Zeiss Universal research microscope and the intensity was regulated using neutral density filters. The photon density flux at each intensity was measured in μmol m^{-2} s^{-1} using a LI-Cor photometer (model LI-185A, Lambda Inc., St. Laurent PQ) and light was varied so that the photon density flux ranged from 0.65 μmol m^{-2} s^{-1} to 150 μm m^{-2} s^{-1}. Single colonies ranging in size from 10 to 23 cells were removed from exponentially growing cultures which had been kept in the dark for over 5 hours, washed in

fresh medium and placed with a drop of medium onto a coverslip. Coverslips were inverted over a ring, 3mm high, which was glued to a microscope slide and which had a small groove filed into it (Figure 11). Temperature was monitored using a microprobe thermometer (Sensortek Inc., Clifton NJ). The tip of the probe (model IT-18) was placed directly into the drop of medium. The lead was placed in the groove of the ring and fastened with a drop of parrafin wax so that the wax formed a smooth surface with the rest of the ring top. Prior to positioning the coverslip, the top of the ring was coated with vaseline in order to seal the preparation and prevent drying out of the specimen. Colonies were observed under 160 x magnification, using a Zeiss universal research microscope.

Tests were run in triplicate and each colony was exposed to the designated light intensity for 2 hours. Observations on colony behavior were noted and the movement of active colonies recorded onto VHS video tape in real time at 30 frames per second. Colony period was measured as the time required for a colony to move from an extended stationary position to the oppositely oriented extended position and then back to its original position. Both the number of motile cells per colony and colony period were measured at approximately half-hour intervals during the 2 hour testing period.

6. Influence of Temperature on Motility. The effect of temperature on the movement of *Bacillaria* was investigated using a similar experimental procedure to that described above except that light intensity was kept constant and temperature was allowed to vary. Light was maintained at an intensity

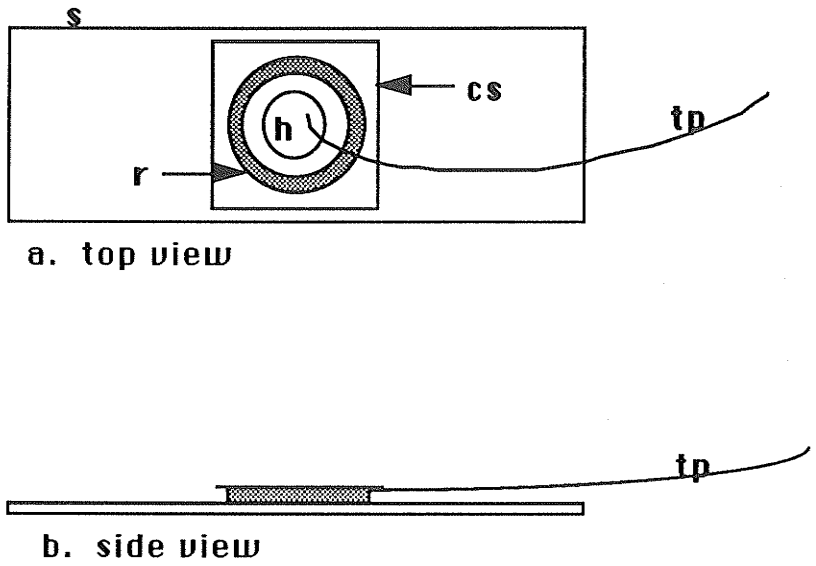


Figure 11. Diagram of hanging drop slide showing the arrangement of slide (s), coverslip (cs), ring (r), hanging drop (h) and temperature probe (tp) in a) top view and b) side view.

having a photon density flux of 0.71 μmol m^{-2} s^{-1}. At this intensity there was no measurable heating of the microscope slide and results from light intensity experiments indicated that this intensity did not inhibit cell movement (Figure 24). Temperature was controlled through the use of an air-conditioned room as well as space heaters. In order to stabilize the temperature around the experimental apparatus, the entire microscope was insulated with a wall of styrofoam. Colonies were exposed to temperatures ranging from 10°C to 35°C.

7. SEM. Cells removed from natural populations as well as clones I_3, I_5, and I_c were examined using scanning electron microscopy. Cleaned frustules were prepared by flooding material removed from culture with 30% H_2O_2 and adding approximately one mg of $KMnO_4$ (van der Werff 1955) so that final concentration was approximately 0.05 mg/mL. This caused a violent reaction during which the temperature rose to approximately 80° C. After completion of the reaction, the material was rinsed several times with distilled water and allowed to dry onto coverslips. The coverslips were mounted on stubs, gold-coated and examined at 25 kV with a Cambridge 100-S90 electron microscope. In order to separate the frustules into their component parts and remove any remaining organic matter a portion of the material removed from cultures was also boiled for several hours in 60% nitric acid. Following this treatment, the material was air-dried, gold-coated and examined with the scanning electron microscope.

RESULTS

1. Growth Rates and Cell Size Reduction. The average daily division rate (k), measured during exponential growth, of three different isolates of *Bacillaria* grown under identical conditions are given in Table 2. All three isolates were established at different times and were found to vary in both cell size and average colony size. In spite of this variation, cell growth occurred at a rate of approximately $k = 1$ division per day for all isolates studied. Figure 12 shows a growth curve for clone I_C grown in test tubes which were inoculated at a density of approximately 500 cells/mL. There is a slight lag period during the first 24 hours, followed by a period of exponential growth. During this period the average daily division rate, k ranged from 1.35 to 0.6 divisions per day. After 5 days, k decreased to approximately 0.4 divisions per day and after 8 days a stationary growth phase was reached.

No auxopsore formation or other type of size regeneration was observed in cells collected from natural populations or grown in culture. In addition, the average cell size in all clones was found to become progressively smaller over time. Clone I_2 (isolated in September 1986) was originally measured to have an average cell length of 101 μm and an average cell width (girdle view) of 6.8 μm. By April, 1988, the cells had reduced in size to a length of 29 μm and a width of 4 μm (Figure 13), after which point the culture died. This size reduction represents a decrease in length of 72% and a decrease in width of 43% over 1.5 years. The reduction in cell length for clone I_2 is approximately 0.13μm per generation. According to the cell division scheme represented in

Table 2. Average daily division rate (k) of *Bacillaria* isolates grown at 20° C and under a lighting regime of 12 hours light : 12 hours dark.

Isolate	I_3	I_5	I_c
Isolation Date	16/7/87	29/10/87	29/8/87
Experiment Date	26/4/88	12/5/88	16/8/88
Culture Age	285 days	202 days	359 days
***k* value (divsions/day)**	0.91	1.00	0.92
Mean Cell Length	68 µm	84 µm	75.5 µm
Mean Colony Size	30 cells (SD = 16.8)	14 cells (SD = 7.7)	25 cells (SD = 11.4)
Mean Total Cell Length per Colony	2040 µm	1176 µm	1888 µm

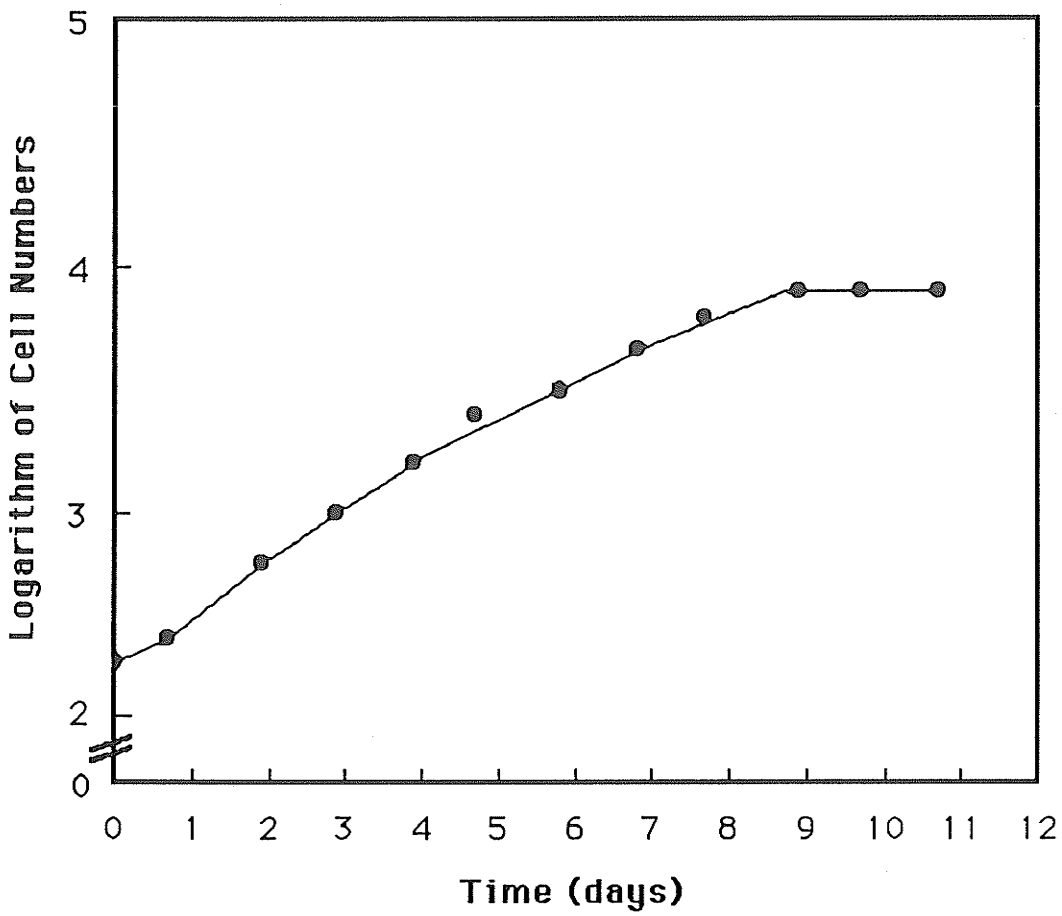


Figure 12. Growth curve for *Bacillaria paradoxa* (clone I_3) grown at 20°C and under a lighting regime of 12 hours light : 12 hours darkness.

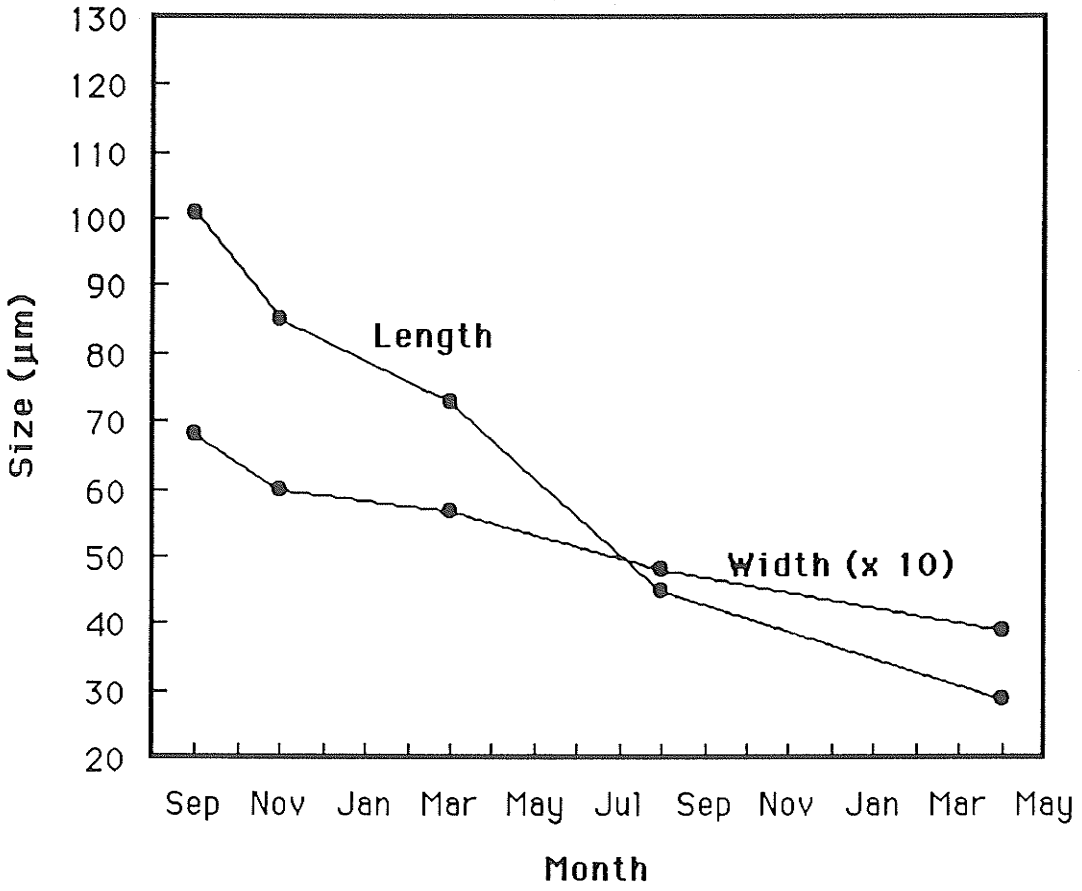


Figure 13. Decrease in length and width of *Bacillaria paradoxa* cells (clone I_2) grown in culture between September 1986 and April 1988.

Figure 8, this value should correspond to twice the thickness of the girdle bands. It is clear from SEM pictures (Figure 16) that the girdle bands of *Bacillaria* consist of more than one component. However, it is not yet known how these components are positioned relative to one another or in what manner they may influence cell division. A similar size reduction trend was observed in the cells of clones I_3, I_5, and I_c. Cells smaller than 85 µm in length were not observed in samples collected from Delta Marsh during the summer of 1987.

The degree of cell size reduction which occurs in diatoms depends largely on the spatial relationships between the different frustule components. It is usually difficult, however, to determine both the exact arrangement of the girdle bands and the manner in which they are joined to the valves (Round 1972; Crawford 1981). *Bacillaria* cells from culture, as well as those from natural populations, appear to have their girdle bands composed of several parts (Figures 14 and 16). The outermost layer is characterized by having one edge which is toothed. It is not clear whether the girdle bands are internal, external or aligned to the valve mantles (i.e. the curved edges of the valves) but they are considerably thinner than the valves, appearing transparent in scanning electron micrographs (Figure 16).

Bacillaria cells grown in culture exhibited few changes in frustule morphology. Cells collected from Delta Marsh were found to possess rows of pores which were open only to the inside of the valve, while external pore openings were found to be occluded with silica (Figure 17). This was also found to be true for all clones grown in culture (Figures 6, 7, 14,15, 16 and 18).

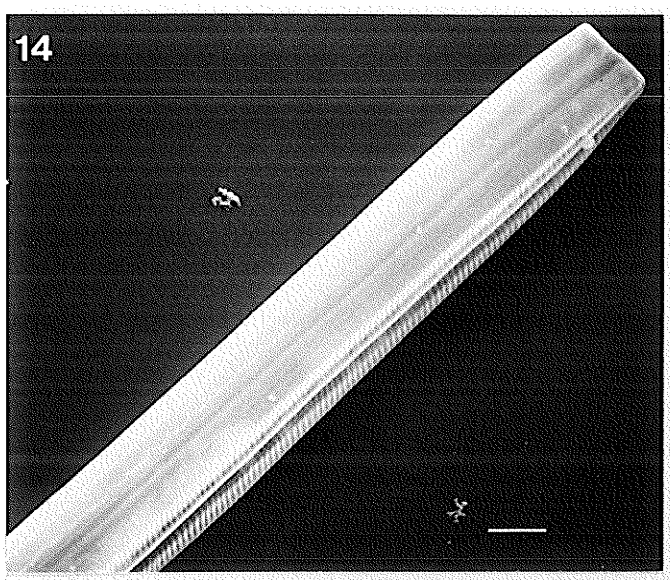
Figure 14. Girdle view of *Bacillaria paradoxa* (clone I_5). Note the toothed edge of the outermost layer of the girdle band. Scale bar = 4.2 μm.

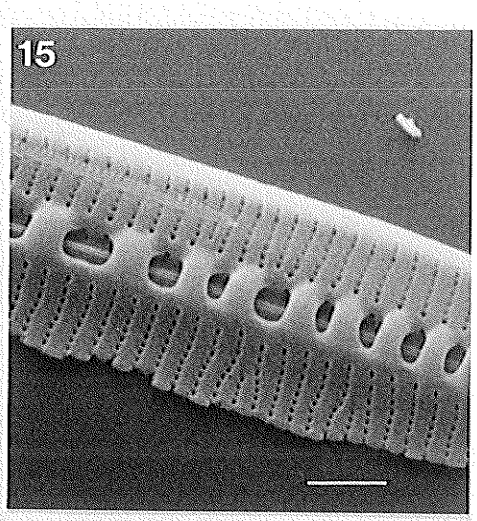
Figure 15. Internal valve view of *Bacillaria paradoxa* (clone I_3) showing incomplete rows of pores. Scale bar = 1.8 μm.

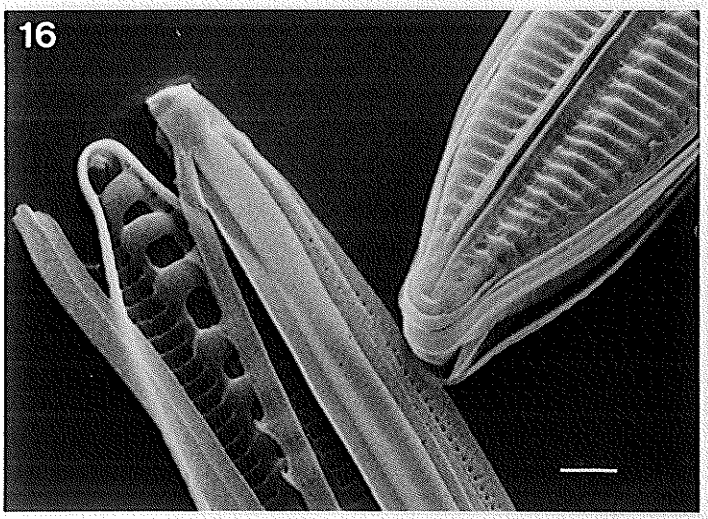
Figure 16. Valve view and girdle view of *Bacillaria paradoxa* (clone I_3) showing the different components of the girdle bands. Scale bar = 1.5 μm.

Figure 17. External valve view of *Bacillaria paradoxa* collected from Delta Marsh. Scale bar = 1.7 μm.

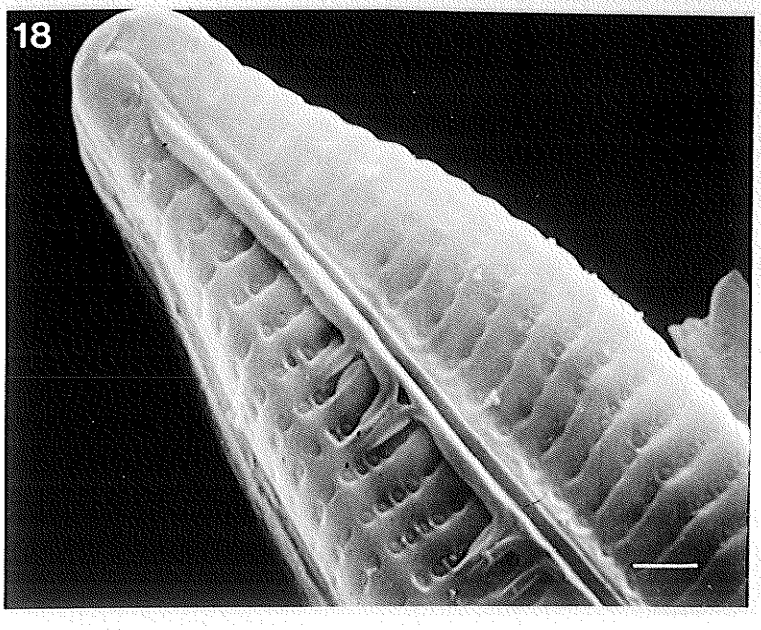
Figure 18. External valve view of *Bacillaria paradoxa* (clone I_3) with a silica raphe protrusuion showing variation in size and shape. Scale bar = 0.9 μm.


14


15


16


17


18

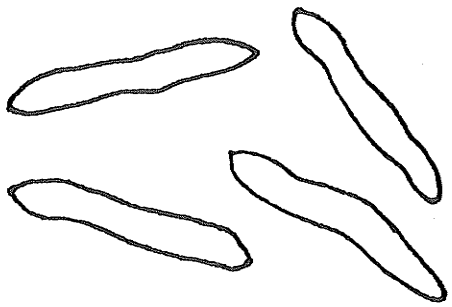
In addition, in both natural and cultured populations these rows, or striae, did not always form completely, with short rows sometimes occurring in between longer ones (Figures 15 and 17). This may occur as cells undergo reduction in size. Changes in raphe morphology were found to occur in some cells of clone I_3 (Figure 18), with the siliceous protrusion next to the raphe fissure being quite variable in size and shape. Thickened portions occurred in some areas, extending onto the valve surfaces and holes were found to occur in other areas. An overall change in frustule shape was also observed in cells which had reached a length of 35 μm (this represents a size reduction of approximately 65%). At this point, frustules were found to vary in shape and thickness in girdle view and have a curved appearance in valve view (Figure 19). Similar frustule shapes have been observed in cultured *Bacillaria* cells by R.W. Drum (personal communication).

In spite of dramatic size reductions and changes in frustule shape, cells were consistently found to retain some degree of motility up until their death. However, it was found that once cells reached a length of less than 45 μm, colonies did not extend completely and most cells, if motile, were observed to shuffle back and forth only for short distances, showing irregular periods. Unlike colonies composed of larger cells, cell movement did not occur in any apparent sequence which caused the overall shape of the colonies to be very irregular.

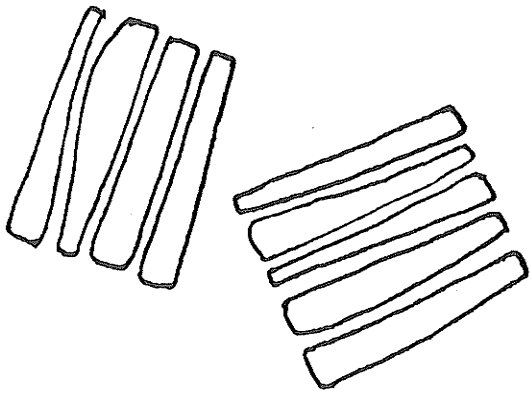
Figure 19. Tracing from a light micrograph of *Bacillaria paradoxa* (clone I_2) frustules of cells from culture showing the change in shape which accompanied size reduction:

a. valve view; b. girdle view.

Scale bar = 23 μm.



19a



19 b

2. Cell Division Rhythms. A rhythm in cell division was found to occur in cultures of *Bacillaria* grown under a lighting regime of 12 hours light: 12 hours darkness (Figure 20). Although it was found that cell division occurred throughout the dark and light periods, a peak generally occurred 6 hours after the onset of the dark period. During the peak in cell division, approximately 7% of cells counted were found in a paired condition (each pair was scored as one cell). The total percentage of paired cells was reduced towards the end of the experiment and this may be due to some of the cultures experiencing a decline in growth. When cultures were grown under a lighting regime of 12 hours dark : 12 hours light and then kept in continuous darkness for 60 hours, daily peaks in cell division were continued to be observed (Figure 21). Although these peaks occurred at approximately the same time each day as that for cultures grown under alternating light conditions, there was a decrease in the total number of paired cells counted. During the period which corresponded to 48 to 60 hours of continuous darkness, only an average of 0.4% of cells counted were found to be paired. After 10 hours of being exposed to light again, approximately 1% of the cells counted were found to be paired.

3. Observations on Motility. The motile behavior of a *Bacillaria* colony is usually regarded as consisting of rhythmic expansions and contractions. However, colonies subjected to only a minimum of disturbance for several days were observed not to display this type of behavior continuously. Following a dark treatment of 14 hours, approximately 77% of colonies

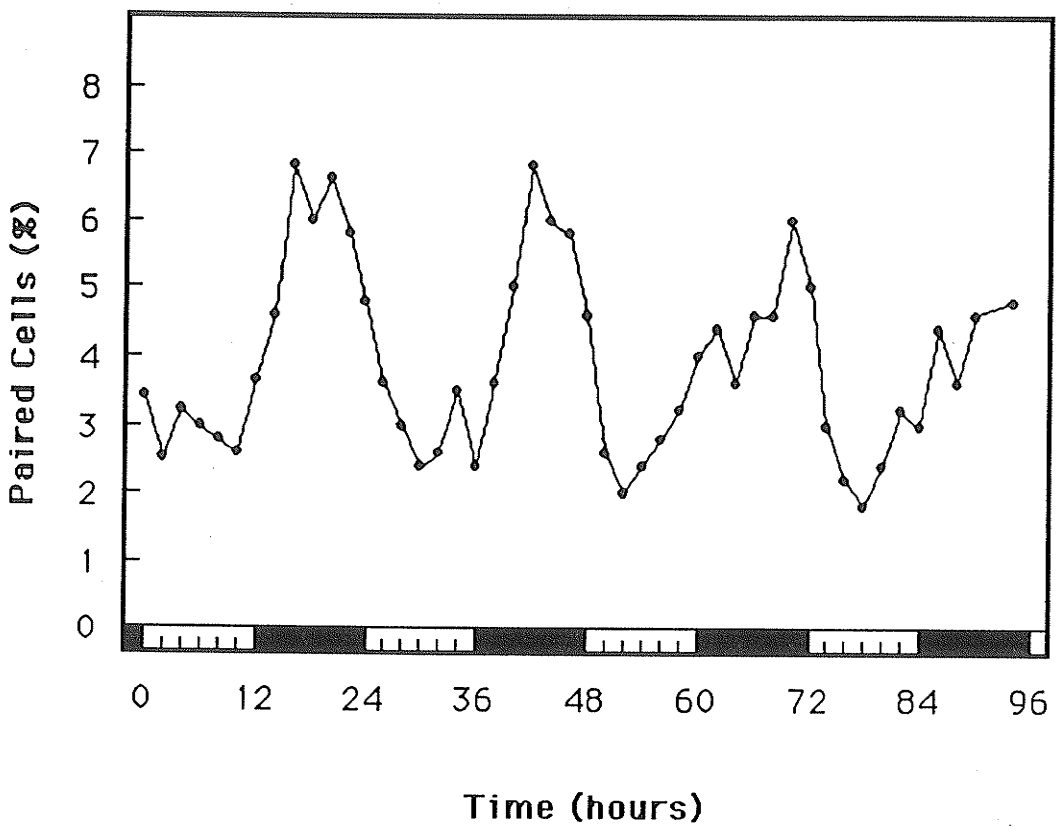


Figure 20. Rhythm in cell division of *Bacillaria paradoxa* grown in culture under a lighting regime of 12 hours light : 12 hours darkness. (Solid bars indicate periods of darkness and open bars indicate periods of light.)

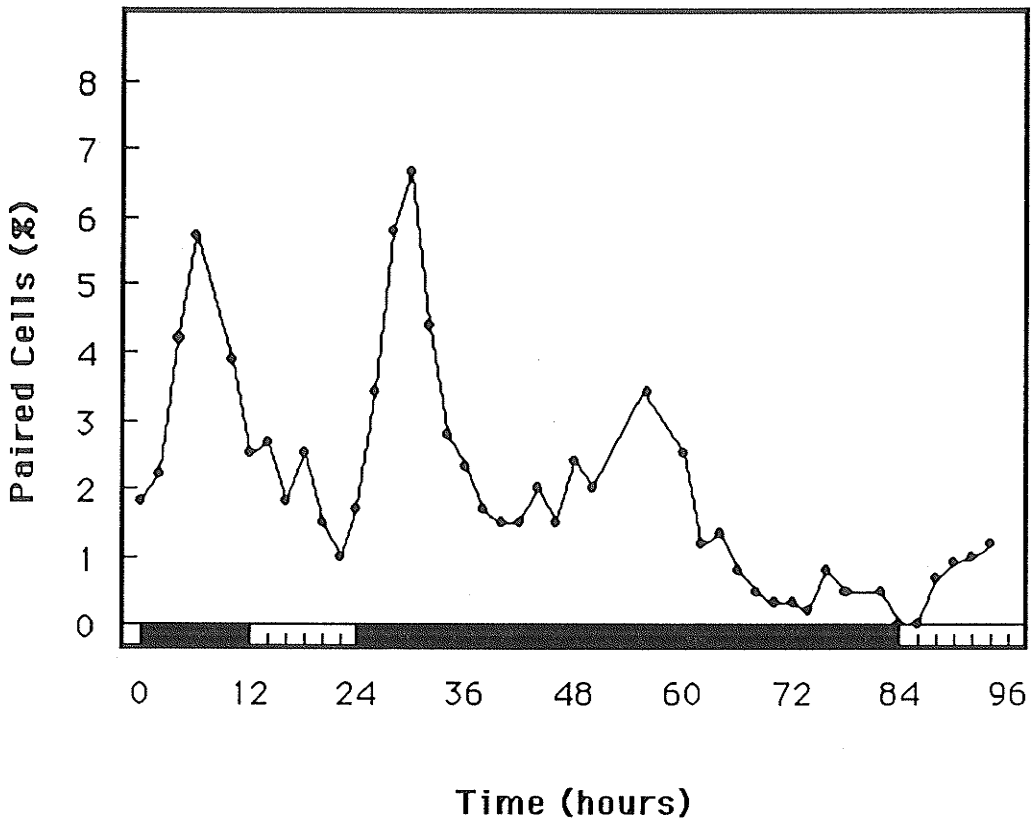


Figure 21. Rhythm in cell division of *Bacillaria paradoxa* grown in culture under conditions of darkness for 60 hours.

observed (clone I_C) were found to exhibit some movement within 5 minutes of being exposed to light with a photon flux density of 30 μmole/m^2/sec. The remaining colonies were found to be mostly aligned, contracted and non-motile, with a higher incidence of aligned colonies occurring among those colonies which were clumped together in groups. Most colonies continued to be active during the first hour of light exposure, after which time the number of active colonies gradually began to decrease as a result of colonies staying in extended positions. After two hours of light exposure, less than 1% of colonies observed were in an aligned and contracted configuration, approximately 20% of colonies were actively motile and approximately 80% were found to stay in a completely extended or nearly extended position. Throughout the remainder of the light period, the majority of colonies continued to stay extended, and after 10 hours of light exposure approximately 95% of colonies were found in this position. The other 5% of colonies showed reduced oscillatory behavior or remained non-motile in only partially extended positions. This type of behavior was observed in colonies both from culture and natural populations. Measurements of the time required for colonies to return to aligned and non-motile positions in the dark were not made.

Funk (1914) observed colonies exposed to the natural alternation of day and night behaving in a similar manner, but noted that colonies which remained extended did not show any degree of movement. However, careful examination of extended colonies revealed that some limited movement does occur. Cells in completely extended colonies often appeared as if they

were trying to move against some type of restraint. This resulted in colonies either bending into curves or rotating from side to side and sometimes flipping over. Cells, which were able to move, did so in short jerks rather than in a smooth gliding motion. Only a small number of cells within a colony, if any, showed movement at one time. Completely motionless colonies were also observed. It was possible to induce extended colonies to move by shaking and tapping the Petri dishes in which they were held. The subsequent gliding motion of cells generally appeared smooth and less jerky than that of cells left undisturbed. The degree and duration of movement depended upon the strength of the stimulus. Colonies returned to extended positions and cell movement essentially ceased usually within 60 seconds, depending on the nature of the stimulus. Colonies found in non-motile contracted states after 14 hours of dark treatment could not be induced to move using mechanical vibrations.

Preliminary observations of individual *Bacillaria* colonies removed from natural populations revealed that they were capable of maintaining continuous movement for over one hour. In these studies, colonies were transferred to drops of fresh medium, air temperature was allowed to range between 23°C and 25°C, and light was provided by a 12V 60W tungsten filament microscope lamp (Wotan, Germany) which was run between 4V and 5V. A decrease in the operating voltage of a tungsten lamp reduces the temperature of the filament and therefore changes the spectral distribution of its emission. At 12V the color temperature of the lamp used in this study is normally 3050 °K, producing light mainly in the red to infra-red portion of

the spectrum, with a peak near 900 nm. When run at 4V, the color temperature drops to approximately 2250 ^{o}K (manufacturer's specifications) and the spectral distribution moves further into the infra-red, peaking near 1200 nm (Lawson 1972). Using appropriate microscope settings, light provided by the lamp at 4V was measured to have a photon-flux density of approximately 7 μmole/m^2/sec. Video tapes of the movement of *Bacillaria* colonies under these conditions revealed that it is highly regular, with colonies consistently forming single rows of cells during each extension. The mean period of movement over an interval of 30 minutes was found to be 54.5 seconds with a standard deviation of 3.6 seconds for a colony consisting of 13 cells and 55.3 seconds with a standard deviation of 2.6 seconds for a colony of 22 cells. The velocity of cells relative to each other was measured to be approximately 13 μm/second. Cells within extended colonies remained attached to one another along their ends which overlap. During maximum extension, cells overlapped by a distance equal to approximately 15% of a single cell's length and therefore in one extension, or half cycle, each cell was capable of moving a maximum distance corresponding to 170% of its total length. Once an individual cell had moved this distance it remained motionless for approximately 12.5 seconds before resuming to glide in the opposite direction. Cell movement often first resumed near the mid-region of a colony, followed by the sequential resumption of cell movement in both colony halves. Therefore, during each colony contraction, a wave of cell start-ups occurred, originating near the centre of the colony and moving out towards both ends. The delay in start-ups was found to be less than

1.5 seconds or 2.7% of the total period. This pattern of cell movement was sustained under the above conditions for periods of over one hour. In some cases, cell movement was observed to first resume near one of the end regions of a colony. In colonies composed of over 40 cells, there often appeared to be more than one area in which cell start-ups originated, causing different parts of the colony to consistently expand and contract at different times.

Colonies grown in culture were generally observed to behave in a similar manner to colonies from natural populations, under appropriate conditions. Observations were also made of colonies in which only a portion of the cells were motile and in which cells did not extend to their maximum distance. Although unfavorable light and temperature conditions have been found to produce this type of behavior, it may also be related to the age of the cells within a colony. It was found that not every cell within a colony needed to be motile in order for regular oscillations to occur. In some cases, groups of cells were observed to actively expand and contract at one end of a colony while cells at the other end remained aligned and non-motile. In other cases, groups of motile cells were separated from each other by groups of non-motile cells. In these situations, groups of active cells would appear to oscillate in unison, independently from each other or as part of a colony moving in its characteristically co-ordinated manner, i.e., synchronization appeared to sometimes persist even if non-motile cells were found within a colony. Cells which did not move their maximum distance while oscillating often consistently moved to the same point along a neighboring cell. It was

also found that cells in the process of dividing were capable of continued movement and full extension.

Under certain conditions colonies were found to become contracted, aligned and non-motile. This occurred in colonies exposed to high light intensities, darkness, temperatures below 10°C and above 30°C and vigorous shaking.

4. Motility Rhythms. A rhythm in the appearance of motile colonies was found to exist in cultures of *Bacillaria* grown under a lighting regime of 12 hours light :12 hours darkness (Figure 22). During the entire dark period, an average of 17% of colonies counted were found to be motile, while during the light period an average of 96% of colonies were found to be motile. The majority of colonies were found to respond to changes in light conditions within the first two hours as is indicated by the sharp increase in the percentage of motile colonies found after two hours of light exposure and the sharp decrease in the percentage of motile colonies found after two hours of darkness. Variations in the percentage of motile colonies counted during periods of darkness may reflect the existence of a nocturnal peak in cell motility. However this may also be a result of inadequate shielding of light. Cultures of *Bacillaria* grown under alternating light and dark conditions and then exposed to continuous darkness, no longer showed a rhythm in the appearance of motile colonies (Figure 23). During 60 hours of darkness, the number of motile colonies always remained below 15%. A small increase in the percentage of motile colonies occurred at the 38-hour mark. This point

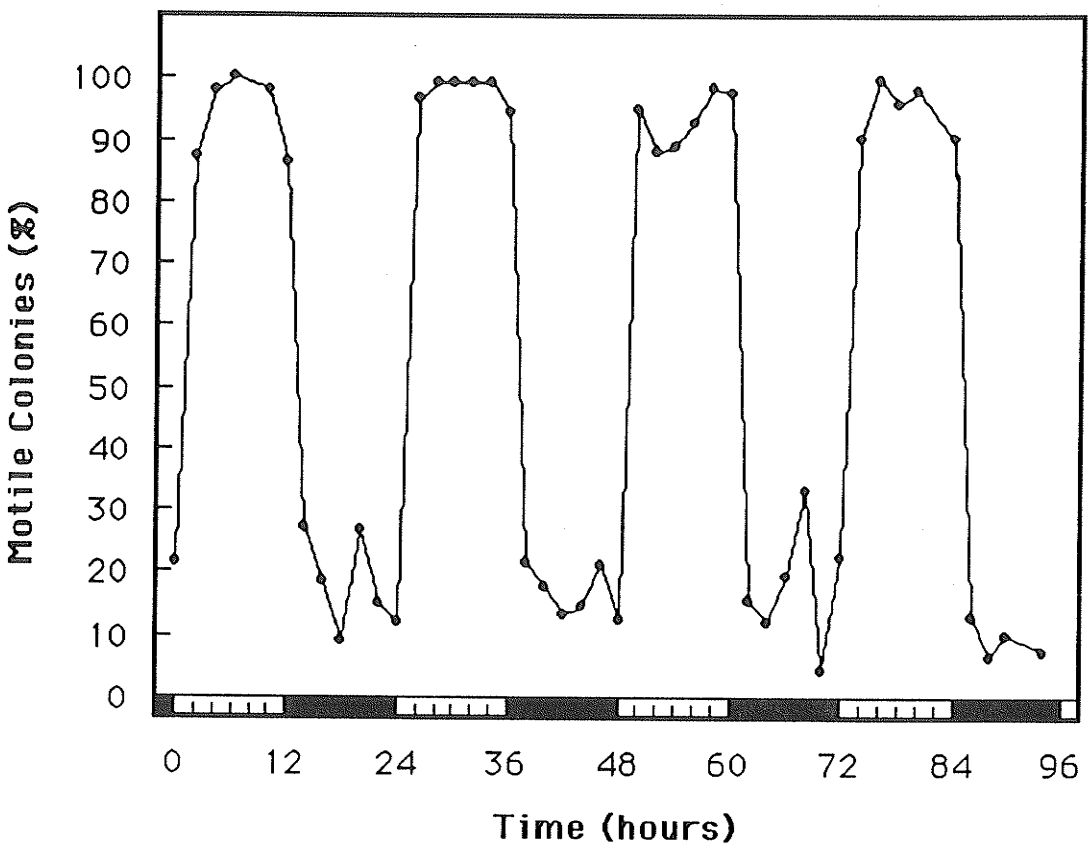


Figure 22. Rhythm in the percentage of motile *Bacillaria paradoxa* colonies occurring in culture when grown under a lighting regime of 12 hours light : 12 hours darkness.

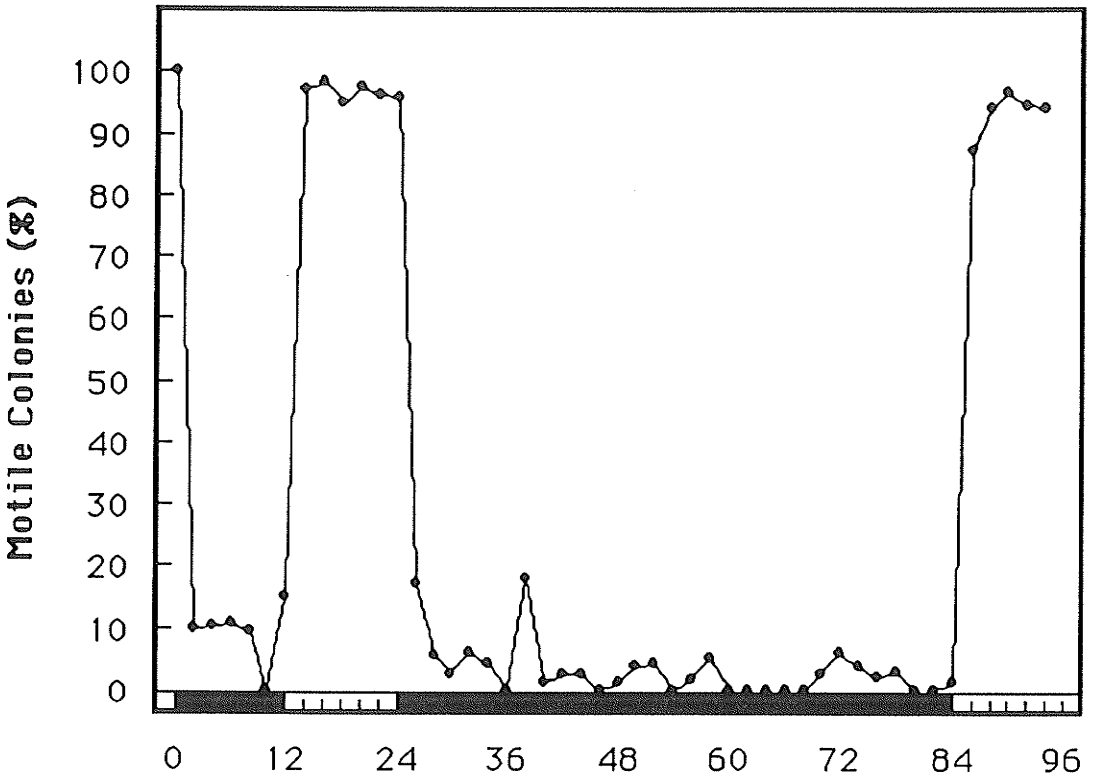


Figure 23. Effect of continuous darkness on the percentage of motile *Bacillaria paradoxa* colonies occurring in culture.

corresponds to the time at which the majority of colonies would be expected to be motile if an endogenous rhythm existed. However, the total number of motile colonies was found to be 16.2% at this point, a value which is less than the average number of motile colonies normally found during periods of darkness in an alternating light/dark regime. In order to test the viability of cells after an extended dark period, cultures were again exposed to 12 hours of light. Similar to the results of the first experiment, it was found that after the first two hours of light exposure approximately 87% of colonies were motile and that during the rest of the light period an average of 94% of colonies were motile.

5. Influence of Light Intensity on Motility. The effect of light intensity on the motile behavior of *Bacillaria* (clone I_3) colonies consisting of cells approximately 60 μm in length is shown in Figure 24. Colonies were removed at least 5 hours into the dark period and after one hour of exposure to light provided by a tungsten filament lamp producing photon density fluxes greater than 17 μmole/m^2/s, the percentage of motile cells per colony was found to drop by an average of 50%. At very high light intensities (150 μmole/m^2/s) colonies became aligned and non-motile within minutes and chloroplasts moved from normally peripheral positions to more central ones. Similar chloroplast movement was also observed in cells exposed to light with photon density fluxes of 17.6 and 49.3 μmole/m^2/s but this did not necessarily result in each cell becoming non-motile. The percentage of motile cells in colonies exposed to light providing 7.4 μmole/m^2/s and

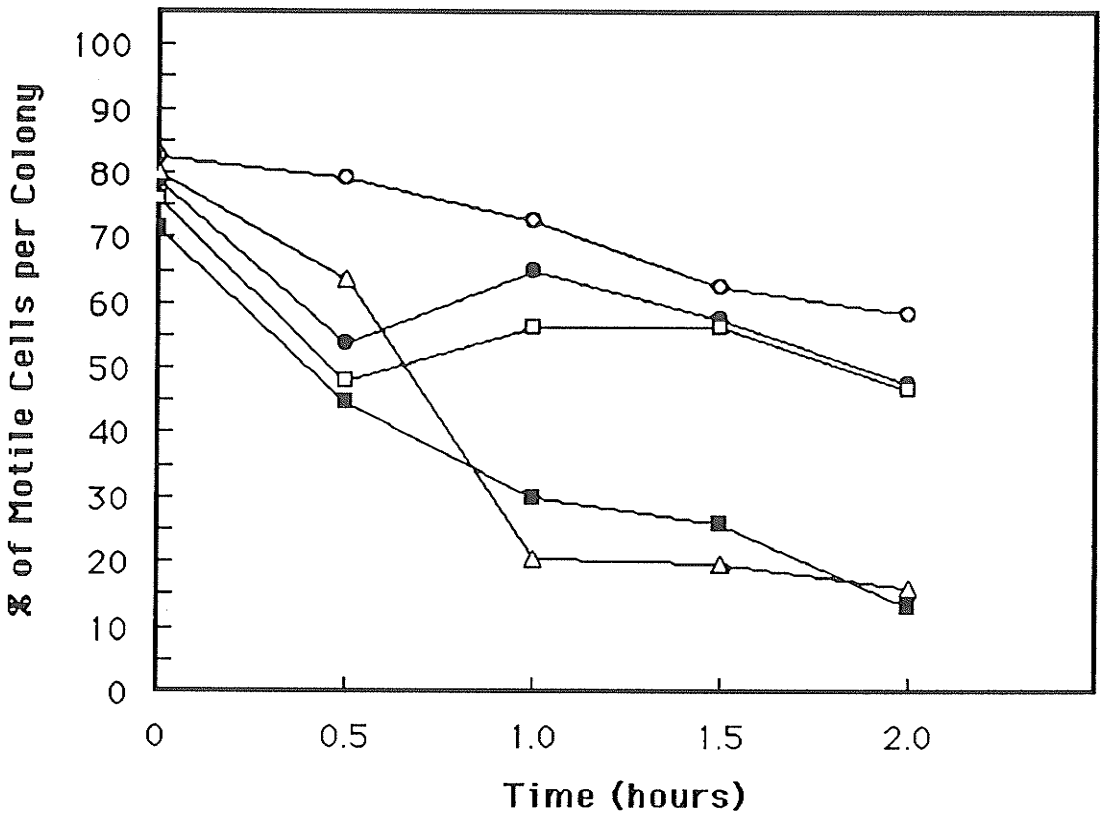


Figure 24. Effect of light intensity on the percentage of motile cells per *Bacillaria* colony. (○, 0.6 μmole/m^2/s; ●, 2.7 μmole/m^2/s; □, 7.4 μmole/m^2/s; ■, 17.6 μmole/m^2/s; Δ, 49.3 μmole/m^2/s.)

2.7 $\mu mole/m^2/s$ was found to decrease during the first 30 minutes of exposure, followed by a period of relative increase. However, after 1.5 hours of exposure the percentage of motile cells again decreased, reaching an average of about 47.2% after two hours. This suggests that cells are capable of recovering from or adapting to the ill effects of high light intensity to some degree. At intensities of 0.65 $\mu mole/m^2/s$ a gradual decrease in the percentage of motile cells occurred, dropping from an average of 82.3% to 58.4% in two hours.

For each light intensity investigated, it was found that each colony tended to become aligned as its number of motile cells decreased. In most cases, this alignment first occurred in the mid-region of the colony while cells which remained active were usually found at either one or both ends of the colony. This type of behavior differs from that of colonies observed under laboratory conditions. Such colonies were found to remain in mainly extended positions after one hour of light exposure (see Results, section3). This difference in behavior may be a result of the type of light to which the colonies were exposed. In the laboratory, illumination was provided by fluorescent lamps which emit light primarily in the visible portion of the spectrum, while in the above study, illumination was provided by a tungsten microscope lamp which emits light mainly in the infra-red region of the spectrum. Colony period measured at 30 minute intervals throughout each treatment was found to be 72 seconds with a standard deviation of 1.2 seconds. The average length of colony extension, the percentage of motile cells and cell velocity were all found to decrease over time. As cells

decreased their amplitude of oscillation, some loss in the degree of co-ordination between cells was also evident. This generally occurred in colonies in which only a relatively small number of cells remained active. In such colonies, cells appeared to move in no distinct pattern, with active cells moving in different directions at the same time. It should be noted that for all colonies only an average of only 77.6% of cells were found to be active at the beginning of each experiment (Figure 24). This may be related to such things as the health and age of individual colonies as well as to the fact that observations were made on colonies from clone I_c which had been grown in culture for over one year, the effects of which may influence motility.

6. Influence of Temperature on Motility. The motile behavior of *Bacillaria* was observed in colonies which were collected from natural populations and within several days exposed to a temperature range of 10°C to 35°C over a period of several hours using a light intensity of 7 μmole/m^2/second. It was found that colonies became non-motile and aligned at temperatures near 10°C and above 30°C. As temperatures increased from 10°C, colonies were found to gradually become more active, with cells showing an increase both in the amplitude of their oscillations and in their relative velocities. Maximum cell velocities were generally reached at temperatures near 25°C. As temperatures increased to 35°C, colonies no longer extended completely and cells gradually became less active, moving smaller distances during consecutive oscillations. However, it was found that within a single colony, groups of cells often responded to increasing temperatures at different rates.

As a result, groups of non-motile and aligned cells occurred in the same colony as groups of almost fully active cells. Stacks of cells were often found to build up in the mid-regions of colonies, with cells located at the ends being the last to become non-motile. Despite the decrease in the number of motile cells per colony, the overall rhythm of colony movement was maintained, i.e., active cells were found to move in their characteristic synchronous manner even if separated by groups of non-motile cells. At 35°C colonies were completely aligned and showed little sign of movement. Colonies kept at 35°C to 40°C for approximately 15 minutes were found to become fully active again as temperatures were allowed to decrease to 25°C. Resumption of movement was found to be gradual, beginning with one to a few cells slowly shifting back and forth against neighboring cells. It also occurred at different rates, resulting in some cells remaining aligned while others extended completely. Once cells were sufficiently cooled, however, all cells within the colonies were found to extend completely in their characteristic synchronous manner with the same frequency exhibited prior to high temperature exposure.

The influence of constant temperature on the number of motile cells per *Bacillaria* (clone I_3) colony is shown in Figure 25. Colonies remained active only at 20°C and 25°C, maintaining an average colony period of 1.2 and 0.8 minutes respectively. At 10°C and 15°C, motility essentially ceased within the first half hour of exposure, with cells gradually reducing the amplitude of their oscillations and eventually becoming aligned. At 30°C and 35°C, colonies became almost completely aligned within 10 minutes of exposure.

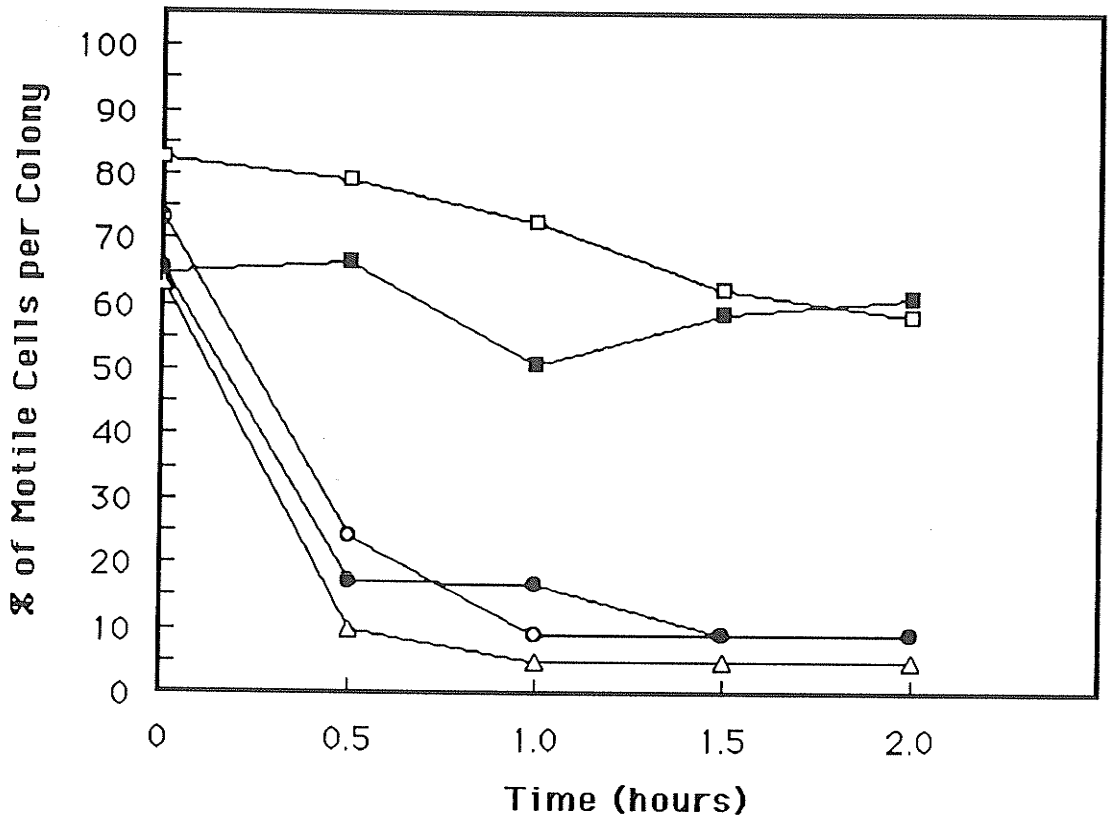


Figure 25. Effect of temperature on the percentage of motile cells per *Bacillaria* colony (clone I_3). (o, 10°C; ●, 15°C; ❑, 20°C; ■, 25°C; Δ, 30°C.)

DISCUSSION

Although diatoms have been successfully grown in culture since the late 19th century, many of the large culture collections maintain relatively few species of diatoms (Eppley 1977; Czarnecki 1987). This is especially true for freshwater diatoms, a fact which can cause considerable difficulty for researchers wishing to experiment with species that are not regularly available from local sites. *Bacillaria paradoxa* was found to be a species not initially available from culture collections and available only sporadically from natural algal populations in the prairie provinces. In response to the lack of available diatom cultures, David Czarnecki of Loras College in Dubuque, Iowa has recently established a large freshwater diatom collection which currently represents 161 taxa from 43 genera, including *Bacillaria paradoxa* and in 1987 a description of this collection was published listing all available cultures.

Colonies of *Bacillaria* isolated from Delta Marsh and obtained from the Loras College culture collection were found to grow very well in culture, with no loss of motility and few, if any, conspicuous morphological changes, aside from cell size reduction, occurring. A cursory comparison of growth rates between *Bacillaria* cells grown in a solely inorganic medium and cells grown in an inorganic medium supplemented with soil extract indicated that the presence of soil extract results in an increased growth rate. This type of growth response to the addition of an organic substance has been observed for numerous diatoms (Lewin and Guillard 1963; Patrick and Reimer 1966;

Prakash *et al.* 1973). Although the response to organic substances derived from different sources is almost identical, the manner by which it is brought about is not clear. Many diatoms have vitamin requirements which may be met by the addition of organic matter into the media. However, in this study, *Bacillaria* was grown in an organic medium to which the vitamins B_{12}, thiamine and biotin were already added. It has also been suggested that organic substances have considerable metal-complexing capacity, allowing trace metals to be more readily utilized by diatoms (Prakash *et al.* 1973; Eppley 1977). Alternately, the presence of organic matter in the medium may be required for the growth of bacteria which may in turn provide substances which lead to the improved growth of diatoms in culture (Pringsheim 1946; Kain and Fogg 1958; Round 1981; Czarnecki 1987).

Cultures of *Bacillaria* grown in test tubes containing 15 ml of medium were generally found to reach a stationary phase of growth within 14 days after inoculation (Figure 12). This necessitated the transfer of a portion of the culture material to tubes containing fresh medium on almost a weekly basis. However, the inconvenience of frequent transfers was overshadowed by the ease of handling colonies for study afforded by small volumes of culture material. A variety of factors can contribute to the end of exponential growth in culture including the exhaustion of nutrients, a limited CO_2 supply, a change in pH, the reduction of light intensity due to self-shading, and autoinhibition due to the accumulation of toxic substances produced by the algae themselves (Fogg 1975). The chemical changes which occurred in

cultures of *Bacillaria* as they progressed from exponential growth to a stationary phase were not examined. However, there is no doubt that a reduction in light intensity due to self-shading did occur but whether this was sufficient to limit exponential growth is not known. It seems quite likely that the rate of diffusion of CO_2 into the culture from the air may be limiting since, aside from the periodic shaking of test tubes several times daily, cultures were essentially stagnant.

The exponential growth rate of *Bacillaria* showed a small variation between clones, with values ranging between 0.9 and 1.0 divisions per day (Table 2). Clonal variations in the growth responses of diatoms have been reported for a number of different species, with the magnitude of deviations between subclones often increasing with conditions unfavorable for growth (Holt and Smayda 1974; Seaburg *et al.* 1981; Edenhofner and Schmid 1984). It has been suggested that these variations in growth responses may be related to the history of stock cultures prior to pre-conditioning for experimental use (Seaburg *et al.* 1981). In this study, growth rates were compared between clones, which although grown under identical conditions, were maintained in culture for varying lengths of time. The effect of culture age is partially reflected in the differences observed in cell size and colony size (Table 2). These differences, along with others undetected, may have had an influence on the growth response of individual clones. For example, clones with a larger average cell size were found to divide more quickly and form smaller colonies. This suggests that there may be a limit on the maximum length

attainable by colonies. Table 2 shows that for clones I_3 and I_5 the mean colony length was approximately 2000 µm, while for clone I_c the mean colony length was 1200 µm. Although smaller diatoms generally grow more quickly than larger species, the trend does not necessarily hold true for comparisons within species (Eppley 1977), as was observed in this study.

Although the average cell size of all *Bacillaria* clones grown in culture was found to reduce over time, no auxospore formation or other type of size regeneration was observed. Auxospore formation in pennate diatoms usually takes place in cells that are reduced to a certain minimum size; above and below this size auxospore formation will not occur. If it does not, cells will continue to divide but will become smaller and smaller, showing various abnormalities and eventually dying out (Geitler *in* Fritsch 1956). This was observed in clone I_2 which died out after reaching a length of 29 µm. Generally, the size of diatom cells capable of sexualization has been found to be between 30% and 40% of the maximum valve length within a species-specific size range (Drebes 1977). Therefore, it is expected that auxospores would develop from *Bacillaria* cells which range from 45 µm to 60 µm in length. However, studies have shown that sexual reproduction depends not only on a certain critical size but also on specific environmental conditions which vary depending on the species (Drebes 1977). Factors such as temperature, light intensity, daylength, salinity and nutrition have all been found to play a role in the formation of auxospores (Steele 1965; Drebes 1966, 1977; Holmes 1966; Mizuno and Okuda 1985). Generally the conditions which

promote vegetative growth also allow the sexualization of diatoms but in some instances a sudden change in temperature and/or light intensity is first required (Drebes 1977). Cultures of *Bacillaria* in this study were not subjected to any such changes and this may have prevented the formation of auxospores.

The phenomenon of cell size reduction in diatoms raises many interesting questions since, as pointed out by Round (1972), the mechanism of cell division is assumed to be similar for all diatoms despite the fact that they do not all show a decrease in size along the apical axis. Round (1972) and Crawford (1981) have presented several different schemes for diatom division which do not assume that all components of the silica frustule are rigid and that use the position of valves and girdle bands to account for size reduction or the lack thereof. However, the exact arrangement of girdle bands and the manner in which they are joined to the valves is often hard to determine, presenting some difficulties in gaining a further understanding of the events leading up to cell size reduction in diatoms.

Cultures of *Bacillaria* (clone I_3) grown under a lighting regime of 12 hours light : 12 hours darkness were found to exhibit a rhythm in cell division which peaked approximately 6 hours after the onset of the dark period. This type of rhythmic behavior in cell division has been recorded for a number of different algae, including *Gonyaulax*, *Euglena* and *Nitzchia* (von Denffer 1949; Sweeney and Hastings 1958; Edmunds 1966). By definition, a circadian rhythm is a rhythm in behavioral or physiological activity which has a period of approximately 24 hours that is maintained under conditions

of constant irradiance and temperature. Not all rhythms showing a 24 hour period under conditions of alternating light and dark are, therefore, necessarily circadian. In the case of cell division, it is often common to have a generation time of approximately 24 hours. Therefore, if partial synchrony of division occurs, the resulting rhythm in cell division can mimic a circadian rhythm. In *Gonyaulax*, the generation times for different populations were found to always be multiples of 24 hours, yet cell division occurred only at the expected time in each 24 hour period. Therefore, the observed rhythm in cell division is not due to the synchronization of generation times since that would yield rhythms having periods greater than 24 hours (Sweeney and Hastings 1958). The generation time of *Bacillaria* (clone I_3) was measured to be 0.91 days and therefore, in order to distinguish between partial synchrony of cell division and an endogenous circadian rhythm, cultures were grown under constant conditions. Since many organisms have been found to lose their rhythmicity in bright, continuous light (Harris and Wilkins 1976), cultures were kept in continuous darkness. Although this method has been successfully used for other algae (Sweeney and Hastings 1957), it also presents some limitations since *Bacillaria* is a photosynthetic organism and continuous darkness will eventually lead to energy exhaustion. It was found that during 60 hours of darkness *Bacillaria* (clone I_3) did continue to show peaks in cell division at approximately 24 hour intervals, although, as expected, these peaks became successively smaller over time (Figure 21). These results are very similar to those found for *Gonyaulax* (Sweeney and

Hastings 1957), suggesting that the observed rhythm in cell division may be an endogenous circadian rhythm. However, the after effects of a light-dark cycle sometimes persists for more than 24 hours (Sweeney 1980) and consequently it is necessary to investigate further aspects of this rhythm before concluding that it is circadian. Other characteristic properties of circadian rhythms include the almost complete independance of period on changing temperature, as well as, an observable shift in phase in response to single or repeated exposures to light or to darkness.

Cultures of *Bacillaria* (clone I_3) grown under a lighting regime of 12 hours light : 12 hours darkness were also found to exhibit a rhythm in cell motility, with the majority of colonies being active during the light periods and inactive during the dark periods (Figure 22). Certain benthic diatoms, both from intertidal and freshwater sediments, show a similar type of rhythm in vertical migration (Round and Eaton 1966; Round and Palmer 1966; Round 1979). In order for these cells to maintain themselves on the sediment surface in the presence of continued sedimentation or other disturbances, they must be continually motile. Therefore, a rhythm in vertical migration can be considered to represent a rhythm in cell motility. This has been confirmed by laboratory studies in which rhythms in both the speed of movement and net distance travelled have been found for migrating diatoms, with peaks in movement corresponding to periods during which cells would have been found on the sediment surface (Round 1978; Happey-Wood and Jones 1988). Migration rhythms have been found to continue in the laboratory under alternating light conditions, continuous light and continuous darkness,

suggesting that they are endogenous. In some cases, this is further supported by the fact that the upward migration of diatoms has been found to occur at least 2 hours before natural dawn and therefore is not necessarily triggered by light (Round and Eaton 1966). Cultures of *Bacillaria* (clone I_3) grown in continuous darkness did not show a rhythm in cell motility (Figure 23). Colonies remained mostly aligned and contracted during the entire dark period and did not become motile until re-exposed to light. In addition, cultures grown in an alternating light/dark regime did not exhibit any motility prior to light exposure, i.e., there was no anticipation of "dawn". These results suggest that the rhythm in motility observed in *Bacillaria* is not endogenous but may be due to some type of photokinetic response. It should also be noted that although the effect of continuous light on *Bacillaria's* motility rhythm was not thoroughly investigated, it was found that if colonies obtained from culture at the end of a 12 hour light period were kept illuminated rather than allowed to enter the next dark period, they continued to show typical light behavior.

Exposure to light in culture causes *Bacillaria* colonies to remain in mainly extended positions although they can be easily induced to move through some form of mechanical disturbance. It is likely that in natural situations such colonies remain active since they generally will not be isolated from disturbance. *Bacillaria* is often found in association with sediments and it can be speculated that the purpose of its motility in light is to avoid being buried by shifting sediments, thus allowing maximum light exposure for photosynthesis. Colonies found contracted after an extended

dark period were not induced to move through mechanical disturbance. Therefore, if light is the only stimulus which will induce colonies to move after a dark period, then it is necessary, for continued movement, that colonies are not covered in sediment during one night's exposure to such an extent that light is no longer is able to penetrate to them in the morning.

Positive photokinetic responses, i.e., increased movements due to light, are known to occur in several different diatoms (Nultsch 1970, 1971). Cells generally accelerate their movements up to an optimum light intensity, after which, movements decrease and may eventually cease. This type of response was also found to occur in *Bacillaria* (clone I_c) colonies. Maximum rates of movement were obtained at light intensities which provided approximately 0.6 μmole/m^2/second, above this, movement gradually decreased, until at intensities providing 150 μmole/m^2/second, cells became aligned and non-motile. It should be noted that the sensitivity of diatoms to light depends partly on their metabolic needs and therefore, values of the number of photons per unit area per unit time obtained for maximum rates of movement may be only test-specific. The response of organisms to light also depends on wavelength. Nultsch (1971) measured the photokinetic action spectrum of the diatom *Nitzchia communis* and found that maximum motility occurred near 670 nm, with the range of activity extending to 800 nm. Tests in this study were performed using a tungsten filament lamp which produced light mainly in the red to infra-red portion of the spectrum. Although this includes wavelengths known to produce maximum rates of activity in other diatoms, it essentially eliminates wavelengths less than

500 nm, which may also play a significant role in motility. Positive and negative phototactic responses have also been found to occur in motile diatoms (Nultsch 1971). Two types of tactic responses have been distinguished, photo-topotaxis, in which movement towards or away from a light source occurs and photo-phobotaxis, in which a reversal in the direction of movement occurs, regardless of the direction of the light, due to a sudden decrease or increase in light intensity. These types of responses are usually evaluated by observing the migratory behavior of cells on a microscope slide or on an agar plate. The evaluation of phototactic responses in *Bacillaria* is difficult due to the regular reversal in direction of motile cells, as well as, the fact that individual cells can only move a limited distance in each direction, never gliding past their neighboring cells.

Motile diatoms have been found to remain active at temperatures of 0°C through to 35°C, although Hopkins (1963) found cells to be most active between 10°C and 17°C. *Bacillaria* (clone I_C) colonies were found to become non-motile below 15°C but remained active up to temperatures near 30°C. Although the temperature induced immotility was found to be reversible at both extremes, the maximum length of time for which cells could be exposed and still become motile again is not known. Colonies exposed to extremes at both ends of the temperature scale were consistently found to become aligned as motility ceased, suggesting that both cell motility and the positioning of cells relative to one another are influenced by temperature extremes in the same manner.

One of the characteristic features of *Bacillaria* colonies is the fact that

cells always remain attached to one another as they glide back and forth along each other. Since the work of Drum and Pankratz in 1965, in which cross-sections of *Bacillaria* frustules were examined, it has generally been believed that neighboring cells are joined together by interlocking silica protrusions which appear as hooks in cross-section (Figures 4 and 5). However, Drum and Pankratz admit to never actually seeing the hooks of adjacent cells interlocked, attributing this to cell displacement during electron microscope preparation. Later, Drum *et al.* (1966) suggested as an alternative, that the silica protrusions may simply fit into the outer raphe grooves of adjacent cells without interlocking. Examination of *Bacillaria* frustules at high magnification with scanning electron microscopy shows that the silica protrusions occurring next to the raphes curve in such a way as to almost touch the valve faces (Figures 6,16, 17 and 18). This, along with the fact that broken raphe protrusions were never observed, despite cells being separated by boiling acid, suggests that the protrusions do not interlock but rather, fit into the grooves of adjacent cells as proposed by Drum *et al.* (1966). This interpretation is consistent with the behavior of colonies which have been observed to bend into almost complete circles while maintaining cell to cell attachment (see Results, section 3). However, this interpretation of colony configuration also raises anew the question as to what holds adjacent cells together and prevents them from gliding completely past one another.

Jarosch (1958) observed that extended colonies, constrained at both ends, would bend into arcs and then rapidly snap back into straight lines once the constraints were removed. This led him to suggest that the substance holding

cells together was very elastic. Funk (1914) and Kamiya (1959) believed that cells were attached by extra-membraneous cytoplasm but no evidence for such cytoplasm has been found for any motile diatoms including *Bacillaria* (Drum and Pankratz 1965). One means of cell to cell attachment that has not been considered for *Bacillaria* is raphe mucilage. Although most chain forming diatoms do not possess raphe systems, several electron microscopy studies have been done on the filamentous and raphed diatom *Navicula confervacea* var. *confervacea* (Rosowski 1980; Rosowski *et al.* 1983). Strands of mucilage, perpendicular to the valve surface, were found to occur along the entire paired raphe system of this diatom and it has been speculated that these strands attach end on end, maintaining the valve to valve connections. Marginal spines have also been found to occur between adjacent valves but it is believed that they mostly function to restrict lateral movement and contribute little to cell adherence. Similar strands of mucilage have been found to occur in the raphes of motile diatoms (Drum and Hopkins 1966; Edgar and Pickett-Heaps 1982; Edgar 1983) and it is likely that, although as of yet not demonstrated, they also occur in *Bacillaria,* playing a role both in cell adherence and motility.

The organic features of diatom cells are not preserved in specimens that are chemically cleaned and it is often necessary for them to be critical-point dried in order to observe such features. However, examination of acid and peroxide cleaned *Bacillaria* frustules did show that all external pore openings were occluded with silica (Figures 6, 16, 17 and 18) and therefore, any mucilage excreted is done mainly through the raphes. Cytochemical

investigations of raphe mucilage from motile diatoms indicate that it is an acid mucopolysaccharide which is soluble once secreted, being easily removed during fixation and dehydration processes and that it originates from cytoplasmic vesicles near the raphes (Edgar and Pickett-Heaps 1982). Although usually seen in the form of strands, Rosowski *et al.* (1983) point out that not too much emphasis should be placed on their size and shape since the mucilage is highly hydrated and it is probably modified in shape and position during dehydration and critical-point drying processes. The strong adhesive powers of raphe mucilage are well documented (Drum and Hopkins 1966; Harper and Harper 1967) and if one considers two *Bacillaria* cells, each excreting mucilage through their adjacent raphes, it is likely that the mucilage from each cell will stick together, perhaps even coalescing, to form a continuous link between the cells. It could then be speculated that if the raphe protrusions fit closely into their corresponding grooves so that a tube of silica is created around the mucilage, the mucilage link between cells would be protected from dissolving rapidly into the surrounding medium. Then, as the cells glide past one another, the mucilage in the exposed portions of the raphe would have an opportunity to dissolve, preventing an excessive build-up of mucilage between the cells.

The flow of mucilage in each raphe can be assumed to occur at an equal and steady rate in one of two directions or, to not occur at all. Based on this assumption, the characteristic movement of *Bacillaria* cells can only take place if, 1) the flow of mucilage in adjacent raphes occurs in opposite directions and that these directions are reversed after colonies reach

maximum extension, or if, 2) mucilage flows in only one pair of adjacent raphes, with flow occurring in the same direction for all cells and reversing after colonies reach maximum extension (Figure 26). It is not possible to distinguish between theses two possibilities by simply observing motile cells within an active colony since the only frame of reference is the colony itself. However, Jarosch (1958) reported that the relative velocity of a living *Bacillaria* cell against a dead one was half the relative velocity of two living cells and this strongly suggests that mucilage flow occurs in both sets of raphes in active cells.

Cells within *Bacillaria* colonies never glide completely past one another but always remain attached and overlapped for a distance which represents at least 15% of a single cell's length for all clones examined. Examination of chemically cleaned frustules has shown that there are no siliceous structures on the valve surfaces which could act to prevent cells from gliding past this point (Figures 2, 6, 7, 14 and 16). It can also be seen that the raphe fissures, through which mucilage is excreted, extend along approximately 99% of the entire cell length and presumably, therefore, do not limit cell movement. It is not known if an organic structure is responsible for stopping cells from continued movement since, as stated earlier, organic features are not preserved in chemically cleaned specimens. However, organic structures are usually associated with specialized pores (Lee, 1980) and no evidence was found for such features in *Bacillaria*.

The fixed distance through which cells move, rather than reflecting a structural restraint, may reflect a limitation of the motility mechanism.

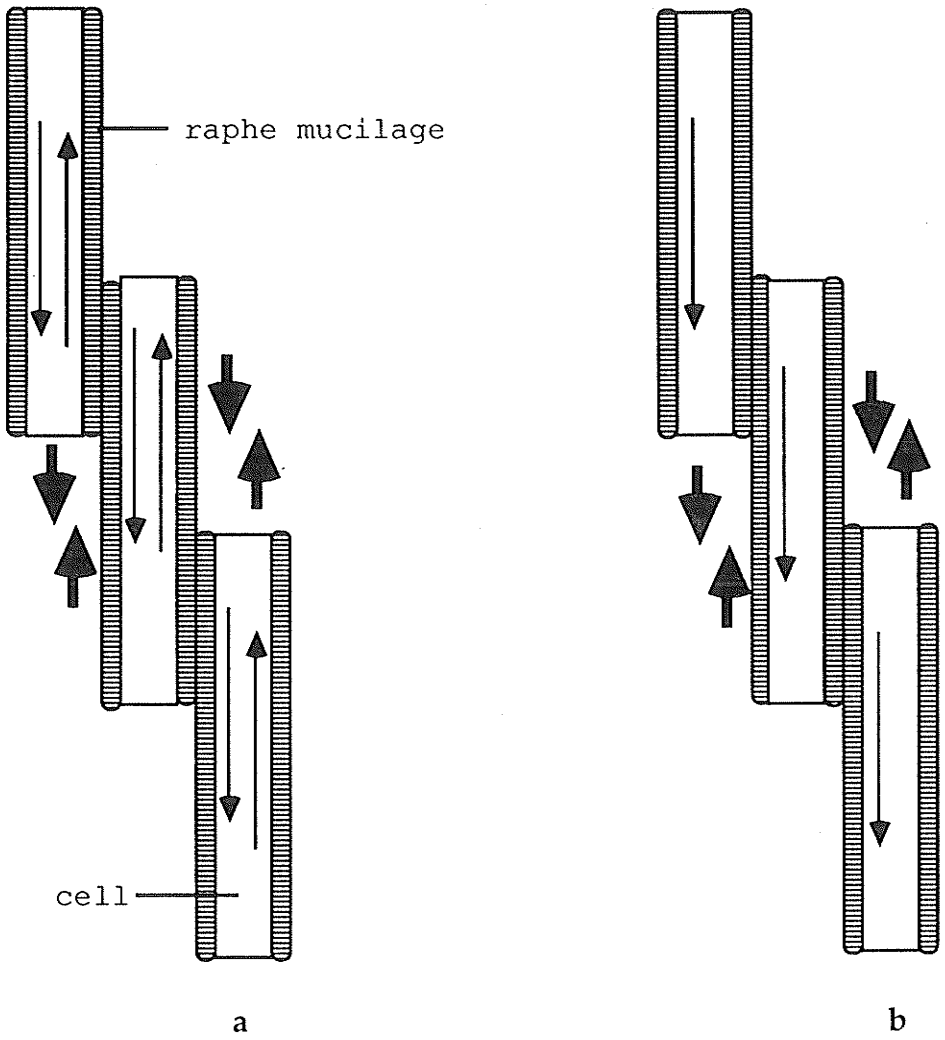


Figure 26. Schematic drawings of a 3-celled *Bacillaria* colony indicating the required direction of mucilage flow if a) both raphes are active and if b) only one raphe is active. (Long, thin arrows indicate direction of mucilage flow and short, fat arrows indicate direction of relative movement between cells.)

Although the exact cellular mechanism for diatom gliding has not been established, it is clear that both mucilage excretion and microfilament action are involved. It is believed that mucilage is excreted into the raphe through the exocytosis of polysaccharide containing vesicles located near the central and apical raphe endings. The amount of mucilage released, therefore, depends, in part, on the size and number of vesicles in which exocytosis occurs. According to the microfilament model for diatom motility (Edgar and Pickett-Heaps 1983), the movement of only a few strands of mucilage are required for cell locomotion to occur. However, according to the capillarity model (Gordon and Drum 1970; Gordon 1987), a continuous supply of raphe mucilage is required. Based on the latter model and the fact that diatoms are essentially unaffected by inertial forces, it can be speculated that motile diatoms move distances directly proportional to the amount of mucilage excreted. Single motile diatoms have often been observed to pause for brief periods of time after which they either continue along in the same way or reverse direction (Edgar 1979). In the case of *Bacillaria*, it can be speculated that as colonies move from complete extension to complete extension, cells release only enough mucilage to allow them to glide to a point where they still overlap at their ends. After a brief pause, cells again glide the same distance but in the opposite direction. This change in direction may be a result of equal amounts of mucilage being alternately excreted at opposite ends of the cells (Gordon 1987).

The limit set on the distance travelled by *Bacillaria* cells in one oscillation may also be related to the activity of the microfilament bundles

found near the raphes. Little is known about the mechanics or biochemistry of microfilamentous systems in diatoms but similar systems in other non-muscle cell types are known to require chemical energy in the form ATP (adenosine triphosphate) and are thought to be regulated by a variety of mechanisms. According to the microfilament model for diatom motility, the flow of mucilage strands in the raphe, and therefore cell movement, will stop if the activity of the microfilament bundles is interrupted. The capillarity model does not require any mechanochemical involvement of the microfilament bundles. Their presence is interpreted as possibly being for control of the release of the mucilage. They may move in such a way as to either cover both pores at the raphe endings, preventing mucilage excretion, or uncover both pores, resulting in no net motion. Therefore, cessation of movement in the microfilament model depends on the inactivation of the microfilamentous system while in the capillarity model cessation of movement in a previously motile cell requires activation of the microfilament system in order to cover or uncover both pores.

Prior to reversing direction, cells in active *Bacillaria* colonies exhibit no movement for a time period of approximately 12 seconds at room temperature. During this time, the psuedo-filamentous configuration of the colony is maintained and cells do not appear to drift apart from each other. The properties of some mucopolysaccharides suggest that it is possible for raphe mucilage to firmly hold non-motile adjacent cells together as well as allow relative shifting between motile cells. These polymers are high molecular weight heteropolysaccharides formed by the polymerization of

repeating disaccharide units made up of hexosamine and a hexuronic acid linked by glycosidic bonds (Varmer and Varmer 1983). Some acid mucopolysaccharides, such as hyaluronic acid, are known to have anomalous viscosity, i.e., their viscosity changes with the rate of shear (Phelps 1972). In the case of hyaluronic acid, viscosity is almost infinitely high at very low shear rates while at high shear rates the viscosity collapses and the polymer lines up with the streamlines of flow. If raphe mucilage has a similar type of viscosity, it may provide a rigid enough bond under conditions of non-motility to prevent cells from separating, while not resisting their motion once sufficient force is produced.

Aside from the regular pause in cell movement that occurs between colony oscillations, non-motility in *Bacillaria* is known to occur under two other sets of conditions. If cells are exposed to sufficiently high light intensities, produced either naturally or by fluorescent lamps, they generally maintain extended positions until light intensity is reduced or until colonies are mechanically disturbed. This inhibition of the motility mechanism may be related to mucilage production, vesicle exocytosis, microfilament activity or a combination of all three. As in active colonies, the connection between adjacent cells is maintained to such a degree as to prevent any drifting, even for periods of time exceeding 12 hours. The fact that the majority of colonies become non-motile only in fully extended positions and that some cells appear as if they are trying to move against some kind of restraint, suggests that once the motility mechanism is interrupted, as normally happens between oscillations, it is unable to generate enough force under these

conditions to overcome the high viscosity of the raphe mucilage. If *Bacillaria* colonies are exposed to very high light intensities, darkness, upper and lower temperature extremes, vigorous shaking they become non-motile as well as aligned. Under these conditions it is likely that the motility mechanism completely shuts down. As the motility mechanism of one cell shuts down, it may reduce the velocity of its neighboring cells. This will increase the viscosity of the raphe fluid and if friction is proportional to the amount of cell overlap then adjacent cells will become aligned until sufficient force is produced to overcome the high viscosity of the raphe mucilage. If mucilage strands are moved through the raphes by microfilament action then perhaps the alignment of non-motile cells is related to the positioning and length of microfilament bundles found beneath the raphes.

The characteristic movement of active *Bacillaria* colonies requires that each cell starts and stops gliding almost simultaneously. There is only a small delay in start-up between cells and this delay appears to travel from cell to cell throughout an entire colony. Behavior of this type strongly suggests that some means exists whereby cell movement can become co-ordinated. If it is assumed that mucilage moves through both sets of raphes in motile cells, then the direction of movement needs to be co-ordinated in such a manner that mucilage flows in the same direction in each pair of raphes of the same valve, with that direction being opposite to the direction of mucilage flow in the raphe pair of the opposite valve. Cells which move from a position of complete extension must secrete mucilage from the apical pores located at the cell ends which overlap. Therefore, if a single cell within a colony is

considered, mucilage is secreted at opposite ends from alternate valves. From this model it follows that the direction of mucilage flow, if it occurs, can only do so in opposite directions in adjacent raphe pairs. Mucilage may also be secreted at other points along the raphes but this should not initially contribute to any net movement since cells which are completely extended are not in contact with a substrate for most of their length. As cells glide past one another, mucilage needs also to be secreted from the second raphe in a pair in order for cells to completely extend in the opposite direction. If the flow of mucilage is the result of capillarity forces (Gordon 1970, 1987) and assuming that there is a constant supply of it, then the access of vesicles to the raphes and the release of their contents needs to be controlled in order to co-ordinate cell movement. It has been speculated (Gordon 1987) that vesicle access to raphes may be controlled by the sliding of microfilaments relative to one another. However, in the above discussion it was noted that according to this model, the transition from a motile to a non-motile cell requires the microfilament system to actively close or open both pores. If this system is inhibited and if the inactive microfilaments remain in positions similar to those prior to inhibition, then gliding may continue as long as mucilage is still secreted. But it has been shown that gliding motility in diatoms is inhibited by drugs that are known to disrupt actin filaments in other cell types (Webster *et al.* 1985). Although this suggests that microfilament bundles do not actively regulate pore access, they may still play a role in the regulation of the movement of the mucilage containing vesicles or in their discharge.

If it is assumed that the flow of mucilage through the raphes is due to

the displacement of mucilage strands by microfilaments (Edgar and Pickett-Heaps 1984), then co-ordination between cells requires the regulation of microfilament activity, as well as mucilage secretion. Microfilament sliding must begin almost simultaneously in adjacent cells and must continue long enough so that at least some strands are carried almost to the cell ends. If adjacent cells are held together only by raphe mucilage then there will be a microfilament to mucilage to microfilament connection between these cells. It is possible that the microfilament activity in the bundles of one cell may induce the microfilaments of adjacent cells to also become active and if there is no inhibition of the motility mechanism through a colony, it can be conceived that the oscillations of all the cells will become entrained. In *Bacillaria,* therefore, the means by which cells communicate to one another may be raphe mucilage. Although this model provides for a connection between microfilament bundles of adjacent cells, it is not known if there is a means by which microfilament bundles in opposite halves of a single cell may be connected or what may cause them to move synchronously and the capillarity model has the same short coming. In order to discern between the validity of the capillarity and microfilament models of gliding motility it is necessary to gain a further understanding of the nature of raphe mucilage, especially of its physical properties, as well as, the mechanics and biochemistry of the microfilamentous system found in diatoms.

Motility for an organism such as *Bacillaria* may be important for several reasons. Although *Bacillaria* is photosynthetic, it lives primarily in the benthos where light generally penetrates only to a few millimeters. The

ability to move through the sediment becomes very important, therefore, if photosynthesis is to continue. Colonies that become part of the plankton or that remain on the sediment surface are exposed to relatively bright light. Laboratory studies indicate that under these conditions colonies tend to remain in mainly extended positions. This response may be important in reducing the rate at which colonies sink through the water column and may also maximize photosynthetic rates. If colonies experience a reduction in light intensity, by sinking lower in the water column or by becoming covered in sediment, they may respond by once again becoming motile. The aligned and non-motile response of colonies to temperature and light extremes would result in a more rapid rate of sinking and may allow colonies to find more suitable conditions for continued growth.

In order to understand the movements of *Bacillaria* colonies it is necessary to understand the means by which they attach to one another. Although it has been speculated in this thesis that attachment is accomplished via raphe mucilage, this has yet to be confirmed. Therefore, further SEM studies using critical point drying techniques, which preserve the organic features of cells, may provide important information as to the existence and/or nature of mucilage bonds. The behavior of *Bacillaria* colonies is such that non-motile and motile conditions can be predicted with a high degree of certainty. This is not as easily done with single motile diatoms and therefore, studies of *Bacillaria* which involve the use of transmission electron microscopy to examine the configurations of microfilament bundles and polysaccharide vesicles of cells in different motile

states may also yield important results for the further understanding of the motility mechanism in all motile diatoms.

BIBLIOGRAPHY

ADMIRAAL,W. 1984. The ecology of estuarine sediment-inhabiting diatoms. In: Round, F. E. and Chapman, D.J., (Eds.). *Progress in Phycological Research. Vol. 3.* Biopress Ltd., Bristol, pp. 269 - 322.

ALLEN, R.D. 1981. Motility. *J. Cell Biol.* 91(3): 148s-155s.

BEAVER, J. 1981. Apparent Ecological Characteristics of Some Common Freshwater Diatoms. Rexdale, Ontario: Plankton Taxonomy Unit, Limnology and Taxonomy Section, Ministry of the Environment.

BRAND, L.E., SUNDA, W.G., GUILLARD, R.R.L. 1983. Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. *Limnol. Oceanogr.* 28(3): 1182-1198.

BRUCE, V.G. AND PITTENDRIGH, C.S. 1956. Temperature independance in a unicellular 'clock'. *Proc. natn. Acad. Sci. U.S.A.* 42: 676-682.

BUCK, J. AND BUCK, E. 1976. Synchronous fireflies. *Sci. Am.* 234(5): 74-85.

BURKHOLDER, P.R. 1934. Movement in the Cyanophyceae. *Quart. Rev. Biol.* 9: 438-459.

CALJON, A.G. 1983. *Brackish-water Phytoplankton of the Flemish Lowland.* Dr. W. Junk Publishers, The Hague. (Developments in Hydrobiology no. 18).

CAPPUCCINELLI, P. 1980. *Motility of Living Cells.* Chapman and Hall, London; 80pp.

CARPENTER, E.J., REMSEN, C.C. & WATSON, S.W. 1972. Utilization of urea by some marine phytoplankters. *Limnology and Oceanography* 17: 265-269.

CASTENHOLZ, R.W. 1964. The effect of daylength and light intensity on the growth of littoral marine diatoms in culture. *Physiologia Plantarium* 17: 951-963.

CASTENHOLZ, R.W. 1982. Motility and Taxes. *In*: Carr, N.G. and Whitton, N.G., (Eds.) *The Biology of Cyanobacteria.* Univ. California Press, Berkeley, pp. 413-439.

CHU, S.P. 1946. The utilization of organic phosphate by phytoplankton. *J. mar. Biol. Ass. UK.* 26: 285-295.

COOKSEY, B. AND COOKSEY, K.E. 1980. Calcium is necessary for motility in the diatom *Amphora coffeaeformis. Plant Physiol.* 65: 129-131.

CRAWFORD, R.M. 1981. Some Considerations of Size Reduction in Diatom Cell Walls.*In* Ross, R. (Ed.) *Proceedings of the Sixth Symposium on Recent and Fossil Diatoms*. Otto Koeltz Science Publishers, Koenigstein, pp. 253-266.

CZARNECKI, D. B. 1987. The Freshwater Diatom Culture Collection at Loras College, Dubuque, Iowa. *Notulae Naturae of The Academy of Natural Sciences of Philadelphia No. 465*. 16 pp.

DARLEY W.M. & VOLCANI, B.E. 1969. Role of silicon in diatom metabolism. A silicon requirement for deoxyribonucleic acid synthesis in the diatom *Cylindrotheca fusiformis* Reimann and Lewin. *Expl. Cell Res.* 58: 334-342.

DENFFER, VON, D. 1949. Die planktonische Massenkultur pennatur Grundiatomeen. *Arch. Mikrobiol.* 14: 159-202.

DOETSCH, R.N. and HAGEAGE, G.J. 1968. Motility in prokaryotic organisms: problems, points of view, and perspectives. *Biol. Rev.* 43: 317-362.

DREBES, G. 1966. On the life history of the marine plankton diatom *Stephanopyxis palmeriana* (Grev.) Grunow. *Helgol. Wiss. Meeresuntus.* 13: 101-114.

DREBES, G. 1977. Sexuality.*In* Werner, D. (Ed.) *The Biology of Diatoms.* Blackwell Scientific Publications, California, pp. 250-283.

DROOP, M.R. 1957. Auxotrophy and organic compounds in the nutrition of marine phytoplankton. *Journal of General Microbiology* 16: 286-293.

DRUM, R.W. and HOPKINS, J.T. 1966. Diatom locomotion: an explanation. *Protoplasma* 62: 1-32.

DRUM, R.W. and PANKRATZ, H.S. 1964. Pyrenoids, raphes and other fine structures in diatoms. *Amer. J. Bot.* 51: 405-418.

DRUM, R.W. and PANKRATZ, H.S. 1965. Locomotion and raphe structure of the diatom *Bacillaria. Nova Hedwigia* 10: 315-317.

DRUM, R.W., GORDON, R., BENDER, R. and GOEL, N.S. 1971. Weakly coupled diatomic oscillators - *Bacillaria's* paradox resolved. *J. Phycol. Suppl.* 7: 13.

DRUM, R.W., PANKRATZ, H.S. & STOERMER, E.F. 1966. *In* Helmcke, J.G. and Kreiger, W. (Eds.) *Diatomeenshalen im elektronenmikroskopischen Bild,* Cramer, Lehre,Teil VI.

DURBIN, E.G. 1974. Studies on the autecology of the marine diatom *Thalassiosira nordenskiöldii* CLEVE. I. The influence of daylength, light intensity, and temperature on growth. *J. Phycol.* 10(2): 220-225.

EDENHOFNER, H.K. & SCHMID, A.M. 1984. Effects of daylength and temperature-cycles on the growth of the aerophilic diatom *Hantzschia amphioxys*. *Arch. Hydrobiol.* 101(1/2): 73-88.

EDGAR, L.A. 1979. Diatom Locomotion: computer assisted analysis of cine film. *Br. Phycol. J.* 14: 83-101.

EDGAR, L.A. 1983. Mucilage secretions of moving diatoms. *Protolpasma* 118: 44-48.

EDGAR, L.A. and PICKETT-HEAPS, J.D. 1982. Ultrastructural localisation of polysaccharides in the motile diatom *Navicula cuspidata*. *Protoplasma* 113: 10-12.

EDGAR, L.A. AND PICKETT-HEAPS, J.D. 1983. The mechanism of diatom locomotion. I. An ultrastructural study of the motility apparatus. *Proc. R. Soc. Lond. B* 218: 331-343.

EDGAR, L.A. and PICKETT-HEAPS, J.D. 1984. Diatom Locomotion. *In* Round, F.E. and Chapman, D.J., (Eds.) *Progress in Phycological Research. Vol. 3.* Biopress Ltd., Bristol, pp. 47-88.

EDGAR, L.A. AND ZAVORTINK, M. 1983. The mechanism of diatom locomotion. II. Identification of actin. *Proc. R. Soc. Lond. B* 218: 345-348.

EDMUNDS, L.N. JR. 1966. Studies on synchronously dividing cultures of *Euglena gracilis* Klebs (strain Z) III. Circadian components of cell division. *J. cell. Physiol.* 67: 35-44.

ENGLEMANN, W. 1879. Über die Bewegungen der Oscillarien und Diatomeen. *Bot. Ztg.* 37: 49.

EPPLEY, R.W. 1977. The growth and culture of diatoms. *In* Werner, D. (Ed.) *The Biology of Diatoms.* Blackwell Scientific Publications, California, pp.24-64.

FOGG, G.E. 1975. *Algal Cultures and Phytoplankton Ecology. 2nd Ed.* Madison: University of Wisconsin Press, Madison.

FRITSCH, F.E. 1956. *The Structure and Reproduction of the Algae. Volume 1.* Cambridge University Press, Cambridge.

FUNK, G. 1914. Beobachtungen über Bewegungen von Bacillariaceenkolonien und deren Abhängigkeit von äußeren Reizen. *Mitteilungen aus der Zoologischen Station zu Neapel.* 22: 45-58.

FUNK, G. 1919. Notizen über Meeresdiatomeen. *Ber. Deutsch. Bot. Ges.* 37: 187-192.

GALLAGHER, J.C. 1983. Cell enlargement in *Skeletonema costatum* (Bacillariophyceae). *J. Phycol.* 19: 539-542.

GERISH, G. and WICK, U. 1975. Intracellular oscillations and release of cyclic AMP from *Dictyostelium* cells. *Biochem. Biophys. Res. Comm.* 65(1): 364-370.

GOLDSBOROUGH, L.G. AND ROBINSON, G.G.C. 1984. Simple bioassay for photosystem-II inhibitors in water using *in vivo* chlorophyll fluoresence. *Weed Research* 24: 351-358.

GORDON, R. 1987. A retalitory role for algal projectiles, with implications for the mechanochemistry of diatom gliding motility. *J. theor. Biol.* 126: 419-436.

GORDON, R. AND DRUM, R.W. 1970. A capillarity mechanism for diatom gliding locomotion. *Proc. natn. Acad. Sci. U.S.A.* 67: 338-344.

GUILLARD, R.R.L. 1968. B12 specificity of marine centric diatoms. *Phycologia* 4: 59-64.

GUILLARD, R.R.L. 1973. Division Rates. *In*: Stein, J.R. (Ed.) *Handbook of Phycological Methods*: Cambridge University Press, Cambridge, pp.289-312.

GUILLARD, R.R.L. and CASSIE, V. 1963. Minimum cyanocobalamin requirements of some marine centric diatoms. *Limnology and Oceanography* 8: 161-165.

GUILLARD, R.R.L. and KILHAM, P. 1977. The ecology of marine planktonic diatoms. *In* Werner, D. (Ed.) *The biology of diatoms.* Blackwell Scientific Publishers, California pp. 372-469.

HALFEN, L.N. 1979. Gliding movements. *In* Haupt, W. and Feinleib, M.E. (Eds.) *Physiology of Movements. Encyclopedia of Plant Physiology* 7: 250-267.

HAPPEY-WOOD, C.M. AND JONES, P. 1988. Rhythms of vertical migration and motility in intertidal benthic diatoms with particular reference to *Pleurosigma angulatum. Diatom Research* 3(1): 83-93.

HARPER, M.A. and J. F. HARPER. 1967. Measurements of diatom adhesion and their relationship with movement. *Br.Phycol. Bull.* 3(2): 195-207.

HARRIS, P.J.C., AND WILKINS, M.B. 1976. Light-induced changes in the period of the circadian rhythm of carbon dioxide output in *Bryophyllum* leaves.*Planta* 129: 253-258.

HARVEY, H.W. 1953. Note on the absorption of organic phosphorus compounds by*Nitzchia closterium* in the dark. *J. Marine Biol. Ass., UK.* 31: 475-476.

HASLE, G.R. 1974. The mucilage pore of pennate diatoms. *Beih. Nova Hedwigia* 45: 167-194.

HASTINGS, J.W. AND SWEENEY, B.M. 1958. A persistent diurnal rhythm of luminescence in *Gonylaulax polyedra. Biol. Bull.* 115: 440-458.

HENDEY, N.I. 1964. *An introductory account of the smaller algae of British coastal waters. Part V. Bacillariophyceae (diatoms).* Fish. Invest.Sev. IV. H.M.S.O., London.

HESS, B. and A. BOITEAUX. 1971. Oscillatory phenomena in biochemistry. *Ann. Rev. Biochem.* 40: 364-370.

HOEK, C. VAN DEN, ADMIRAAL, W., COLIJN, F. and JONGE, V.N. DE. 1979. The role of algae and seagrass in the ecosystem of the Wadden Sea: a review. *In*: W.J. Wolff, (Ed.). *Flora and vegetation of the Wadden Sea,* 9-118. (Report 3 of the Wadden Sea Working Group, Texel, The Netherlands.)

HOLMES, R.W. 1966. Short-term temperature and light conditions associated with auxospore formation in the marine centric diatom, *Coscinodiscus concinnus* W. Smith. *Nature* 209: 217-218.

HOLT, M.G. & SMAYDA, T.J. 1974. The effect of daylength and light intensity on the growth rate of the marine diatom *Dentonula confervacea* (CLEVE) GRAN. *J. Phycol.* 10(2): 231-237.

HOPKINS, J.T. 1963. A study of the diatoms of The Ouse Estuary, Sussex. I. The movement of the mud-flat diatoms in response to some chemical and physical changes. *J. mar. Biol. Ass., U.K.* 43: 653-663.

HOPKINS, J.T. 1966. The role of water in the behavior of an estuarine mud-flat diatom. *J. mar. biol. Ass. U.K.* 46: 617-626.

HOPKINS, J.T. 1967. The diatom trail. *J. Queckett Micr. Cl.* 30: 209-217.

HOPKINS, J.T. AND DRUM, R. W. 1966. Diatom motility: an explanation and a problem. *Br. Phycol. Bull.* 3: 63-67.

HOUPT, P.M. 1980. Observation of the motile behavior of diatoms. *Microscopy* 34: 53-58.

HUSTEDT, F. 1930. Bacillariophyta (Diatomeae). *In* Pacher, A., (Ed.). *Die Süßwasser Flora Mitteleuropas. 2 ed.* Jena G. Fischer, pp.466.

HUSTEDT, F. 1957. Die Diatomeen flora des Flußsystems der Weser im Gebiet der Hansestadt Bremen. *Abhandl Naturwiss. Ver. Bremen.* 34: 181-440.

JACKSON, J.D. 1905. Movements of diatoms and other microscopic plants. *Amer. Nat.* 461: 287-291.

JAROSCH, R. 1958. Zur Gleitbewegung der niederen Organism. *Protoplasma* 50: 277-289.

JAROSCH, R. 1962. Gliding. *In* Lewin, R.A. (Ed.) *Physiology and Biochemistry of Algae.* Academic Press, New York, pp. 573-581.

JÖRGENSEN, E.G. 1953. Silicate assimilation by diatoms. *Physiologia Pl.* 6: 301-315.

JÖRGENSEN, E.G. 1955. Variations in the silica content of diatoms. *Physiologia Plantarium* 8: 840-845.

JÖRGENSEN, E.G. 1977. Photosynthesis. *In* Werner, D. (Ed.) *The biology of diatoms.* Blackwell Scientific Publications, California.

KAIN, J.M. and FOGG, G.E. 1958. Studies on the growth of marine phytoplankton. I. *Asterionella japonica. . J. mar. biol. Ass. U.K.* 37: 397-413.

KAMIYA, N. 1959. Protoplasmic Streaming. *Protoplasmatologia* 8(32).

KUENZLER, E.J. 1966. Glucose-6-phosphate utilization by marine algae. *Journal of Phycology* 1: 156-164.

LAWSON, D. 1972. *Photomicrography.* Academic Press Inc., London. 494 pp.

LAUTERBORN, R. 1896. *Untersuchungen über Bau, Kernteilung und Bewegung der Diatomeen.* Englemann, Leipzig, 165 pp.

LEE, R. E. 1980. *Phycology.* Cambridge University Press, Cambridge.

LEWIN, J.C. 1953. Heterotrophy in diatoms. *J. gen. Microbiol.* 9: 305-313.

LEWIN, J.C. 1955. Silicon metabolism in diatoms. II. Sources of silicon for growth of *Navicula pelliculosa*. *Plant Physiology* 30: 129-134.

LEWIN, J.C. 1957. Silicon metabolism in diatoms. IV. Growth and frustule formation in *Navicula pelliculosa*. *Canadian Journal of Microbiology* 3: 427-433.

LEWIN, J.C. and GUILLARD, R.R.L. 1963. Diatoms. *Annual Review of Microbiology* 17: 373-414.

LEWIN, J.C. and LEWIN, R.A. 1960. Auxotrophy and Heterotrophy in Marine Littoral Diatoms. *Canadian Journal of Microbiology* 6(2).

LIEBISCH, W. 1929. Experimentelle und kritische Untersuchungen über die Pektinmembran der Diatomeen unter besonderer Berücksichtigung der Auxosporenbildung und der Kratikularzustände. *Z. Bot.* 22: 1-65.

LILLIE, R.D. 1965. *Histopathologic Technic and Practical Histochemistry*. McGraw-Hill Book Company, New York.

LONERGAN, T.A. 1983. Regulation of cell shape in Euglenagracilis. I. Involvement of the biological clock, respiration, photosynthesis and cytoskeleton. *Plant Physiol.* 71(4): 719-730.

LUND, J.W.G. Studies on *Asterionella formosa* Hass. II. Nutrient depletion and the spring maximum. *Journal of Ecology* 38: 15-35.

MACKENZIE, C. and WALKER, M.H. 1983. Substrate contact, mucus and eugregarine gliding. *J. Protozool.* 30: 3-8.

MANSO-MARTINEZ, R. 1982. Podophyllotoxin poisioning of microtubules at steady-state: Effect of substoichiometric and superstoichiometric concentrations of drug. *Mol. Cell. Biochem.* 45: 3-11.

MARTENS, P. 1940. La locomotion des diatomées. *La Cellule* 48: 277-306.

MCCARTHY, J.J. 1972. The uptake of urea by marine phytoplankton. *Journal of Phycology* 8: 216-222.

MCDONALD, J.D. 1869. On the structure of the diatomaceous frustule and its genetic cycle. *Ann. Mag. nat. Hist.* 3: 1-8.

MCINTIRE, C.D. and OVERTON, W.S. 1971. Distributional patterns in assemblages of attached diatoms from Yaquina estuary, Oregon. *Ecology* 52: 758-777.

MCINTIRE, C.D. and MOORE, W.W. 1977. Marine Littoral Diatoms: Ecological Considerations. *In* Werner, D. (Ed.) *The Biology of Diatoms.* Blackwell Scientific Publications, California, pp.333-371

MCLACHLAN, J. 1959. The growth of unicellular algae in artificial and enriched sea water media. *Canadian Journal of Microbiology* 5: 9-15.

MEDLIN, L.K. and CRAWFORD, R.M. 1986. Histochemical and ultrastructural evidence for the function of the labaite process in the movement of centric diatoms. *Br. Phycol. J.* 21(3): 297-301.

MEDLIN, L.K. and WILSON, W.B. 1979. Effects of temperature and salinity on the growth of the benthic diatom *Nitzchia ovalis* Arnott (Bacillariophyceae). *Contr. Mar. Sci.* 22: 127-136.

MIZUNO, M. and OKUDA, K. 1985. Seasonal change in the distribution of cell size of *Cocconeis scutellum var.ornata* (Bacillariophyceae) in relation to growth and sexual reproduction. *J. Phycol.* 21(4): 547-553.

MÜLLER, O. 1889. Durchbrechungen der Zellwand in ihren Beziehungen zur Ortsbewegung der Bacillariaceen. *Ber.dtsch. bot. Ges.* 7: 169-180.

NÄGELI, C. 1860. Die Bewegung im Pflanzenreiche. *Beitr. wiss. Bot.* 2: 1-92.

NANJUNDIAH, V. 1986. How rapidly do uncoupled oscillators desynchronize? *J. theor. Biol.* 121: 375-379.

NICHOLS, W.H. 1973. Growth media - freshwater. *In* Stein,J.R. (Ed.) *Handbook of Phycological Methods.* Cambridge University Press, Cambridge, pp. 7-24.

NULTSCH, W. 1956. Studien uber die Phototaxis der Diatomeen. *Arch. Protistenk.* 101: 1-68.

NULTSCH, W. 1962. Über das Bewegungsverhalten der Diatomeen.*Planta* 58: 22-23.

NULTSCH, W. 1970. Photomotion of microorganisms and its interactions with photosynthesis. *In* Halldal, P. (Ed.) *Photobiology of Microorganisms.* Wiley Interscience, London, pp. 213-249.

NULTSCH, W. 1971. Phototactic and photokinetic action spectra of the diatom *Nitzschia communis. Photochem. Photobiol.* 14: 705-712.

NULTSCH, W. 1974. Movements. *In* Stewart, W.D.P. (Ed.) *Algal Physiology and Biochemistry.* Univ. California Press, Berkely, pp. 864-893.

PAASCHE, E. 1968. Marine plankton algae grown with light-dark cycles. II. *Ditylum brightwellii* and *Nitzchia turgidula*. *Physiologia Plantarium* 21: 66-77.

PALMER, C.T. 1910. The apparatus of locomotion in *Surirella*. *Proc. Del. Co. Instit. Sci.* 5: 146-156.

PALMER, J.D. AND ROUND, F.E. 1967. Persistent vertical-migration rhythms in benthic microflora. IV. The tidal and diurnal nature of the rhythm in the diatom *Hantzchia virgata*. *Biol. Bull.* 132: 44-55.

PATE, J. L. and CHANG, L. E. 1979. Evidence that the gliding motility in prokaryotic cells is driven by rotary assemblies in the cell envelopes. *Current Microbiol.* 2: 59-64.

PATRICK, R. AND REIMER, C.W. 1966. *The Diatoms of the United States. Volume 1*. Monographic Series of the Academy of Natural Sciences of Philadelphia # 13.

PFITZER, E. 1871. Untersuchungen über Bau und Entwicklung der Bacillariaceen (Diatomeen). *Botan. Abhandl.* 2: 1-189.

PFITZER, E. 1871. Untersuchungen über Gallertbidungen der Algen. *Verh. d. Nat. Ver. Heidlegerg* 7: 376-402.

PHELPS, C.F. 1972. *Polysaccharides*. Oxford University Press, London, 16 pp.

PICKETT-HEAPS, J.D., HILL, D.R.A., and WETHERBEE, R. 1986. Cellular movement in the centric diatom *Odontella sinensis*. *J. Phycol.* 22: 334-339.

PRAKASH, A., RASHED, M.A., JENSEN,A., & SUBBA RAO, D.V. 1973. Influence of humic substances on the growth of marine phytoplankton: diatoms. *Limnology and Oceanography* 18: 516-524.

PRINGSHEIM, E.G. 1946. The biphasic or soil-water culture method for growing algae and flagellata. *J. Ecol.* 33: 193-204.

QUENTIN, E. AND HARDELAND, R. 1986. Circadian rhythmicity of protein synthesis and translational control in *Euglena gracilis*. *J. Interdisciplinary Cycle Res.* 7(3): 197-205.

RHEE, G.Y. & GOTHAM, I.J. 1981. The effect of enviromental factors on phyoplankton growth: temperature and the interactions of temperature with nutrient limitation. *Limnol. Oceanogr.* 26: 635-648.

RINCÉ, Y., M.R. PLANTE-CUNY, C. RIAUX, J.M. ROBERT and M.O. MALISSEN. 1981. Comparison between benthic diatom populations in muddy sediments of four localities along the French Western Coast. *In* Ross, R (Ed.) *Proceedings of the Sixth Symposium on Recent and Fossil Diatoms*. Koeltz Books, Koenigstein, pp. 371-384.

ROSOWSKI, J.R. 1980. Valve and band morphology of some freshwater diatoms. II. Integration of valves and bands in *Navicula confervacea var. confervacea. J. Phycology* 16: 88-101.

ROSOWSKI, J.R., HOAGLAND, K.D., ROEMER, S.C. 1983. Valve and band morphology of some freshwater diatoms. IV. Outer surface mucilage of *Navicula confervacea var. confervacea. J. Phycol.* 19: 342-347.

ROUND, F.E. 1972. The problem of reduction of cell size during diatom cell division. *Nova Hedwigia* 23: 291-303.

ROUND, F.E. 1978. On rhythmic movement of the diatom *Amphora ovalis*. *British Phycological Journal* 13: 311-317.

ROUND, F.E. 1979. Occurrence and rhythmic behavior of *Tropidoneis lepidoptera* in the epipelon of Barnstable Harbour, Massachusetts, U.S.A. *Marine Biology* 54: 215-217.

ROUND, F.E. 1981. *The ecology of algae.* Cambridge University Press, Cambridge.

ROUND, F.E. AND EATON, J.W. 1966. Persistent, vertical-migration rhythms in the benthic microflora. III. The rhythm of epipelic algae in a freshwater pond. *J. Ecol.* 54: 609-615.

ROUND, F.E. AND PALMER, J.D. 1966. Persistant, vertical-migration rhythms in benthic microflora. II. Field and laboratory studies on diatoms from the banks of the River Avon. *J. mar. biol. Ass. U.K.* 46: 191-214.

RUSSELL, D.G. and SINDEN, R. E. 1981. The role of the cytoskeleton in the motility of cocidian sporozites. *J. Cell Sci.* 50: 345-359.

SCHULTZ, M. 1865. Die Bewegung der Diatomeen. *Arch. Mikr. Anat.* 1: 376-402.

SEABURG, K.G., PARKER,B.C., WHARTON, JR., R.A. and SIMMONS, JR., G.M. 1981. Temperature-growth responses of algal isolates from Antactic oases. *J. Phycol.* 17: 353-360.

SMITH, G.M. 1938. *Cryptogamic Botany. Vol. 1.* McGraw-Hill, London.

SMITH, H.L. 1888. A contribution to the life history of the Diatomaceae - part II. III. Motion of the diatoms. *Proc, Amer. Soc. Micr.* 9: 126-167.

SMITH, S.D., MCLEOD, B.R., LIBOFF, A.R. and COOKSEY, K. 1987. Calcium cyclotron resonance and diatom mobility. *Bioelectromagnetics* 8: 215-227.

SMITH, W. 1853. *A Synopsis of the British Diatomaceaea, 1.* John van Voorst, London.

SOUTH, G.R. and WHITTICK, A. 1987. *Introduction to Phycology.* Blackwell Scientific Publications, Oxford.

SPANGLE, L. AND ARMSTRONG, T.B. 1973. Gliding motility of algae unaffected by cytochalasin B. *Expl. Cell Res.* 80: 490-493.

STEBBINGS, H. and HYAMS, J.S. 1979. *Cell Motility.* Longman Group Limited, London.

STEELE, R.L. 1965. Induction of sexuality in two centric diatoms. *BioScience* 15: 298.

STEPHENS, G.C. & NORTH, B.B. 1971. Extrusion of carbon accompanying uptake of amino acids by marine phytoplankters. *Limnology and Oceanography* 16: 752-757.

STOERMER, E.F., PANKRATZ, H.S. andBOWEN, C.C. 1965. Fine structure of the diatom *Amphipleura pellucide.* II. Cytoplasmic fine structure and frustule formation. *Am. J. Bot.* 52: 1067-1078.

SULLIVAN, C.W. & VOLCANI, B.E. 1973. Role of silicon in diatom metabolism. III. the effects of silicic acid on DNA polymerase, TMP kinase and DNA synthesis in *Cylindrotheca fusiformis. Biochim. Biophys. Acta* 308: 212-299.

SWEENEY, B.M. 1960. The photosynthetic rhythm in single cells of *Gonyaulax polyedra. Cold Spring Harb. Symp. quant. Biol.* 25: 145-148.

SWEENEY, B.M. 1969. *Rhythmic Phenomena in Plants.* Academic Press, London, 138pp.

SWEENEY, B.M. 1980. How to detect the presence of a circadian rhythm. *In* Gantt, E. (Ed.) *Handbook of Phycological Methods: Developmental and Cytological Methods.* Cambridge University Press, Cambridge, pp. 231-238.

SWEENEY, B.M. AND HASTINGS, W.H. 1958. Rhythmic cell division in populations of *Gonyaulax polyedra. J. Protozool.* 5(3): 217-224.

TANNER, C.E. 1981. Chlorophyta life histories. *In* Loban, C.S. and Wynne, M.J. (Eds.) *The Biology of Seaweeds*. Blackwell Scientific Publications, Oxford, pp. 218-247.

VAN DER WERFF, A. 1955. A new method of concentrating and cleaning diatoms and other organisms. *Int. Verh. Theor. Angew. Linol. Verh.*, 12: 276-277.

VARMA, R. AND VARMA, R.S. 1983. *Mucopolysaccharides (Glycosaminoglycans) of Body Fluids in Health and Disease*. Walter de Gruyter & Co., Berlin.

VESK,M. and JEFFREY, S.W. 1977. Effect of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanopyxis turris*. *J. Phycol.* 13: 271-279.

VOLKNANDT,W. AND HARDELAND, R. 1984. Circadian rhythmicity of protein synthesis in the dinoflagellate,*Gonyaulax polyedra* : A biochemical and radioautographic investigation. *Comp. Biochem. Physiol.* 77B(3): 493-500.

WEBSTER, D.R., COOKSEY, K.E., RUBIN, R.W. 1985. An investigation of the involvement of cytoskeletal structures, and secretion in gliding motility of the marine diatom, *Amphora coffeaeformis*. *Cell Motility* 5: 103-122.

WENHAM, F.H. 1856. Cilia in unicellular plants. *Quart. J. micr. Sci.* 4: 157-160.

WERNER, D. 1977. Silicate metabolism. in: Werner, D. (Ed.) *The biology of diatoms*. Blackwell Scientific Publications, California, pp. 111-149.

WESSELLS, N.K., SPOONER, B.S., ASH, J.F., BRADLEY, M.O., LUDUENA, M.A., TAYLOR, E.L., WRENN, J.T. and YAMADA, K.M. 1970. Microfilaments in cellular and developmental processes. *Science* 171: 135-143.

WILLIAMSON, R.E. 1981. Actin in motile and other processes in plants. *Can. J. Bot.* 58: 766-772.

YAHARA, I., HARADA, F., SEKITA, S., YOSHIHIRA,K. and NATORI, S. 1982. Correlation between effects of 24 different cytochalasins on cellular structures and cellular events and those on actin in vitro. *J. Cell Biol.* 92: 69-78.

YPEY, D.L., CLAPHAM, D.E. and R.L. DEHAAN. 1979. Development of electrical coupling and action potential synchrony between paired aggregates of embryonic heart cells. *J. Memb. Biol.* 51(1): 75-96.