THE RELATIONSHIP BETWEEN IRRADIANCE (QUANTITY, QUALITY AND PHOTOPERIOD), SINKING RATE AND CARBOHYDRATE CONTENT IN TWO MARINE DIATOMS

by

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ABSTRACT

Diatoms are physiologically able to control their sinking rates through the selective accumulation of lighter ions in the vacuole (an energy-requiring process). The extent to which a diatom needs to physiologically control its sinking rate will depend on its excess density (i.e. the degree to which the cell is denser than seawater). The excess density of a diatom, and thus the amount of energy required to maintain a low sinking rate, might change with the carbohydrate content of the cells. The purpose of this work was to simultaneously measure sinking rate and carbohydrate content in 2 marine diatoms (*Thalassiosira weissflogii* and *Ditylum brightwellii*) to see if sinking rate is affected by irradiance driven changes in carbohydrate content.

In the first set of experiments, carbohydrate content was varied by growing cells on light/dark cycles under high and low light. Sinking rate measurements (SETCOL method) were made at the end of the light period and the end of the dark period. Sinking rates were measured on live and heat-killed cells in order to compare the inherent sinking rate of the cell (determined by ballast) to the physiologically-determined sinking rate. In the second set of experiments, carbohydrate content was varied by growing cells under continuous red, white or blue light.

Sinking rate was not positively correlated to carbohydrate content in *T. weissflogii* or *D. brightwellii* grown on light/dark cycles under low or high white light. Sinking rate in *D. brightwellii* was under physiological control in all experiments, but in *T. weissflogii* physiological sinking rate control was intermittent. *D. brightwellii* showed diel changes in buoyancy, with higher sinking rates at the end of the dark period than at the end of the light period, when cells were positively buoyant. *T. weissflogii* had higher sinking rates when grown under red light than under white or blue light, but there were no differences in carbohydrate content. *D. brightwellii* contained twice as much carbohydrate when grown under red light than under white or blue light, but there were no differences in sinking rate.

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GENERAL INTRODUCTION

Diatoms are ecologically important organisms, responsible for approximately 25% of primary production worldwide (Willen 1991). Much of this production occurs in temperate coastal waters during the spring bloom, a period of rapid diatom growth. Due to their silica frustule, diatoms are denser than seawater and have an inherent tendency to sink. They are able to adjust their sinking rate in response to environmental conditions such as changes in nutrient concentrations or light (Smayda 1970). In many cases, the ability of a diatom to control its sinking rate is dependent on the physiological status of the cell. The position and abundance of diatoms in the water column is important to the organisms that eat them, and affects succession patterns in the upper mixed layer. Once a bloom goes into decline, diatoms clump together to form faster sinking aggregates. The resultant mass transport of diatoms out of the upper mixed layer is a major sink in the global carbon cycle.

The processes of bloom formation, aggregation and sedimentation will be reviewed first. The physical and biological factors affecting sinking rate will then be considered, followed by an examination of the evidence for and against energy dependent control of sinking rate. It is proposed that the inconsistencies in the evidence arise from irradiance driven changes in carbohydrate content which alter the density of the cell.

ECOLOGY OF THE SPRING BLOOM

The bulk of diatom production occurs during the spring bloom. Through the winter the mixed layer is deep, and diatoms do not receive enough light to grow. With the relative calm and increased irradiance of the spring, the upper waters warm up and thermally stratify, the depth of the mixed layer moves above the critical depth (depth at which total photosynthesis equals total respiration of primary producers in the water column, Parsons *et al.* 1984a), and the resultant increase in photosynthesis allows cells to grow and multiply (Mann 1992). It is during this time of active growth, when light and nutrients are abundant at

the surface, that it is advantageous for diatoms to minimize their sinking rate. The bloom will last as long as the growth rate exceeds losses due to grazing and sedimentation.

As the season progresses, nutrients at the surface become limiting and sinking rates start to increase and become more variable (Waite *et al.* 1992a). It is advantageous for diatoms to find a compromise position where they are deep enough to obtain adequate nutrients, but not too deep so that they can receive enough light for photosynthesis. Without their ability to sink, diatoms would be unable to optimize their position in the water column. Eventually, a chlorophyll maximum will form near the bottom of the euphotic zone, a result of decreased diatom sinking rates due at least in part to the change in density of the water and higher concentrations of nutrients in this region (Steele and Yentsch 1960).

The extent and duration of a diatom bloom affects the success of organisms that eat diatoms as well as patterns of phytoplankton succession in the upper mixed layer. Diatoms are eaten by copepods and as aggregates they become a food source for larger organisms in the water column (Dagg 1993, Lampitt *et al.* 1993a), as well as for benthic organisms when they sink (Billett *et al.* 1983). The character of the mixed layer changes once the diatoms sink out, with the diatom based food chain replaced by a more complex recycling community in which the primary producers are mostly flagellates, and biomass is much lower (Smetacek 1985).

AGGREGATION

Since the sinking rate of an individual healthy diatom cell is typically very small (e.g. 0.1 m d^{-1}), especially relative to the vast depths of the ocean, mass sedimentation of diatoms is usually preceded by the formation of diatom aggregates (Riebesell 1989) which sink faster because sinking rate increases exponentially with size (Stoke's Law, Appendix 2). The aggregate formation rate depends on the number of collisions between cells, and their "stickiness" (i.e. the probability of adhesion upon collision). In addition to cells sticking to

other cells, which happens when the cells themselves are sticky, aggregation can also occur between cells and particulate mucus.

It was only recently that the importance of particulate mucus to diatom aggregation was realized. Also called transparent exopolymeric particles (TEP), these sticky particles of mucus are between 5 and 50 μm in diameter, and occur freely suspended in the pelagic ocean at concentrations between 1000 and 15000 ml^{-1} (Kiorboe and Hansen 1993). These particles are formed from dissolved carbohydrates exuded primarily by diatoms (Passow *et al.* 1994). It is not known whether diatoms produce extracellular carbohydrates to facilitate aggregation, whether they serve to provide a substrate for bacteria, or whether they just leak out of the cell (Passow *et al.* 1994). While the production of mucus has been shown to increase when cells become senescent (Hoaglund *et al.* 1993), no relationhsip has been found between cell stickiness and growth stage or environmental concentrations of nutrients (Kiorboe and Hansen 1993).

The aggregation of a diatom bloom can occur in as little as 24 hours. Diatoms within the aggregates are still actively photosynthesizing, and the aggregates do not necessarily sink out immediately (Alldredge and Gotschalk 1989). It is not clear what purpose is served by mass aggregation; apart from removing diatoms from the surface waters, the aggregate could provide a zone of higher nutrients due to excretion from the associated microbial community. If higher nutrients are the primary advantage gained from aggregation, then the greatest benefit would come to the cell which gets caught up with the aggregate temporarily, but unattaches before it is dragged out of the euphotic zone (Alldredge and Gotschalk 1989.)

SEDIMENTATION

The period following the spring bloom is usually the time of greatest flux of organic matter to the benthos, with the volume of marine snow increasing up to 20 fold (Lampitt *et al.* 1993b, Davies and Payne 1984, Alldredge and Gotschalk 1989, Billett *et al.* 1983, Olesen 1993). The relatively high contribution of diatoms to export flux compared to other

components such as zooplankton fecal pellets occurs partly because diatoms sink out "en masse" when grazers are still relatively rare (Smetacek 1985). The carbon taken up by diatoms ultimately comes from the atmosphere, and the sedimentation of diatoms down to coastal sediments, or into the deep ocean represents a potential sink in the global carbon cycle. The significance of this sink is in dispute. While some studies show that as much as 60% of spring bloom biomass can reach the sediments (Passow 1991), it averages 30% in the Greenland Sea (Culver and Smith 1989) and may more typically be between 10-20% in coastal areas (Davies and Payne 1984, Cushing 1992). Part of the variability in export flux arises from species-specific sedimentation patterns (Takahashi 1986). Diatoms rarely reach oceanic sediments intact. Martin *et al.* (1987) found that 15% of primary production sedimented out past 100 m in the ocean, and only 1% reached 4000 m. Diatom carbon that is remineralized in the deep ocean still represents a temporary (up to 1000 years) carbon sink, due to global ocean circulation patterns (Hedges 1992).

In the face of the large scale process of diatom mass sedimentation, the sinking rate and physiology of individual cells would seem irrelevant. The need to understand the global carbon cycle has focussed research on the aggregation and sedimentation processes of diatoms, since changes in sinking rate on an individual level are generally minor and short-lived. However, recent evidence suggests that sedimentation can occur without large scale formation of aggregates, and that aggregate formation is initiated by physiological changes in individual cells. In coastal areas where the distance to the sediments is relatively short, diatoms have been found to reach the bottom without large scale aggregate formation (Waite *et al.* 1992b). As coastal areas are highly productive, such individual fluxes could contribute significantly to the global carbon cycle. The percentage of bloom biomass that sediments out also depends on the species composition of the bloom, since sedimentation is species-specific. Certain bloom diatoms (e.g. *Thalassiosira* spp.) contribute up to 10 times more carbon to the sediments than co-occuring bloom diatoms such as *Chaetoceros* and *Skeletonema* (Riebesell 1989, Waite *et al.* 1992b). The process of aggregate formation itself can be highly species-

specific, with cells apparently able to control when and if they aggregate by changing their surface properties (Passow *et al.* 1994). Individual physiology is also important to the sinking rates of large diatoms in oligotrophic seas. These organisms, such as *Ethmodiscus* spp. and *Rhizosolenia* mats show a vertical migration pattern, sinking down past the nutricline to take up nitrate, then rising back up to the surface at average rates of 85 m d^{-1} to photosynthesize (Villareal and Carpenter 1988, Villareal and Carpenter 1994). This upward transport of nitrogen could be a significant nutrient input to these oligotrophic areas (Villareal *et al.* 1993). These effects of individual species-specific processes on the overall pattern of diatom sedimentation make the study of sinking rates and their physiological controls relevant.

FACTORS AFFECTING SINKING RATE

The sinking rate of a particle in a liquid medium depends on the size, shape and density of the particle, and on the density and viscosity of the medium, as governed by Stokes' Law (see Appendix 2). At first it was thought that the sinking rates of phytoplankton were solely the result of their size and morphology, and that with the help of turbulence, being small and spiny was enough to keep a cell suspended (Smayda 1970). This was supported by the observation that the majority of bloom-forming diatoms are chain-forming, medium to small-sized cells with protuberances like silica spines, chitan microfibrils and gelatinous threads (Smetacek 1985).

The possibility that diatoms had physiological control over sinking rate arose following observations that sinking rate varied with growth stage, and that sinking rates were higher in preserved cells, which are incapable of metabolic control (Gross and Zeuthen 1948, Smayda 1970). Physiological control of sinking rate is thought to occur through changes in the density of the cell. A cell can change its density by changing its bulk composition to favour heavier components (carbohydrate, protein) or lighter components (lipids), or by changing the relative concentrations of light and heavy ions in its vacuole. Bulk composition is less likely to play a regulatory role in sinking rate control because the response time would be slow, and

changes in cell composition in response to factors such as nitrogen limitation or photosynthetic rate would not necessarily occur in the direction required for optimal sinking rate. While sinking rate is ultimately constrained by cell size and shape, physiologically mediated changes in density can produce a range of sinking rates within those constraints.

Most of the excess density of a diatom comes from its frustule, which has a density of about 2.6 g cm^{-3} (Boney 1989). One way the cell can make itself lighter is by changing the ionic composition of its vacuole (Gross and Zeuthen 1948, Anderson and Sweeney 1978). To ensure buoyancy, every 1 μm^3 of silica frustule requires 2.5 μm^3 of cell sap at a density of 1.02 g cm^{-3} (Boney 1989). Although small cells have an inherently low sinking rate due to their size, they have a lot of frustule relative to the size of their vacuole, and therefore they have only a limited ability to change their sinking rate by changing the ionic composition of their vacuole. Large cells have relatively less frustule compared to the size of their vacuole (i.e. a lower surface area to volume ratio), and though they can sink much faster than small cells, they are capable of a much greater range of sinking rates. By changing their density, large diatoms can even become positively buoyant (Villareal 1988).

ENERGETIC CONTROL OF SINKING RATE

Given that changes in cell density can occur through the selective accumulation of lighter or heavier ions in the vacuole, and that this is an energy-requiring process, the ability of a cell to control its sinking rate should depend on its energetic status. Observations that sinking rate tended to increase when cells were limited by light or nutrients suggest that the maintenance of low sinking rates is an active energy-requiring process that does not function properly under stress. The literature on sinking rates reveals that while there is much evidence to support the energy dependence of sinking rate control, there is also evidence that disputes it.

EVIDENCE SUPPORTING ENERGY-DEPENDENT CONTROL OF SINKING RATE

Nutrients

Generally, sinking rates increase with nutrient limitation (Lannergren 1979, Titman and Kilham 1976), although this varies depending on the nutrient. Often an increase in sinking rate does not occur until several days after the nutrient runs out (Bienfang and Harrison 1984). Bienfang *et al.* (1982) found that sinking rates increased the most after silicate depletion, which is particularly interesting since silicate depletion would presumably decrease the amount of frustule which should decrease sinking rate.

Since nutrient-limited cells are not necessarily energy limited, it is hard to relate these data to the possible energetic control of sinking rate. An increase in sinking rate in response to limiting nutrients may be a deliberate acclimation rather than a loss of sinking rate control. Not only would an increase in sinking rate bring the cell into more nutrient-rich environments, it would also decrease the diffusion boundary layer around the cell, facilitating nutrient uptake (Titman and Kilham 1976, Lannergren 1979).

Light

The effect of light is more relevant to energetic control of sinking rate, as energy can come directly from photosynthesis in the form of ATP, as well as from the respiration of carbohydrates produced from photosynthesis. Assuming that energy is needed for sinking rate control, and that sinking rate control always operates to minimize sinking rate (which may not be true), then sinking rate would be expected to increase under low light, during the dark period of a light/dark cycle and during prolonged periods in the dark. Exposure to darkness would immediately deprive the cell of photosynthetic energy, and once reserves had been consumed, very little respiratory energy would be available.

A study of sinking rates in mesocosms found that sinking rate tended to increase on overcast days and decrease when it was sunny (Bienfang 1981a). In the lab, *Ditylum brightwellii* grown under high light had lower sinking rates than cultures grown under low light (Waite *et al.* 1992c). For *D. brightwellii* grown on a light/dark cycle, sinking rates

were highest at night during the period of cell elongation (Eppley *et al.* 1967). Sinking rate has also been shown to increase after cells have been in the dark for about 100 h (Waite *et al.* 1992c).

Inhibitors

Probably the most consistent sinking rate response is the increase in sinking rate that occurs when cells are incapacitated with inhibitors or killed, providing strong support for energetic control of sinking rate. Sinking rates of *D. brightwellii* increased after treatment with ionophores or respiratory inhibitors such as cyanide (Anderson and Sweeney 1977, Waite *et al.* 1992c). After treatment with DCMU, a photosystem II blocker, diel sinking rate variation in *D. brightwellii* no longer occurred (Anderson and Sweeney 1978).

EVIDENCE AGAINST ENERGY-DEPENDENT CONTROL OF SINKING RATE

A high sinking rate under high light is not necessarily evidence against the energetic control of sinking rate since a high sinking rate may at times be beneficial. However, cells that are able to maintain low sinking rates under low light would bring the energetic control of sinking rate into question.

In the field, sinking rates tend to be lower near the chlorophyll maximum where light is low than near the surface, and this decrease in sinking rate is not necessarily due to the increased nutrients or increased water density in this region (Bienfang 1980, Johnson and Smith 1986). Laboratory studies have shown that light is the crucial factor, as cultures growing under high light and high nitrate have higher sinking rates than cultures growing under low light and high nitrate (Bienfang *et al.* 1983). A positive correlation between sinking rate and light has been found for *Chaetoceros gracilis* and *C. flexosum* (Culver and Smith 1989), *T. weissflogii* (Bienfang *et al.* 1983) and *Coscinodiscus concinnus* (Granata 1991). Over a diel cycle, maximum sinking rates were found near the end of the light period, and minimum sinking rates were found near the end of the dark period, for *Coscinodiscus concinnus* (Granata 1991) and *D. brightwellii* (Anderson and Sweeney 1977). Mats of

Rhizosolenia also tended to sink faster in the afternoon than in the morning (Villareal *et al.* 1993).

In all these examples, lower sinking rates were found under lower light, or decreased exposure to light. Given that the data are valid, the most plausible explanation of this observation is that there is either a non-energy dependent way to control sinking rate, or it takes much less energy to maintain a low sinking rate under low light than under high light. While the degree to which sinking rate is energy dependent is likely to vary widely between species, some of the conflicting evidence regarding the energetic control of sinking rate has come from the same species (*D. brightwellii*), indicating that inconsistency in the response of sinking rate to light is not solely due to inter-species variation.

DOES CARBOHYDRATE ACT AS BALLAST IN MARINE DIATOMS?

The data suggest that the ability of a diatom to control its sinking rate depends on more than its energetic status. Since most of the cases where sinking rate control is not strictly energy-dependent involve light, the explanation may lie in some other effect of light upon the cell. As diatoms are photosynthetic organisms, one of the biggest changes that would occur in the cell in response to light would be the accumulation of carbohydrate.

Carbohydrate is the most dense of the major cell constituents, at about 1.5 g cm^{-3}, while protein is around 1.3 g cm^{-3}, and lipid is around 0.86 g cm^{-3} (Boney, 1989). Carbohydrate has the greatest capacity for affecting sinking rate because it is the heaviest, and also the most variable, ranging between 7 - 80% of total cell carbon content (Varum and Myklestad 1984). In comparison, protein content is fairly constant unless nitrogen is limiting. While there is some carbohydrate associated with diatom cell walls, most of the carbohydrate in a diatom is in the form of the reserve polysaccharide β-1,3-glucan, also known as chrysolaminarin. Glucan is a source of both energy and carbon skeletons for amino acid synthesis (Myklestad 1988). Depending on daylength and light intensity, levels of glucan in a cell can increase 3 or 4 fold over the day, presumably causing a significant increase in cell

density. Increases in carbohydrate over the day followed by decreases at night have been seen in both lab cultures (Varum *et al.* 1986) and natural populations (Hama and Handa 1992). Glucan content increases with irradiance and also increases in response to nutrient limitation (Varum and Myklestad 1984). The extent to which a diatom accumulates glucan is species-specific (Myklestad 1988).

As a relatively dense and large component of a cell, carbohydrate content could potentially act as additional ballast in diatoms. When carbohydrate content is low, it would take less energy to maintain the same sinking rate than when carbohydrate content is high. It may be the variation in carbohydrate content and the cell's ability to compensate for it that leads to contradictory evidence for the energetic control of sinking rate.

Carbohydrate content is known to affect the buoyancy of cyanobacteria. Cyanobacteria tend to dominate in environments where growth factors are spatially separated, with light available near the surface, and nutrients available in deeper waters. As an adaptation to such environments, many cyanobacteria vertically migrate, often on a daily basis (Kromkamp and Mur 1984, Villareal and Carpenter 1990). In species that inhabit calm environments, this buoyancy pattern is regulated by gas vesicles and cell turgor pressure. In cyanobacteria whose gas vesicles are too strong to be collapsed by turgor pressure, changes in buoyancy occur through carbohydrate ballasting (Oliver 1994). Cells photosynthesizing near the surface accumulate carbohydrate to the point where the increased density causes the cell to sink out of lighted regions. Once the carbohdyrate reserves are used up, cell density decreases and the cell rises again. Among the species showing this behaviour are *Microcystis aeruginosa* (Kromkamp *et al.* 1988), *Trichodesmium theibautii* (Villareal and Carpenter 1990) *Oscillatoria* and *Spirulina* (van Rijn and Shilo 1985).

Due to their silica frustule, diatoms are denser than cyanobacteria, so changes in their carbohydrate content would have relatively less of an effect on the overall density of the cell. However, since the range of diatom sinking rates is much narrower than that of cyanobacteria, the contribution of carbohydrate to diatom density could be important.

GOALS OF THIS THESIS

The objective of this work is to determine whether carbohydrate acts as ballast in diatom cells, accelerating sinking rates under high light by increasing cell density, and decreasing density under low light so that less energy is required for sinking rate maintenance. Despite the potential for carbohydrate ballasting in diatoms, this is the first study in which both sinking rate and carbohydrate content have been measured.

In the first chapter, carbohydrate content is varied by growing cells on light/dark cycles under high or low light. Sinking rate and carbohydrate content are measured at the end of the light period, when carbohydrate content is high, and at the end of the dark period, when carbohydrate content is low. In another experiment, cells are exposed to prolonged darkness to determine how sinking rate changes as carbohydrate reserves are consumed. These experiments are conducted on live cells with active sinking rate control mechanisms, and on heat-killed cells that have no means of density control.

The second set of experiments manipulate carbohydrate content by changing light quality. Phytoplankton grown under red light tend to accumulate more carbohydrate relative to those grown under blue light (Kowallik 1987). This experiment measures the sinking rate and carbohydrate content of cells grown under red, blue or white light, to see if higher carbohydrate accumulations under red light are accompanied by higher sinking rates.

The above experiments are performed on two species: *Ditylum brightwellii* and *Thalassiosira weissflogii*. These are single-celled cosmopolitan diatoms. Neither is a major bloom former, but they grow well in the lab and are physiologically well-characterized. The larger size of *D. brightwellii* (3500 μm^3) compared to *T. weissflogii* (600 μm^3) will allow an examination of the role of size in sinking rate control.

CHAPTER I

INTRODUCTION

In the ocean, phytoplankton are subjected to variations in the light environment, including diel periodicity in irradiance, changes in irradiance with weather and position in the water column, and prolonged darkness if cells get mixed below the euphotic zone. These changes in irradiance lead to changes in carbohydrate content, which could change cell density enough to affect sinking rate. The changes in irradiance could also affect sinking rate by changing availability of energy for sinking rate control. These two factors have opposite effects on sinking rate (i.e. low light would decrease available energy, but would also decrease carbohydrate ballasting), which may contribute to the inconsistent response of sinking rate to light. By comparing sinking rate and carbohydrate content under various changes in irradiance, I have attempted to determine the relative contributions of carbohydrate ballasting and energy availability to sinking rate control.

DIEL PERIODICITY IN IRRADIANCE

Carbohydrate

The most noticeable change in the oceanic light field is the diel periodicity in irradiance. Carbohydrates are produced only during photosynthesis, resulting in an increase in carbohydrate content during the day, and a decrease at night. In *Skeletonema costatum* grown on photoperiods of different lengths, accumulation of the reserve polysaccharide β-1,3-glucan increased with daylength up to 50% of total cell carbon in an 18 h light period (Varum *et al.* 1986). During the dark period, glucan decreased from 25% of cell carbon (6 h dark) to 4% of cell carbon (18 h dark). Since the reduction in glucan carbon that occurred during the dark period was not accompanied by a decrease in total cell carbon, the glucan carbon must have been used in the synthesis of other cell components rather than being respired (Varum *et al.* 1986). Rates of glucan catabolism are actually higher during the day (20% h^{-1}) than at night (8% h^{-1}) (Myklestad 1988), showing that rates of glucan synthesis must be high during

the day in order for glucan to accumulate. The glucan content of *S. costatum* grown under continuous light was lower and less variable than that grown under light-dark cycles, ranging between 17-25% of total cell carbon (Varum *et al.* 1986).

Sinking Rate

The effect of diel cycles on sinking rate has not been well documented. Even when cultures are grown under diel cycles, sinking rate measurements are usually made at the same time each day. The lack of information may be due to the fact that most sinking rate methods require at least 3-4 h to complete, and the variability in sinking rate may well swamp out any diel oscillation. The sinking rates of *Coscinodiscus concinnus* measured over 3 diel cycles showed an erratic pattern, but generally increased during the light period and decreased during the dark period (Granata 1991). There is conflicting information on diel variation in sinking rate of *Ditylum brightwellii*. Eppley *et al.* (1967) found that the highest sinking rates occurred in the dark during a period of cell elongation. The rhythmic division taking place in these cultures may have affected sinking rate independently of a diel effect, so comparison with non-rhythmic cultures of *D. brightwellii* may not be meaningful. Anderson and Sweeney (1978) found the lowest sinking rates in *D. brightwellii* occurred at the end of the dark period, and the highest sinking rates occurred in the latter half of the light period. These differences in sinking rate could be accounted for by changes in vacuolar ion concentrations. Low sinking rates were accompanied by high Na^+, and low K^+ and Cl^-, relative to when sinking rates were high and Na^+ was low and K^+ and Cl^- were high.

LIGHT INTENSITY

Carbohydrate

Irradiance changes with the weather, time of year and the position of cells in the water column. Given that growth is not limited by nutrients, the carbohydrate content of a cell usually increases with irradiance, though the extent of carbohydrate accumulation is species-specific (Myklestad 1988). A culture of *Thalassiosira weissflogii* grown under high light (593

μmol photons m^{-2} s^{-1}), then transferred to low light (72 μmol photons m^{-2} s^{-1}), had a reduced growth rate within three hours and an 83% reduction in the rate of carbohydrate production. When cells grown at low light were transferred to high light, their carbohydrate content increased 10-fold after 8 h (Post *et al.* 1985).

Sinking Rate

If sinking rate is energy-dependent, high sinking rates would be expected in cells growing under low light. Such was the case for *D. brightwellii*, for which sinking rates of cells grown under high light (110 μmol photons m^{-2} s^{-1}) were 0.028 m d^{-1}, while cells grown under low light (10 μmol photons m^{-2} s^{-1}) sank at 0.043 m d^{-1} (Waite *et al.* 1992c). While cells grown under low light did have a significantly higher sinking rate, the absolute differences in sinking rate were very small.

It is more common to see low sinking rates in cells grown under low light, and higher sinking rates in cells grown under high light. A positive relationship between sinking rate and irradiance was found in phytoplankton assemblages in the Greenland Sea, as well as for *Chaetoceros flexosum* and *C. gracilis* grown in the lab (Culver and Smith 1989). *Coscinodiscus concinnus* grown under moderate light (48 μmol photons m^{-2} s^{-1}) had an average sinking rate of 0.96 m d^{-1}, while under low light (9 μmol photons m^{-2} s^{-1}) the average sinking rate was 0.57 m d^{-1}. Sinking rates of *T. weissflogii* showed a step-function increase with irradiance, from an average sinking rate of 0.1 m d^{-1} at low light (13 and 27 μmol photons m^{-2} s^{-1}) to an average of 0.24 m d^{-1} at higher light (64-850 μmol photons m^{-2} s^{-1}) (Bienfang *et al.* 1983).

PROLONGED DARKNESS

Carbohydrate

A cell that sinks or is mixed below the euphotic zone can remain in darkness for days or weeks before being mixed back up. In the dark, cells are deprived of photosynthetic energy and must rely on reserves to obtain respiratory energy. When *S. costatum* grown

under continuous light was transferred to darkness, respiration consumed 44% of the carbohydrate, 58% of the protein, and 27% of the lipid within a few days (Handa 1969). Another study on *S. costatum* found that after 10 days in darkness, glucan was less than 1% of total cell carbon (Varum *et al.* 1986). While glucan content can decrease to almost zero, total carbohydrate content can only decrease to a certain point, since cell wall carbohydrates are not used for respiration.

Sinking Rate

If energy is necessary for sinking rate control, the loss of both photosynthetic and respiratory energy during prolonged periods in the dark should result in increased sinking rates. For a cell below the euphotic zone, an increased sinking rate would decrease its chances of being mixed back into the euphotic zone and having a renewed chance to grow. After 226 h of darkness, sinking rates of *D. brightwellii* increased 10-fold (Waite *et al.* 1992c). Since sinking rate increases did not occur until after the first 50 hours in the dark, it was hypothesized that the cell had been obtaining energy from reserve carbohydrates, but unfortunately carbohydrate content was not measured in this study. Even over the course of a 3 h sinking rate experiment, sinking rates were higher when the experiment was run in the dark than in the light (Waite *et al.* 1992c).

GOALS OF THIS CHAPTER

Carbohydrate changes predictably with irradiance, increasing during the day and at high light, and decreasing in the dark and under low light. Sinking rate does not vary predictably with light. If sinking rate control was wholly dependent on energy availability, sinking rates would be high under low light and low under high light, which does not always occur. Carbohydrate ballasting would increase sinking rates under high light, and decrease them under low light. These competing influences of carbohydrate ballasting and energy availability may be causing the variability in the response of sinking rate to light. In order to differentiate between sinking rate due to the inherent ballast of the cell (i.e. bulk composition

and frustule), and the sinking rate obtained when physiological sinking rate control mechanisms are active, it is necessary to compare the sinking rates of live cells to cells that have been physiologically incapacitated through treatment with respiratory inhibitors, preservatives, heat, etc.

The goal of this chapter was to measure the carbohydrate content and sinking rate of live and heat-killed cells under diel cycles, high and low, white and blue light, and in prolonged darkness, to determine the extent to which carbohydrate acts as ballast, when physiological sinking rate control mechanisms are in place (live cells) and when there is no physiological control (heat-killed cells).

MATERIALS AND METHODS

GENERAL METHODS

The marine diatoms *Thalassiosira weissflogii* and *Ditylum brightwellii* were obtained from the Northeast Pacific Culture Collection, Department of Oceanography, University of British Columbia (NEPCC No. 636 and 8 respectively). Semi-continuous batch cultures were grown in enriched artificial seawater (ESAW) based on the recipe by Harrison *et al.* (1980) with sodium glycerophosphate replaced with an equimolar concentration of sodium phosphate, ferrous ammonium sulphate with an equimolar concentration of ferric chloride and additions of selenite, nickel and molybdate to acheive 1 nM final concentration.

Cultures were grown at 16°C in a cold chamber on a 14:10 light:dark cycle, in 3 L glass flat-bottom boiling flasks, bubbled continuously with air filtered through a 0.22 μm membrane filter, and stirred with Teflon-coated stir bars at 60 rpm. Illumination was provided by Vita-lite™ fluorescent tubes to give 200 μmol quanta m^{-2} s^{-1} for cultures grown under high light and attenuated with neutral density screening to give 18 μmol quanta m^{-2} s^{-1} for cultures grown under low light. These irradiances were measured in air inside empty culture vessels using a Biospherical Instruments QSL 100 light meter.

Growth rate was measured daily both by taking *in vivo* fluorescence and cell counts about 30 min into the light period. *In vivo* fluoresence was measured with a Turner Designs Model 10 AU fluorometer. Cell counts were made using a Coulter Counter model TAII equipped with a population accessory. Cell volumes were calculated from Coulter Counter measurements using a 100 μm aperture that had been calibrated using 10 μm latex microspheres. All measurements were made on cells in mid-logarithmic growth phase, after acclimation for a minimum of 10 generations for high light cultures and 6 generations for low light cultures.

CELL COMPOSITION

Cell carbon and nitrogen content were determined by filtering samples onto pre-combusted 13 mm Gelman AE glass fiber filters and analyzing them using a Carlo Erba model 60 CNS analyzer. Chlorophyll quotas were measured fluorometrically after extraction in 90% acetone (Parsons *et al.*, 1984b).

Protein measurements were made by filtering samples onto precombusted 25 mm Whatman GF/F filters, which were frozen at -20°C until analysis. Homogenization was done as described by Berges *et al.* (1993), with filters ground in 3% trichloroacetic acid and solublized in 1N NaOH. Protein was assayed using the method of Bradford (1976), using the microassay procedure of the Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, 500-001) and bovine serum albumin (BSA, Sigma Chemical Co. A 7638) as a standard.

Carbohydrate samples were taken by filtering between 50 and 100 mL of culture onto precombusted 25 mm Whatman GF/F filters, which were frozen at -20°C until analysis could be performed. Carbohydrate was extracted by placing the filters in 13 x 100 mm borosilicate glass test tubes with 2.5 mL of 3N H_2SO_4, then placing the tubes in 95°C water for 2 h. The extract was poured off and reserved, and another 2.5 mL was added to the filter, extracted at 95°C for an additional 30 min and then the extracts were pooled. The carbohydrate was assayed by the phenol-sulfuric acid method of Dubois *et al.* (1956), using larger test tubes (16

x 125 mm) and directing the stream of acid onto the surface of the sample to ensure the sample was heated and mixed evenly (Herbert *et al.* 1971).

SINKING RATE

Sinking rates were measured using the SETCOL method of Bienfang (1981b) with modifications as in Waite *et al.* (1992c). The SETCOL was water-jacketed and maintained at 16 ± 0.5°C. Cell counts measured with a Coulter Counter were used as the index of biomass. The population accessory on the Coulter Counter made it possible to determine the number of cells in a range of size classes, from which a relationship between sinking rate and cell size could be determined.

Due to the large number of sinking rate measurements being made simultaneously, 20 mL subsamples of the different fractions were put into glass scintillation vials and preserved with Lugol's solution rather than counting immediately. While the shrinkage caused by Lugol's is a potential complication, most of this shrinkage occurs in the first 24 h (Montagnes *et al.* 1994). Preserved samples were not counted until at least 48 h had elapsed from the time of preservation.

CARBOHYDRATE CONTENT AND SINKING RATE EXPERIMENTS

Three cultures each of *T. weissflogii* or *D. brightwellii* grown under high or low light were subsampled at intervals of between 1 and 6 h for cell counts and carbohydrate. When sampling was done in the dark, care was taken to minimize exposure to light, by sampling into opaque vessels with only enough light for vision.

Sinking rate measurements were made in the last 3 h of the light period and the last 3 h of the dark period. The sinking rate trials at the end of the light period were run under the same light regime that the cells were growing under (18 μmol photons m^{-2} s^{-1} for low light and 200 μmol photons m^{-2} s^{-1} for high light). For sinking measurements made in the last 3 h of the dark period, the trials were carried out in the dark, and care was taken during sampling

to minimize exposure to light. Samples were also filtered for carbon, nitrogen, carbohydrate, chlorophyll *a* and protein analyses.

Trials comparing sinking rate of heat-killed to live cells were conducted the same way as the diel sinking rate trials previously described except that 350 mL of the subsampled culture was put into a 500 mL Erlenmeyer flask and microwaved at medium power for 2.5 min (45°C final temperature) for high light cultures and 3 min (50°C final temperature) for low light cultures. A subsample of the heat-treated cells was stained with Evans blue, a mortal stain that enters a cell when membrane integrity is lost (Crippen and Perrier, 1974), to determine the proportion of cells that were dead. Preliminary experiments showed that sinking rates increased markedly when only 10% of the cells were dead. If less than 5% of the cells were dead, the sample was microwaved for further 15 s intervals until at least 10% of the cells in the sample were dead. Cells were not heat-treated until all were dead because this could be accompanied by a decrease of about 25% in cell volume and up to 75% in carbohydrate. It was important to maintain carbohydrate and volume as near to the control values as possible, in order to make heat-killed sinking rates comparable to sinking rate measurements on live cells. The routine heat treatment typically killed between 20 and 40% of cells, with decreases of between 5-20% in cell volume and 5-75% in carbohydrate content.

Because heat killing is inexact, and the extent of cell volume decrease and carbohydrate loss varied, carbohydrate measurements were made on both the control culture before the sinking rate trial, and on leftover heat-killed and live culture from the middle fraction of the SETCOL to determine the carbohydrate content of heat-killed cells.

PROLONGED EXPOSURE TO DARKNESS -- *D. brightwellii*

Four 3 L cultures of *D. brightwellii* were grown under 200 μmol photons m^{-2} s^{-1} continuous light, bubbled and stirred as previously described. When they reached mid-logarithmic phase they were put in the dark. Subsamples were taken after 0, 100 and 244 h in the dark. At each subsampling time, sinking rates were measured on live and heat-killed cells

in the dark, and samples were taken for carbohydrate, protein, chlorophyll, carbon and nitrogen content.

RESULTS

I. *Thalassiosira weissflogii*

A) WHITE LIGHT

GROWTH RATE

Growth rates of *Thalassiosira weissflogii* grown under high (200 μmol photons m^{-2} s^{-1}) and low light (18 μmol photons m^{-2} s^{-1}) were 1.0 ± 0.05 d^{-1} and 0.21 ± 0.03 d^{-1} respectively (growth vs irradiance curve in Appendix 4).

CARBOHYDRATE CONTENT OVER A DIEL CYCLE

The carbohydrate content of *T. weissflogii* grown under high light increased 3-fold during the light period, and decreased by a similar amount during the dark period (Fig. 1.1). At the end of the dark period, cells grown under low light contained about half the carbohydrate of high light grown cells; by the end of the light period, low light grown cells contained only 15% of the carbohydrate content of high light grown cells. The carbohydrate content of cells grown under low light was lowest just before noon, with an increase of approximately 50% during the light period.

SINKING RATE AND CARBOHYDRATE CONTENT

There was no significant difference in sinking rate of cells grown under low light between the end of the light period and the end of the dark period (Fig. 1.2). Sinking rates of cells grown under high light were significantly higher than those of cells grown under low light. At the end of the dark period, the sinking rates of cells grown under high light were significantly higher than at the end of the light period. These results were similar to those of

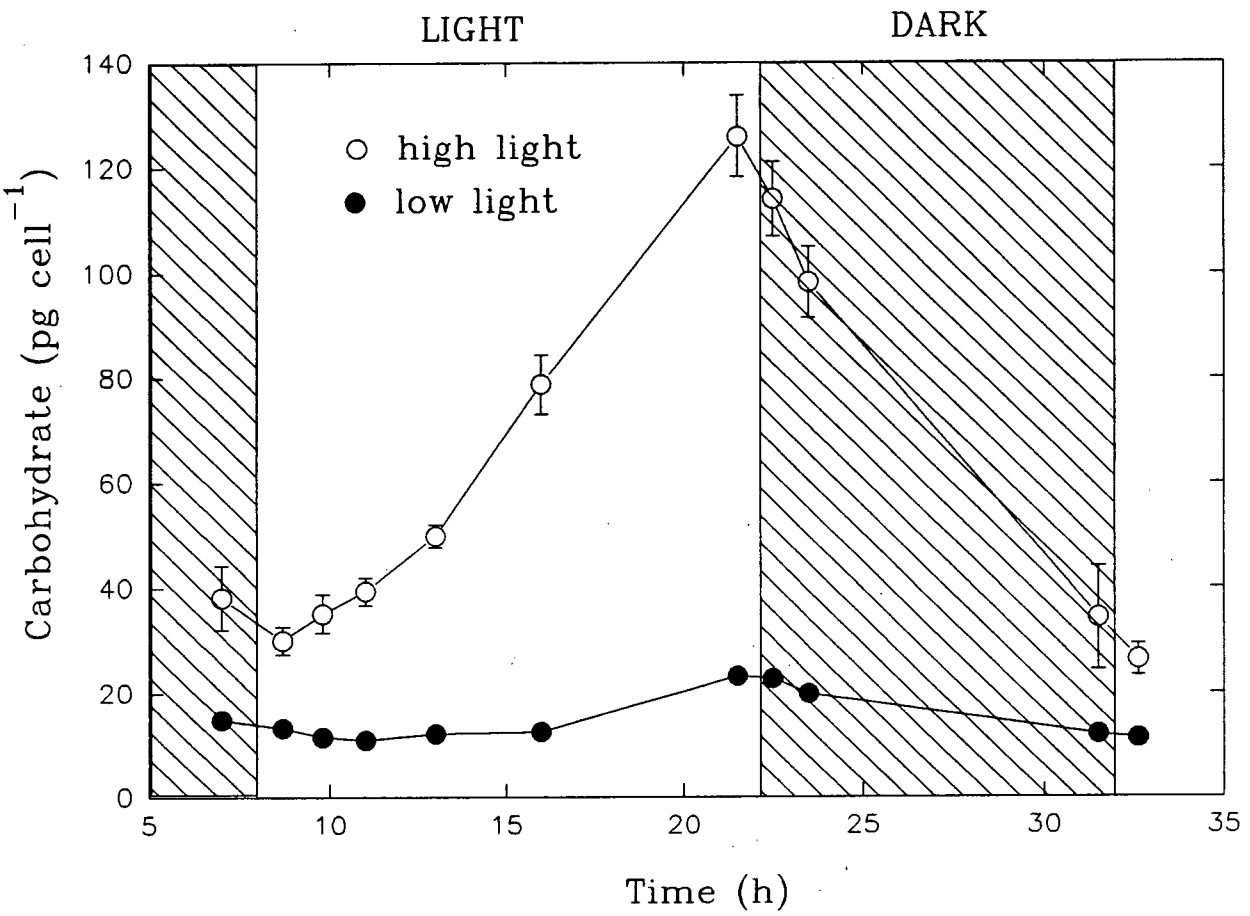

Figure 1.1 Carbohydrate content over a diel cycle (14 L:10 D) for *Thalassiosira weissflogii* grown under high white light (200 μmol photons m^{-2} s^{-1}) or low white light (18 μmol photons m^{-2} s^{-1}). Bars represent the mean of 3 replicate cultures with ± 1 SE, or where not seen are smaller than symbol.

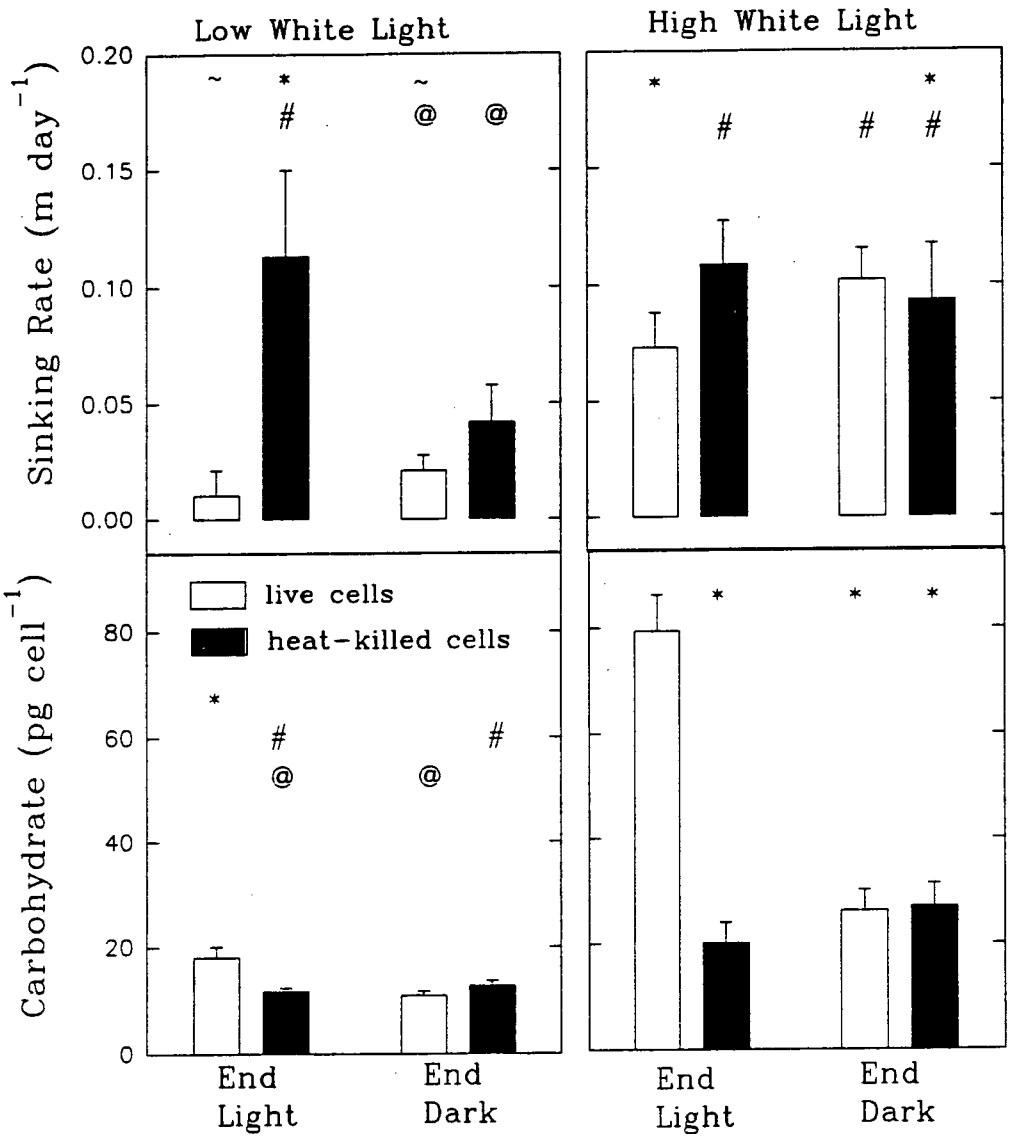

Figure 1.2 Effect of heat-killing on sinking rate (3 h SETCOL) and carbohydrate content of *Thalassiosira weissflogii* grown on a 14:10 light:dark cycle under low white light (18 μmol photons m^{-2} s^{-1}) or high white light (200 μmol photons m^{-2} s^{-1}) and measured at the end of the light period (end light) and the end of the dark period (end dark). Each bar represents the mean of four replicate cultures ± 1 SE. Matching symbols are above bars that are not significantly different; comparison applies to adjacent graphs (i.e. low and high light).

a previous experiment, except that in the previous trial the sinking rates of cells grown under high light were not significantly higher than those of cells grown under low light, and there was no difference in sinking rate of high light grown cells between the end of the light period and the end of the dark period (data in Appendix 1).

At the end of the light period, the sinking rate of heat-killed cells was significantly higher than that of live cells by about 10-fold in low light grown cells, and 30% in high light grown cells. At the end of the dark period, sinking rates of live and heat-killed cells were not different for either low or high light grown cells. There were no differences in the sinking rates of heat-killed cells except in cells grown under low light at the end of the dark period, for which heat-killed sinking rates were 50% lower.

Carbohydrate content was about 4 times higher in high light grown cells at the end of the light period compared to the other treatments. For both low and high light grown cells, carbohydrate content at the end of the light period was lower after heat-killing, decreasing by 30% for low light grown cells and 75% for high light grown cells. At the end of the dark period, the carbohydrate content of heat-killed cells was either not different or just slightly significantly higher than that of live cells. There was a negative correlation between sinking rate and carbohydrate content in live low light grown cells, but no relationship between sinking rate and carbohydrate content in live high light grown cells or in heat-killed cells (Fig. 1.3).

For cells grown under high light, there was no difference in the relationship between sinking rate and cell volume for live or heat-killed cells at either time of day (Fig. 1.4). For cells grown under low light, the sinking rate of live cells was generally lower than that of heat-killed cells at the end of the light period, but at the end of the dark period, the relationship between sinking rate and cell volume of live and heat-killed cells was not significantly different.

In summary, only in low light grown cells at the end of the light period was the sinking rate of heat-killed cells substantially higher than that of live cells. There were no

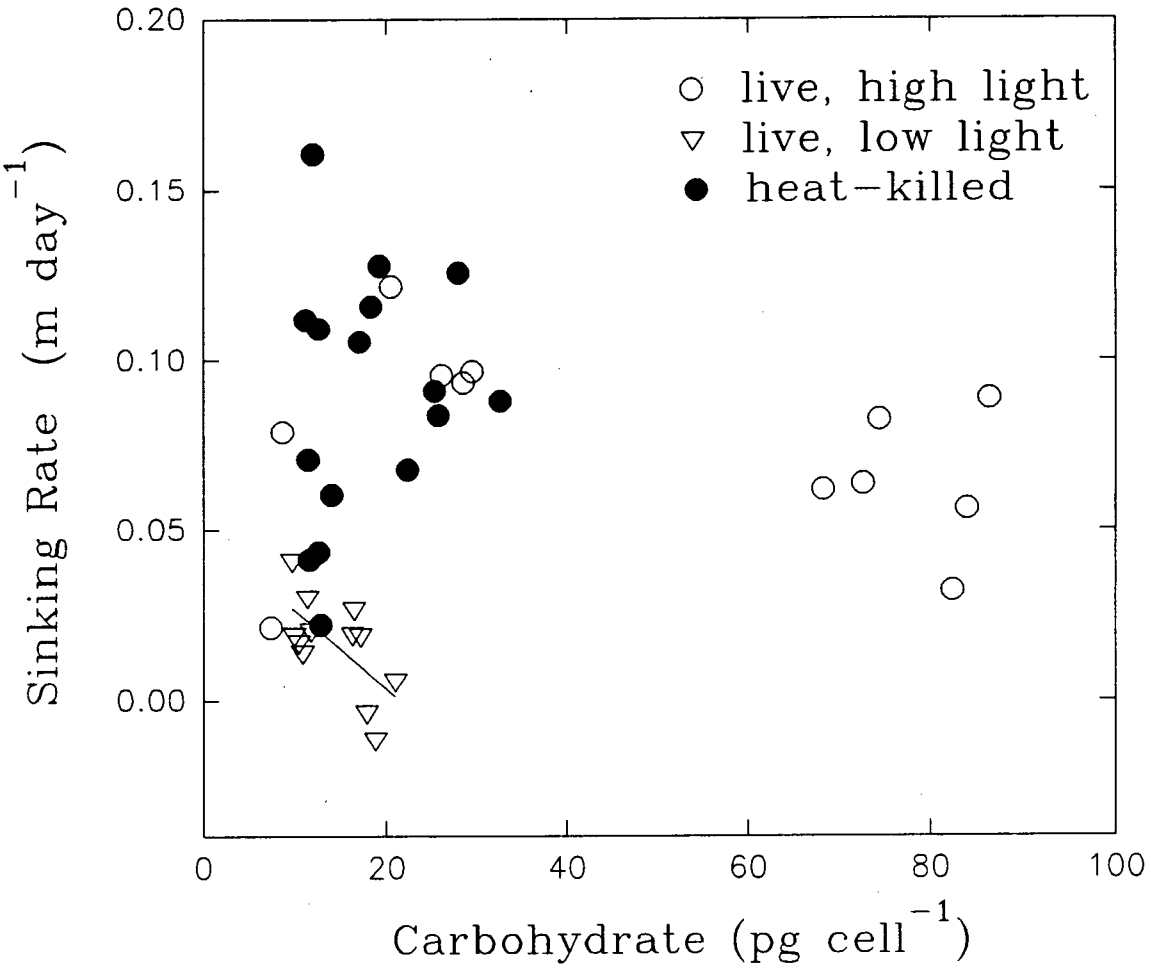

Figure 1.3 The relationship between sinking rate and carbohydrate content in live and heat-killed *T. weissflogii*. Includes data from cultures grown under high and low light, at the end of the light period and the end of the dark period. The relationship was significant for cells grown under low light; the solid line represents a linear regression through the data. Equation of regression line is $Y = -0.00227\ X + 0.049$ ($r^2 = 0.412$).

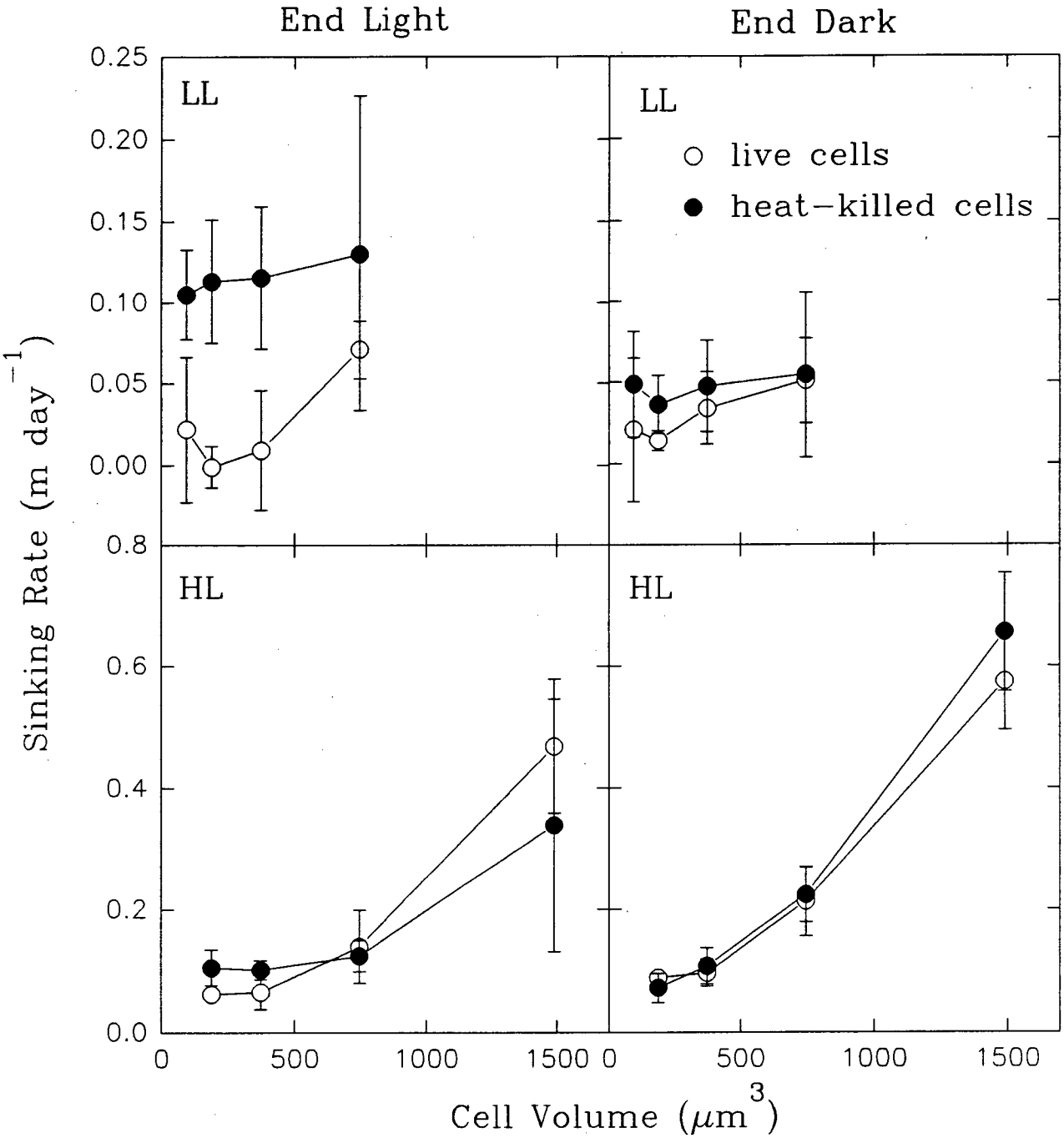

Figure 1.4 The relationship between sinking rate and cell volume in live and heat-killed *T. weissflogii* grown under low white light (LL) (18 µmol photons m^{-2} s^{-1}) or high white light (HL) (200 µmol photons m^{-2} s^{-1}) and measured at the end of the light period (end light) and the end of the dark period (end dark). Values are the mean of 4 replicate cultures ± 1 SE.

differences in sinking rate between heat-killed cells except for low light grown cells at the end of the dark period, whose heat-killed sinking rate was low and not different than their live sinking rate. Sinking rates of high light grown cells were higher than those of low light grown cells.

CELL COMPOSITION

The average cell volume of cells grown under low light was 25% lower than cells grown under high light (Fig 1.5A). There were no significant differences in cell volume between the end of the light period and the end of the dark period for either low or high light grown cells.

Chlorophyll *a* content was about twice as high in cells grown under low light as it was in cells grown under high light. Chlorophyll *a* content decreased significantly from the end of the light period to the end of the dark period by about 15% in low light grown cells and 30% in high light grown cells (Fig. 1.5B).

Protein content decreased by about half from the end of the light period to the end of the dark period for both low and high light grown cells. There were no significant differences in protein content between low and high light grown cells at either time of day (Fig. 1.5C).

Carbon content decreased from the end of the light period to the end of the dark period by about 20% in low light grown cells and by almost half in high light grown cells (Fig. 1.5D). At the end of the light period, high light grown cells contained about 30% more carbon than low light grown cells; at the end of the dark period, the carbon content of high and low light grown cells was not significantly different.

Nitrogen content decreased significantly from the end of the light period to the end of the dark period by about 10% in low light grown cells and by about 30% in high light grown cells (Fig. 1.5E). There was no significant difference in nitrogen content between high and low light grown cells at the end of the light period, or between high and low light grown cells at the end of the dark period.

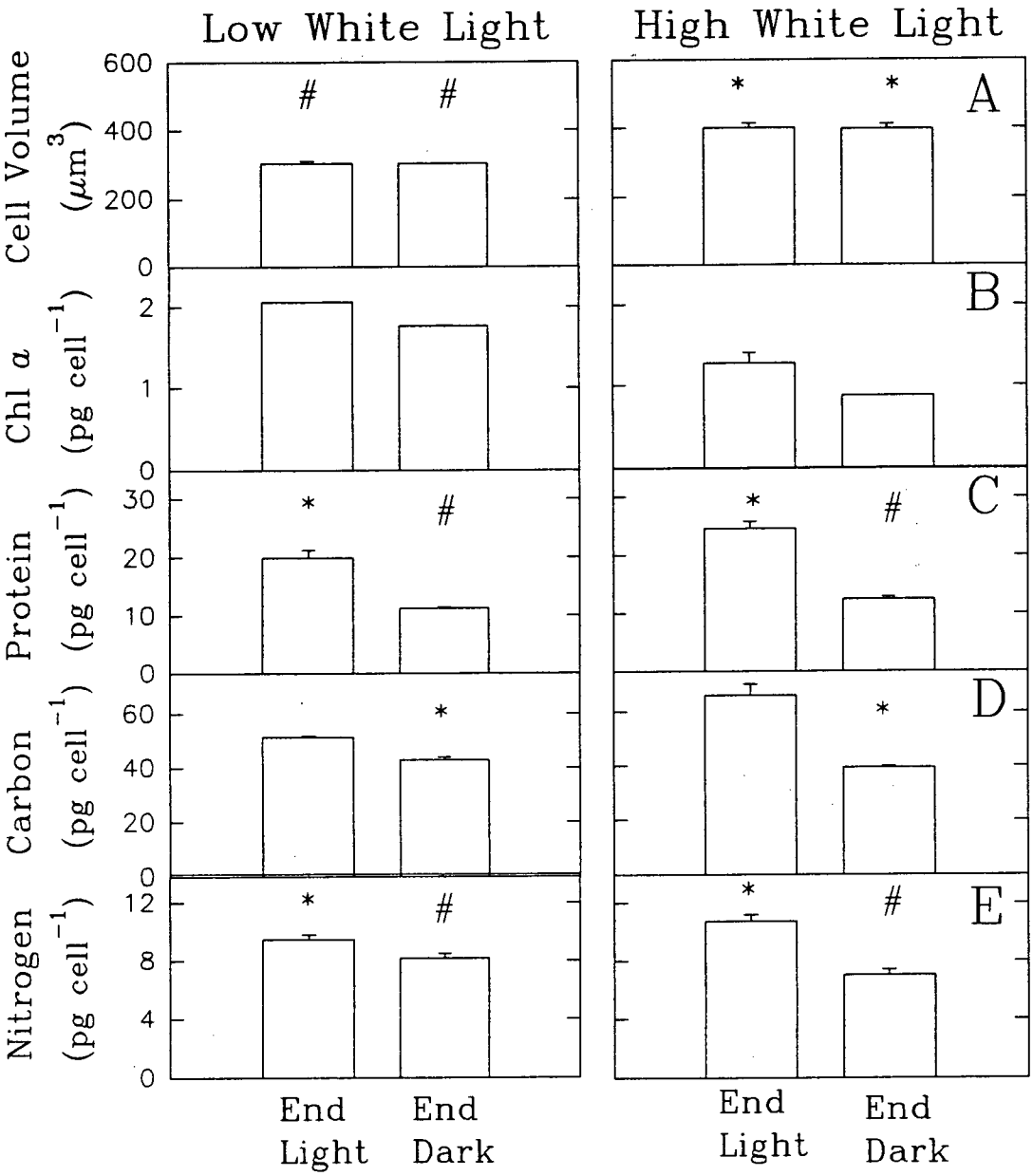

Figure 1.5 Cell composition of *Thalassiosira weissflogii* grown on a 14:10 light:dark cycle at low white light (18 µmol photons m^{-2} s^{-1}) or high white light (200 µmol photons m^{-2} s^{-1}). Samples were taken near the end of the light period (end light) and the end of the dark period (end dark) for A) cell volume, B) chlorophyll *a*, C) protein , D) carbon, and E) nitrogen. Each bar represents the mean of duplicate determinations from 2 separate cultures ± 1 SE, or where not seen are smaller than symbol. Matching symbols are above bars that are not significantly different; comparison applies to adjacent graphs (i.e. high and low light).

B) BLUE LIGHT

GROWTH RATES

Growth rates of *Thalassiosira weissflogii* grown under high and low blue light were $0.88 \pm 0.07\ d^{-1}$ and $0.23 \pm 0.01\ d^{-1}$ respectively.

SINKING RATE AND CARBOHYDRATE CONTENT

There was no difference in sinking rate from the end of the light period to the end of the dark period for cells grown under either low or high blue light (Fig. 1.6). Sinking rates of cells grown under high blue light at the end of the light period were significantly higher than those of low blue light grown cells. Sinking rates of cells grown under high blue light at the end of the dark period were not significantly different from those of cells grown under low blue light at either time of day. The sinking rates of cells grown under blue light were similar to those grown under white light, except that sinking rates of cells grown under high white light at the end of the dark period were significantly higher than the other treatments, while under blue light, sinking rates of high light grown cells at the end of the dark period were not different from any other treatment.

The changes in carbohydrate content from the end of the light period to the end of the dark period for *T. weissflogii* grown under blue light were less pronounced than those for *T. weissflogii* grown under white light (Fig. 1.6). The carbohydrate content of low blue light grown cells decreased by about 10% from the end of the light period to the end of the dark period, while the carbohydrate content of high blue light grown cells decreased by about 50%. High blue light grown cells contained about 5 times more carbohydrate than low blue light grown cells at the end of the light period, and about 3 times the carbohydrate of low blue light grown cells at the end of the dark period.

In summary, cells with the highest carbohydrate content (cells grown under high blue light at the end of the light period) had sinking rates at least 2 times higher than cells with the

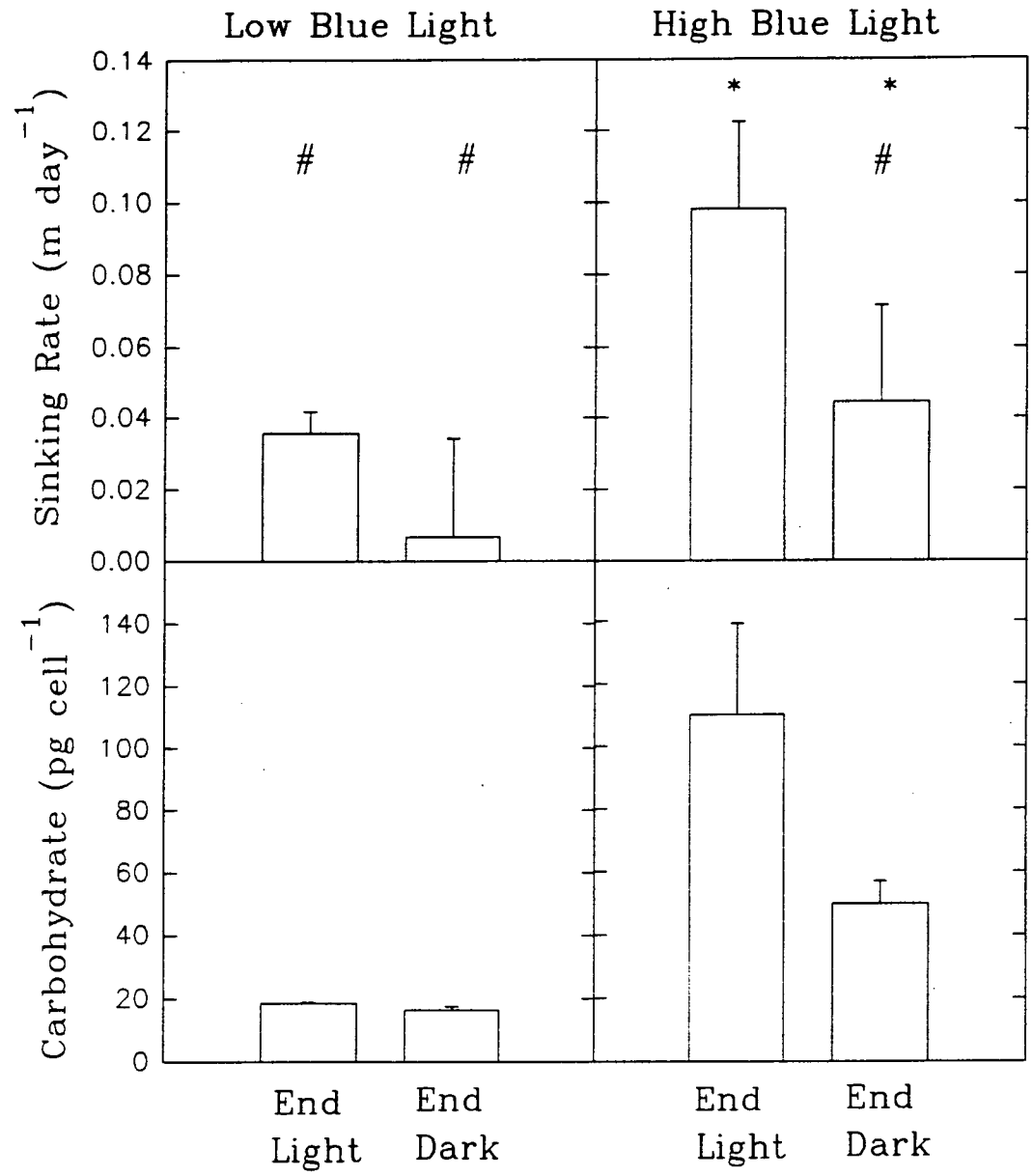

Figure 1.6 Sinking rate (3 h SETCOL) and carbohydrate content of *Thalassiosira weissflogii* grown on a 14:10 light:dark cycle under low blue light (18 µmol photons m^{-2} s^{-1}) or high blue light (200 µmol photons m^{-2} s^{-1}) and measured at the end of the light period (end light) and the end of the dark period (end dark). Each bar represents the mean of three replicate cultures ± 1 SE. Matching symbols are above bars that are not significantly different; comparison applies to adjacent graphs (i.e. high and low light).

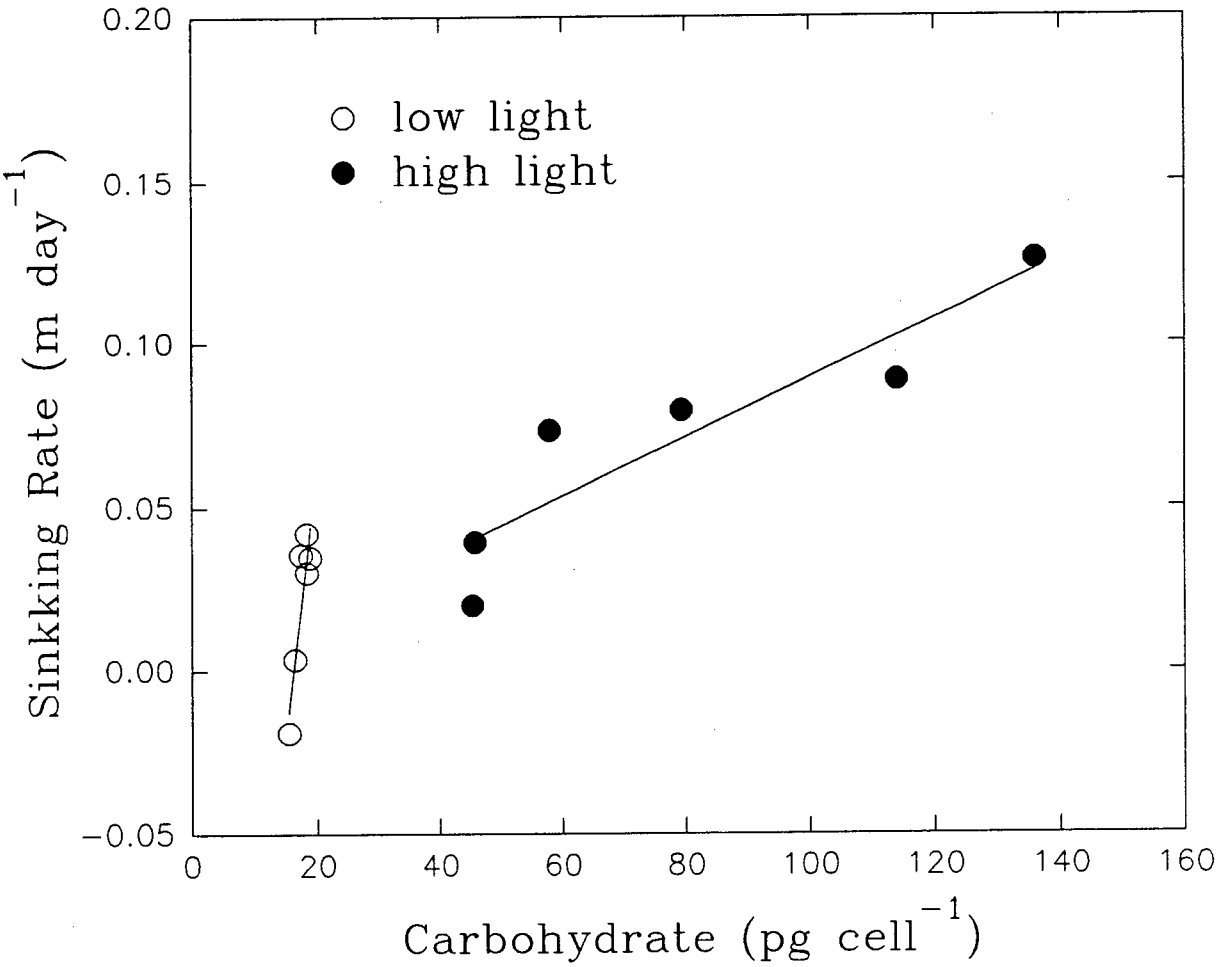

Figure 1.7 The relationship between sinking rate and carbohydrate content in *T. weissflogii* grown under low blue light (18 μmol photons m^{-2} s^{-1}) or high blue light (200 μmol photons m^{-2} s^{-1}). Figure includes data from the end of the light period and the end of the dark period. Solid line represents a linear regression through the data. Equation of the regression line is Y = 0.000902 X - 0.00074 (r^2 = 0.834) for cells grown under high light and Y = 0.01661 X - 0.2701 (r^2 = 0.833) for cells grown under low light.

lowest carbohydrate content (cells grown under low blue light). There was no difference in sinking rate in high blue light grown cells from the end of the light period to the end of the dark period, while carbohydrate decreased by half during the same time. There was a positive correlation between sinking rate and carbohydrate content for cells grown under high and low blue light (Fig. 1.7)

CELL COMPOSITION

The cell volume of *T. weissflogii* grown under blue light was about 50% larger than cells grown under white light in the previously described experiment (Fig 1.8A). Cells grown under high blue light were about 40% larger than cells grown under low blue light. There were no significant differences in cell volume between the end of the light period and the end of the dark period (Fig. 1.8A).

Chlorophyll *a* content of cells grown under low blue light decreased by about 15% from the end of the light period to the end of the dark period, while chlorophyll *a* did not change for cells grown under high blue light (Fig 1.8B).

The protein content of cells grown under low and high blue light decreased slightly from the end of the light period to the end of the dark period (Fig. 1.8C).

The carbon content of cells grown under low blue light was 20 to 50% lower than that of cells grown under high blue light (Fig. 1.8D). There was no significant difference in carbon content of low blue light grown cells from the end of the light period to the end of the dark period, while there was a decrease in carbon content of about 25% from the end of the light period to the end of the dark period in high blue light grown cells.

Nitrogen content of cells grown under high blue light also decreased from the end of the light period to the end of the dark period (Fig. 1.8E). Nitrogen content of cells grown under low blue light was lower than that of high blue light grown cells for the same time of day.

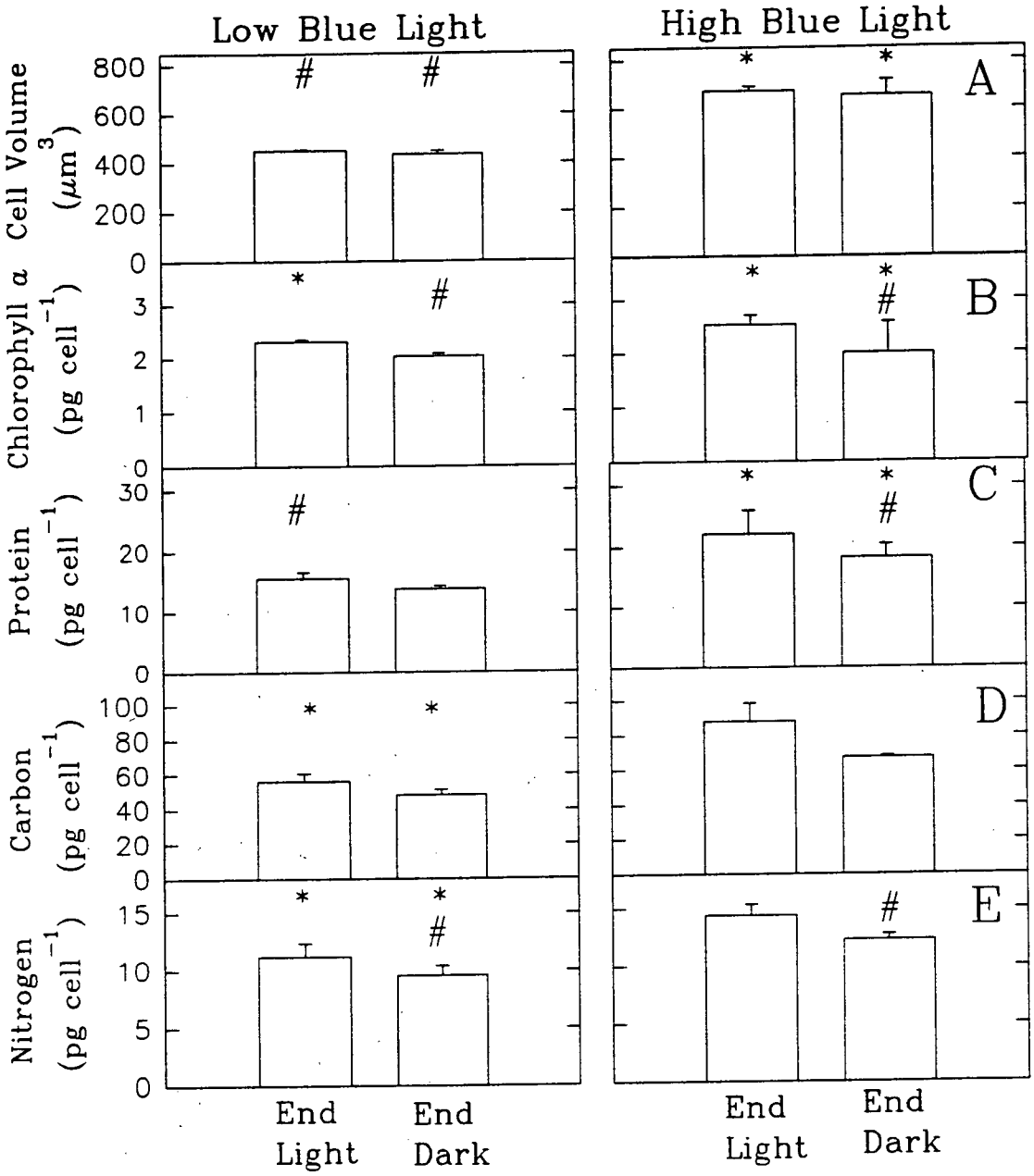

Figure 1.8 Cell composition of *Thalassiosira weissflogii* grown on a 14:10 light:dark cycle at low blue light (18 µmol photons m^{-2} s^{-1}) or high blue light (200 µmol photons m^{-2} s^{-1}). Samples were taken near the end of the light period (end light) and the end of the dark period (end dark) for A) cell volume, B) chlorophyll *a*, C) protein, D) carbon, and E) nitrogen. Each bar represents the mean of duplicate determinations from 3 separate cultures ± 1 SE. Matching symbols are above bars that are not significantly different; comparison applies to adjacent graphs (i.e. high and low light).

The general pattern of decreases in protein, carbon and nitrogen contents from the end of the light period to the end of the dark period was similar to that which occurred under white light.

II. *Ditylum brightwellii*

A) DIEL CYCLES

GROWTH RATE

Growth rates of *Ditylum brightwellii* grown under high (200 μmol photons m^{-2} s^{-1}) and low (18 μmol photons m^{-2} s^{-1}) white light were 0.98 ± 0.11 d^{-1} and 0.25 ± 0.06 d^{-1} respectively.

CARBOHYDRATE CONTENT OVER A DIEL CYCLE

The changes in carbohydrate content over a diel cycle were not as large for *D. brightwellii* (Fig. 1.9) as they were for *T. weissflogii* (Fig. 1.1). In cells grown under high light, carbohydrate increased by about 20% during the light period, then decreased by about 50% during the dark period. The carbohydrate content of cells grown under low light was between 10 and 20% of that of cells grown under high light, and changed by approximately 30% from minimal carbohydrate in the mid afternoon, to maximal carbohydrate at the end of the light period.

SINKING RATE AND CARBOHYDRATE CONTENT

Sinking rates and carbohydrate contents of live and heat-killed cells grown under high and low light at the end of the light period and the end of the dark period are shown in Figure 1.10. The results match those from a previous experiment that did not have a heat-killing component (data in Appendix 1). There was no difference in sinking rate between low light grown cells at the end of the light period, low light grown cells at the end of the dark period, and high light grown cells at the end of the dark period. At the end of the light period, cells

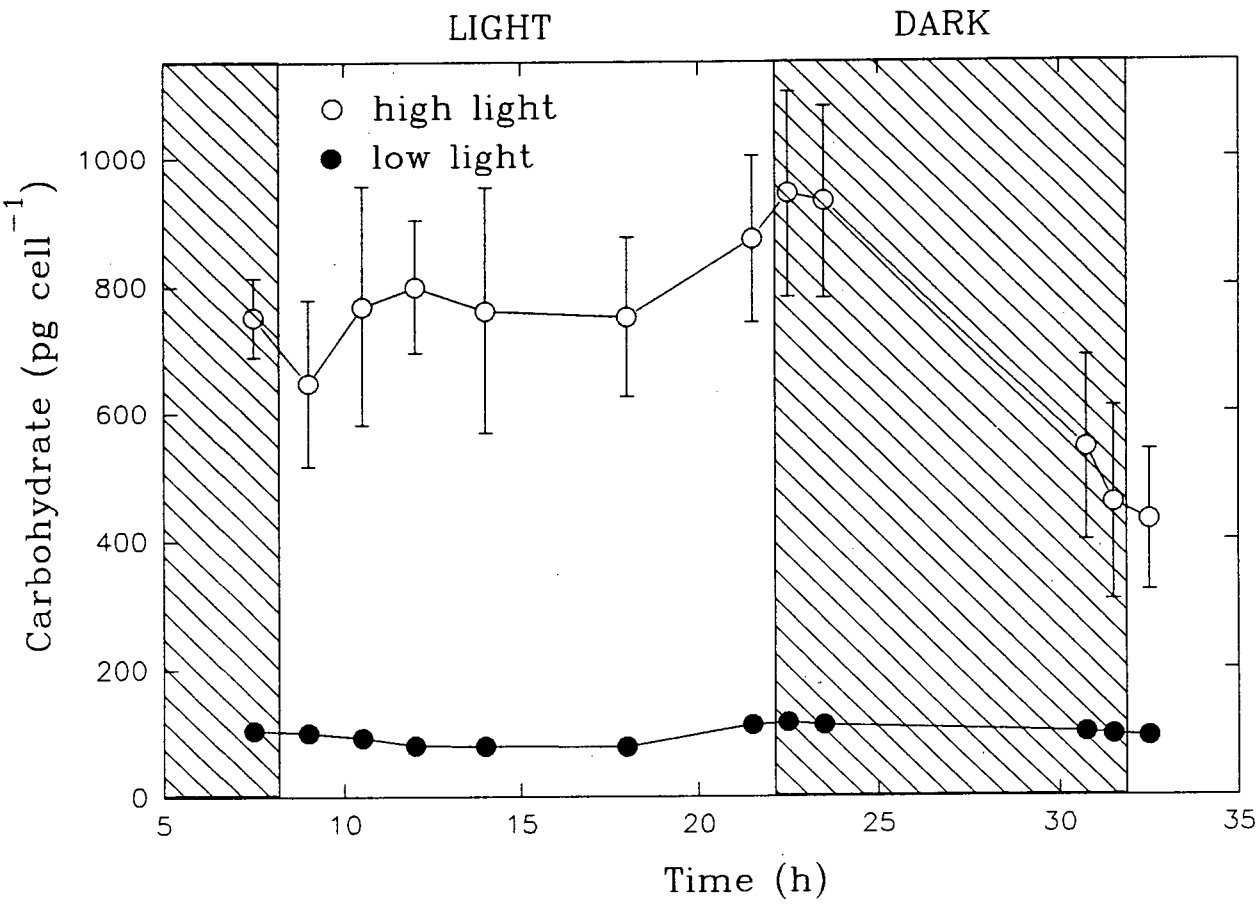

Figure 1.9 Carbohydrate content over a diel cycle (14 L:10 D) for *Ditylum brightwellii* grown under high white light (200 μmol photons m^{-2} s^{-1}) or low white light (18 μmol photons m^{-2} s^{-1}). Bars represent the mean of 3 replicate cultures with ± 1 SE, or where not seen are smaller than symbol.

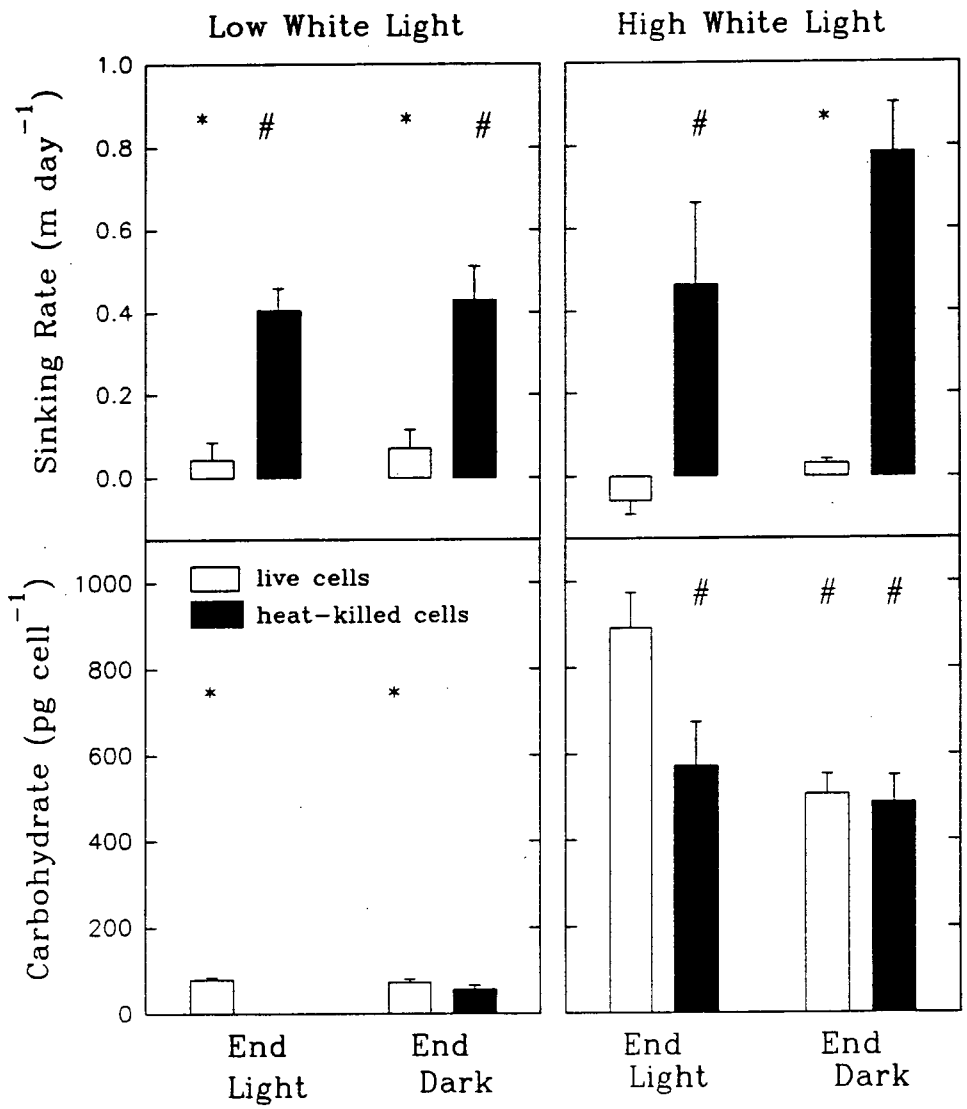

Figure 1.10 Effect of heat-killing on sinking rate (3 h SETCOL) and carbohydrate content of *Ditylum brightwellii* grown on a 14:10 light:dark cycle under low light (18 µmol photons m^{-2} s^{-1}) or high light (200 µmol photons m^{-2} s^{-1}) and measured at the end of the light period (end light) and the end of the dark period (end dark). Each bar represents the mean of four replicate cultures ± 1 SE. Matching symbols are above bars that are not significantly different; comparison applies to adjacent graphs (i.e. high and low light).

grown under high light had a negative sinking rate (i.e. an ascent rate), significantly lower than sinking rates of other live cells.

Sinking rates of heat-killed cells were about 10 times higher than those of live cells. There was no difference in heat-killed sinking rate between low light grown cells at the end of the light period, low light grown cells at the end of the dark period and high light grown cells at the end of the light period. Heat-killed sinking rates of high light grown cells at the end of the dark period were 40% higher on average than the other heat-killed sinking rates.

Cells grown under low light contained 10 to 20% of the carbohydrate of cells grown under high light. There was no significant difference in carbohydrate content of low light grown cells from the end of the light period to the end of the dark period. Low light grown heat-killed cells from the end of the dark period contained about 20% less carbohydrate than the live low light grown cells. Carbohydrate in low light grown heat-killed cells from the end of the light period was not measured.

The carbohydrate content of high light grown cells at the end of the dark period was about half of what it had been at the end of the light period. Heat-killing at the end of the light period reduced the carbohydrate content of high light grown cells by about 35%. There was no difference in carbohydrate content between live or heat-killed cells at the end of the dark period, and heat-killed cells at the end of the light period.

There was a negative correlation between sinking rate and carbohydrate content in live high light grown cells, and no relationship between sinking rate and carbohydrate content in live low light grown cells or heat-killed cells (Fig. 1.11).

The sinking rates of heat-killed cells tended to increase with cell size (Fig. 1.12). Sinking rates of live cells were lower than those of heat-killed cells, and tended to stay constant or decrease slightly with increasing cell volume.

In summary, the cells with the highest carbohydrate content (high light grown cells at the end of the light period) had the lowest sinking rates. Heat-killed sinking rates of low and high light grown cells at the end of the light period were not different despite a 5-fold

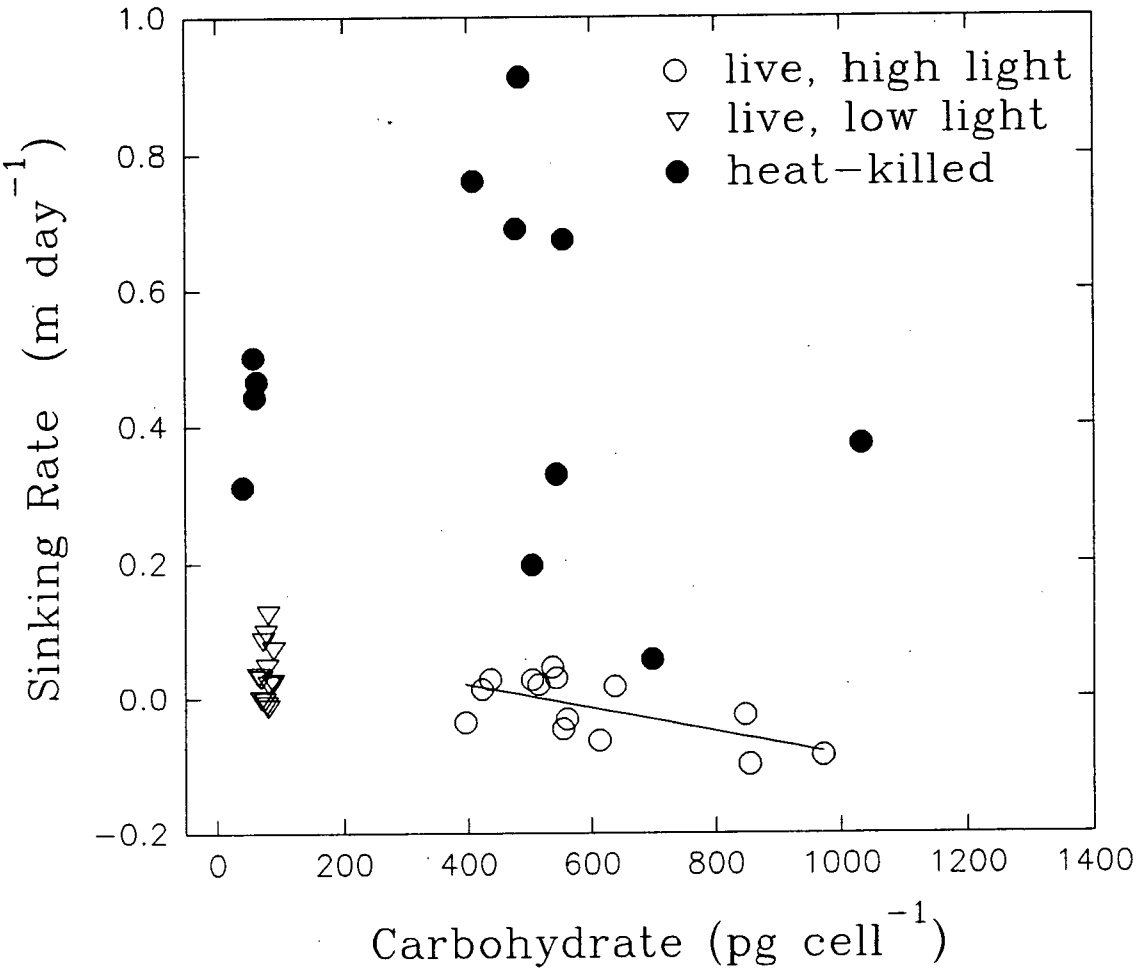

Figure 1.11 The relationship between sinking rate and carbohydrate content in live and heat-killed *D. brightwellii*. Figure includes data from cultures grown under high and low light, at the end of the light period and the end of the dark period. The relationship was significant for cells grown under high light; the solid line represents a linear regression though the data. The equation of the regression line is Y = -0.00017 X + 0.0904 (r^2 = 0.422).

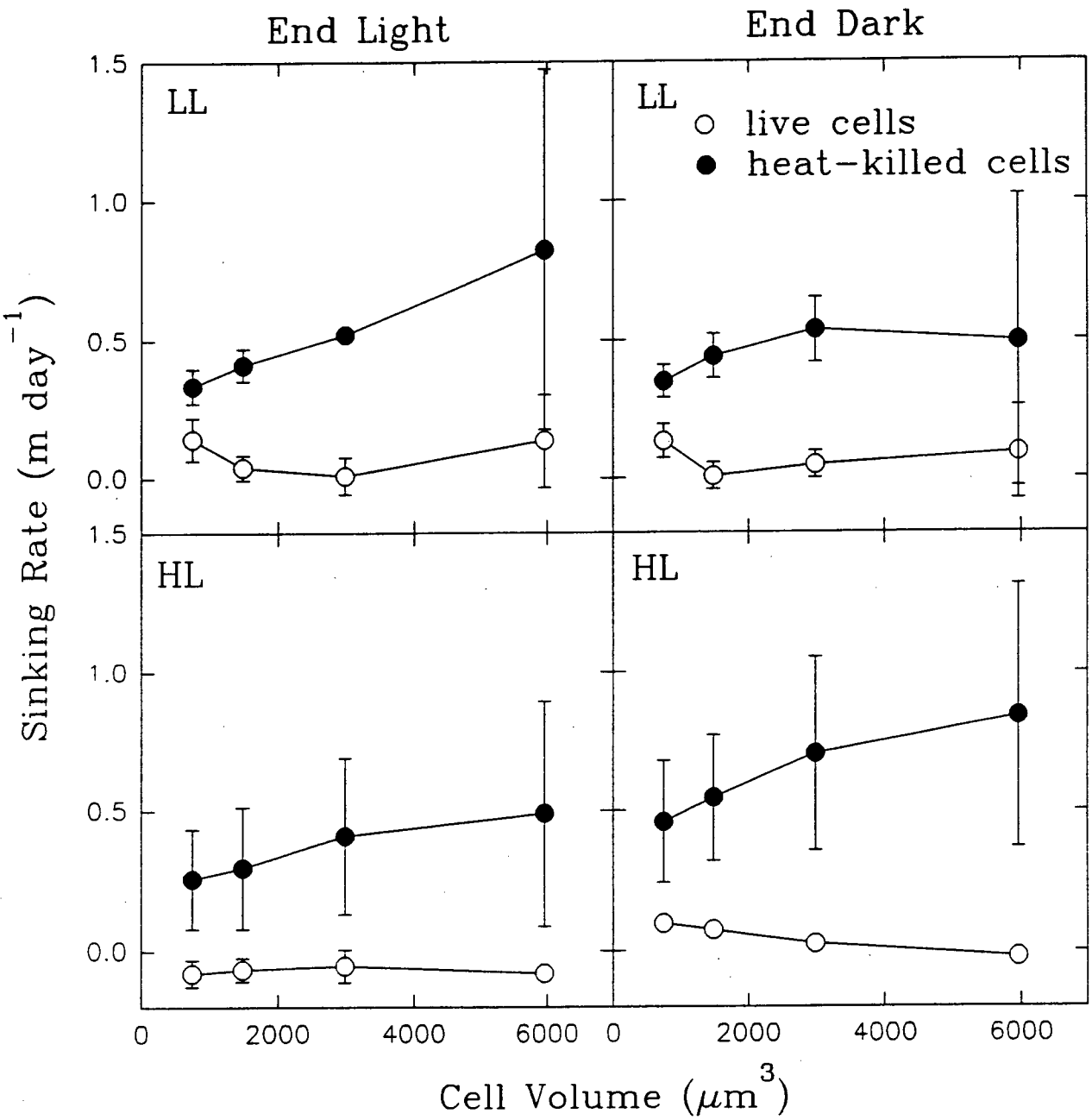

Figure 1.12 The relationship between sinking rate and cell volume in live and heat-killed *D. brightwellii* grown under low white light (LL) (18 µmol photons m^{-2} s^{-1}) or high white light (HL) (200 µmol photons m^{-2} s^{-1}) and measured at the end of the light period (end light) and the end of the dark period (end dark). Values are the mean of 4 replicate cultures ± 1 SE, or where not seen are smaller than symbol.

difference in carbohydrate content of these cells. In cells grown under high light, there was no difference in the carbohydrate content of heat-killed cells between the end of the light period and the end of the dark period; sinking rates of heat-killed cells were higher at the end of the dark period than at the end of the light period.

CELL COMPOSITION

The average cell volume (as determined by a Coulter Counter) of cells grown under high light was about twice that of cells grown under low light (Fig. 1.13A). Cell volume was significantly higher at the end of the dark period than at the end of the light period for both low and high light grown cells, with increases of approximately 5 and 10% respectively.

The chlorophyll *a* content of low light grown cells was about 20% higher than that of high light grown cells (Fig. 1.13B). There was no difference in chlorophyll *a* content from the end of the light period to the end of the dark period in either low light or high light grown cells.

Cells grown under high light contained 2 to 3 times as much protein as cells grown under low light (Fig. 1.13C). Protein content at the end of the light period was not different from that at the end of the dark period in either low or high light grown cells.

Cells grown under high light contained about twice the carbon and 50% more nitrogen than cells grown under low light. There were no differences in carbon or nitrogen content between the end of the light period and the end of the dark period for either low or high light grown cells (Fig. 1.13 D, E).

B) PROLONGED EXPOSURE TO DARKNESS

SINKING RATE AND CARBOHYDRATE CONTENT

Sinking rates of live *D. brightwellii* increased with increasing time in the dark (Fig. 1.14A). Sinking rates of heat-killed cells remained high and did not change with increasing

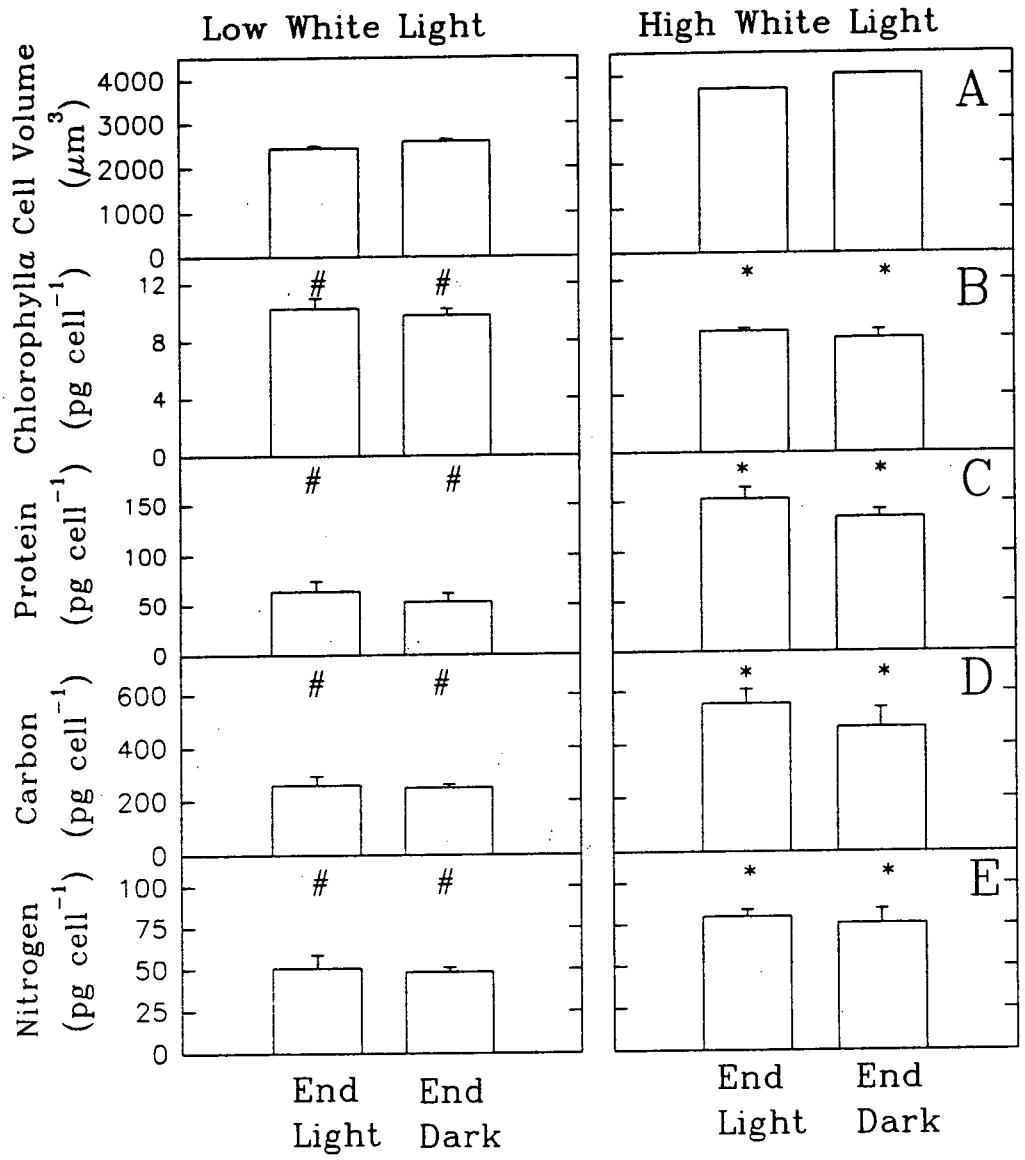

Figure 1.13 Cell composition of *Ditylum brightwellii* grown on a 14:10 light:dark cycle at low white light (18 µmol photons m^{-2} s^{-1}) or high white light (200 µmol photons m^{-2} s^{-1}). Samples were taken near the end of the light period (end light) and the end of the dark period (end dark) for A) cell volume, B) chlorophyll *a*, C) protein, D) carbon, and E) nitrogen. Each bar represents the mean of duplicate determinations from 3 separate cultures ± 1 SE, or where not seen are smaller than symbol. Matching symbols are above bars that are not significantly different; comparison applies to adjacent graphs (i.e. high and low light).

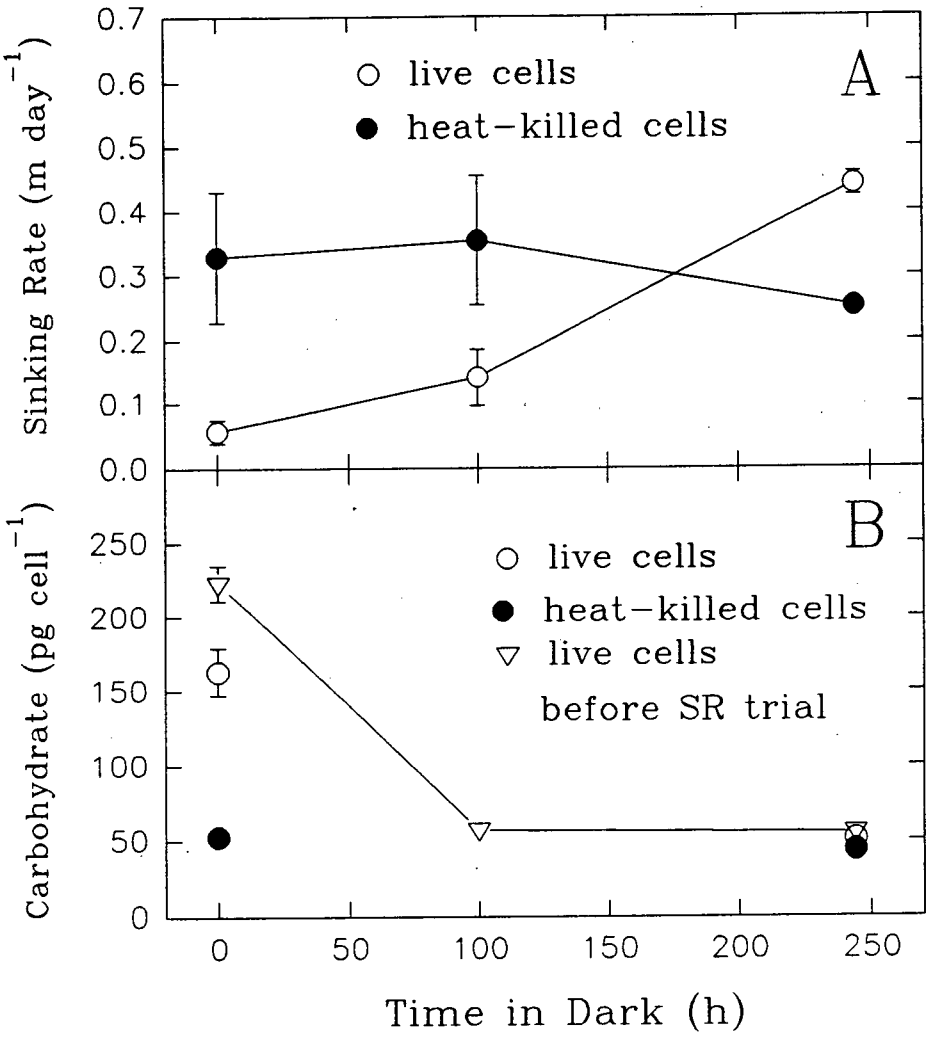

Figure 1.14 (A) Sinking rate (3 h SETCOL) and (B) carbohydrate content of live and heat-killed *D. brightwellii* grown at high white light (200 µmol photons m^{-2} s^{-1}) then placed in the dark for 244 h. Carbohydrate content before the sinking rate trial (live cells) is shown by inverted triangles. Carbohydrate content of live and heat-killed cells after the sinking rate trials (3 h) is shown by open and closed circles, respectively. Carbohydrate content after the sinking rate trial at 100 h was not measured. Values are the mean of 4 replicate cultures ± 1 SE, or where not seen are smaller than symbol.

time in the dark. At 0 and 100 h, sinking rates of live cells were lower than those of heat-killed cells, and by 244 h, sinking rates of live cells were about 50% higher than those of heat-killed cells. Heat-killing reduced the carbohydrate content of the cells to the baseline amount, regardless of the carbohydrate content of the live cells prior to heat-killing. Heat-killed cells had the same carbohydrate content as live cells that had been in the dark for at least 100 h. There was no correlation between sinking rate and carbohydrate content for live or heat-killed cells (Fig. 1.15). The maximum carbohydrate content of cells grown under continuous light was about 4 times lower than that of cells grown under diel cycles (data not shown).

The relationship between sinking rate and cell volume for live and heat-killed cells after 0, 100 and 244 h in the dark is shown in Figure 1.16. The pattern for heat-killed cells is similar for all 3 dark periods. For live cells, sinking rate decreased with increasing cell volume at 0 h, stayed the same with increasing cell volume at 100 h and increased with increasing cell volume at 244 h.

Cell volume decreased by about 50% over the course of the experiment (Fig. 1.17A). On a per cell basis, protein, carbon and nitrogen content decreased by half after 244 h in the dark, while chlorophyll *a* content did not change (Fig. 1.17). There were no changes in protein, carbon or nitrogen per unit cell volume; chlorophyll *a* content per unit cell volume increased (Fig. 1.18).

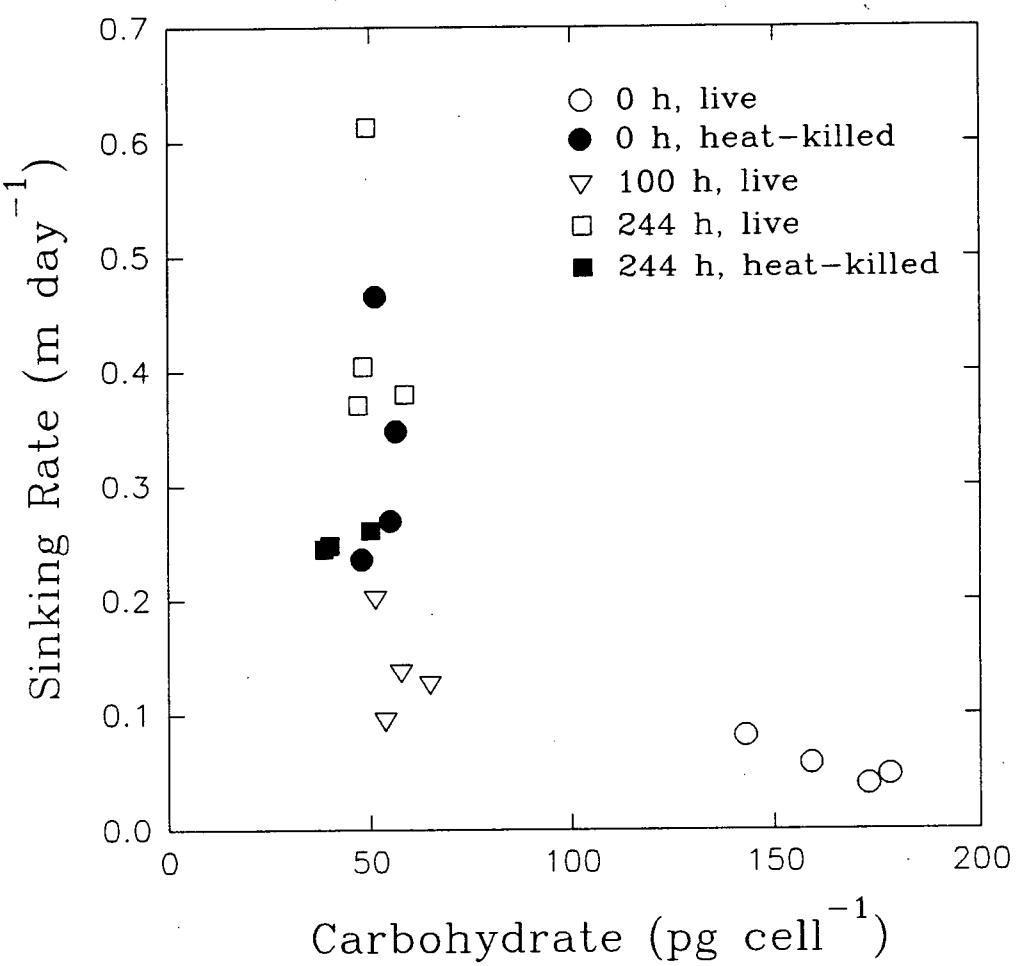

Figure 1.15 The relationship between sinking rate and carbohydrate content after 0, 100 and 244 h in the dark for live and heat-killed *D. brightwellii*.

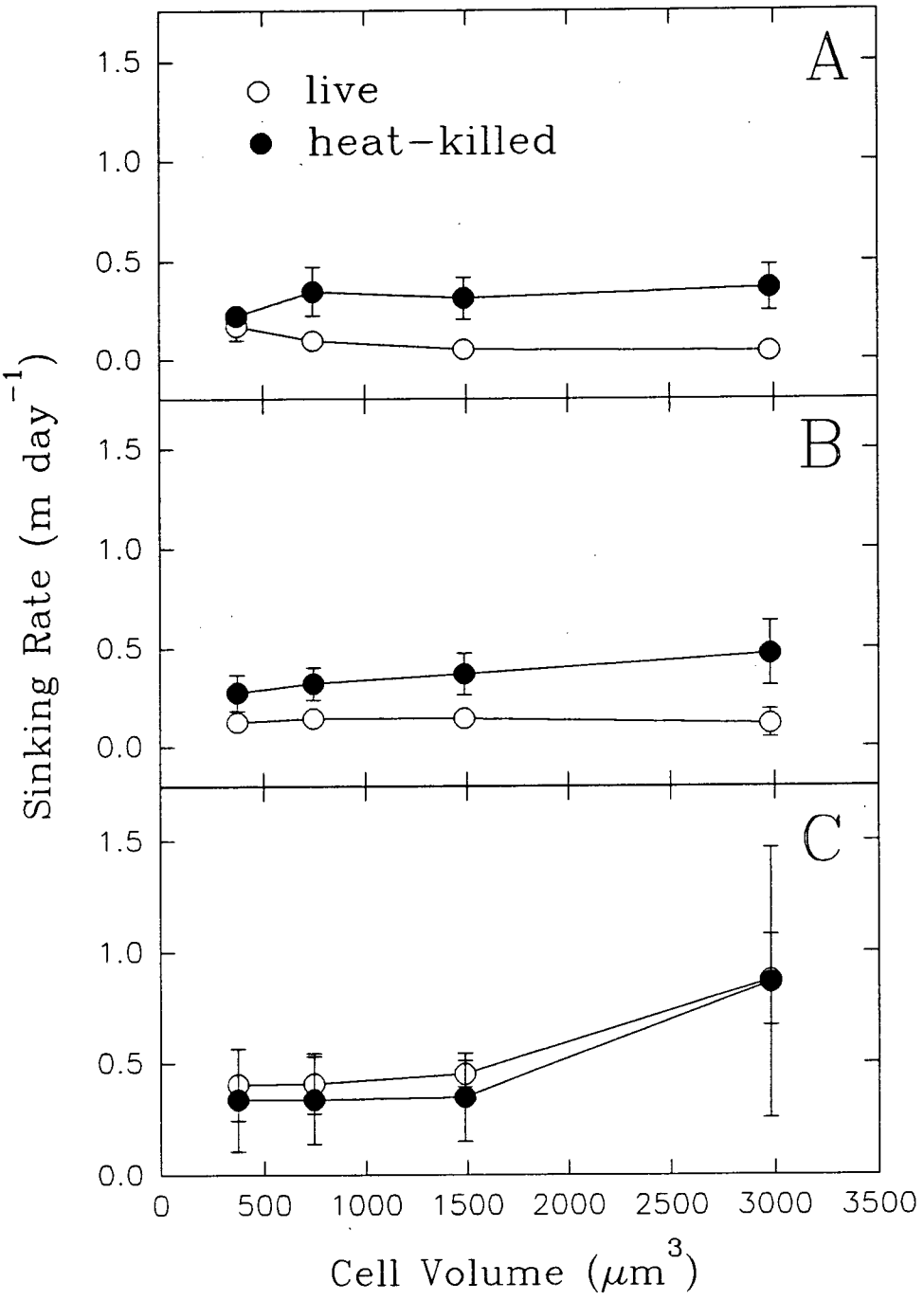

Figure 1.16 The relationship between sinking rate and cell volume in live and heat-killed *D. brightwellii* grown at high white light (200 µmol photons m^{-2} s^{-1}) and left in the dark for (A) 0 h, (B) 100 h and (C) 244 h. Values are the mean of four replicate cultures ± 1 SE, or where not seen are smaller than the symbol.

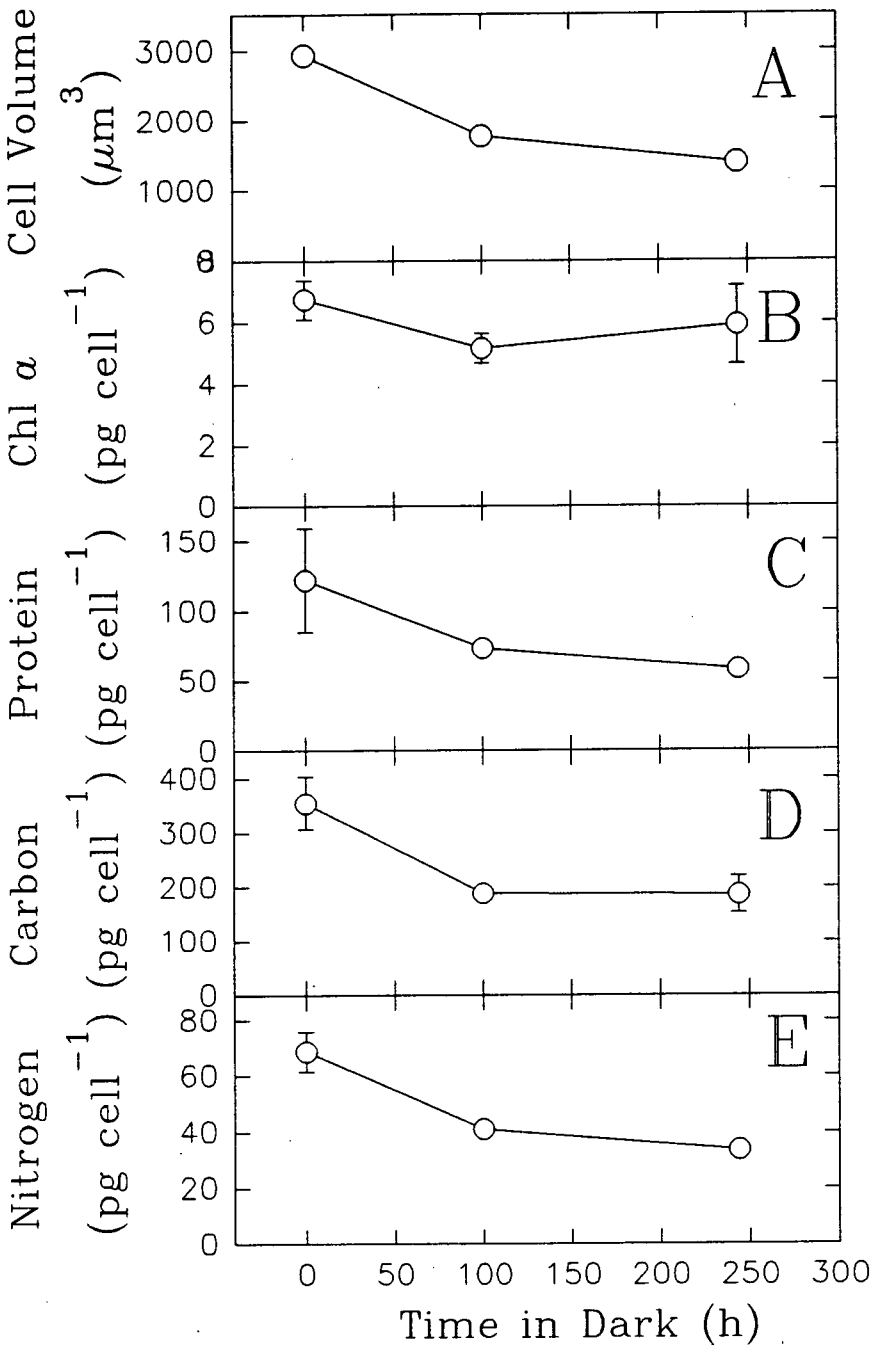

Figure 1.17 Cell composition (per cell) of *D. brightwellii* grown at high white light (200 µmol photons m^{-2} s^{-1}) then placed in the dark for 244 h. A) cell volume, B) chlorophyll *a*, C) protein, D) carbon, and E) nitrogen. Values are the mean of 4 replicate cultures ± 1 SE, or where not seen are smaller than symbol.

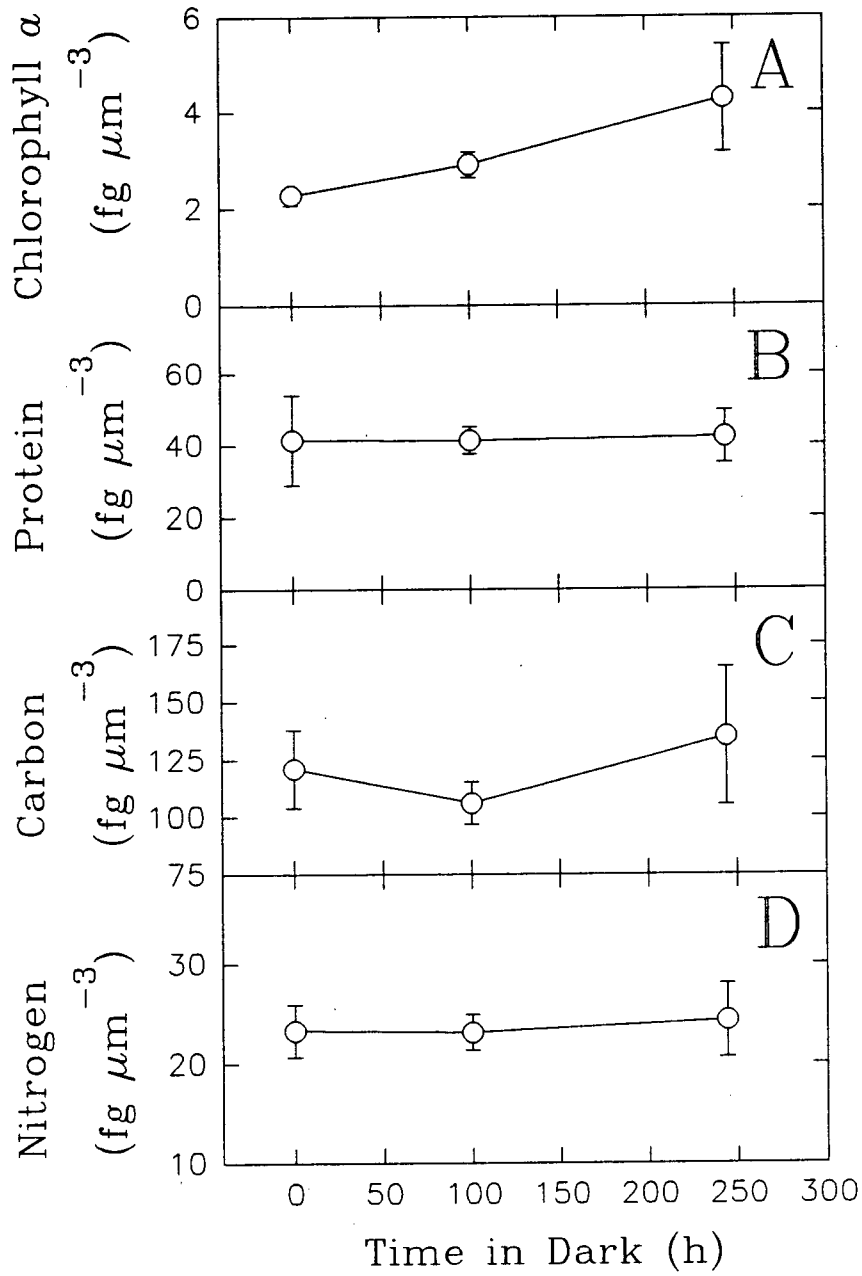

Figure 1.18 Cell composition (per unit cell volume) of *D. brightwellii* grown at high white light (200 μmol photons m^{-2} s^{-1}) then placed in the dark for 244 h. A) chlorophyll *a*, B) protein, C) carbon, and D) nitrogen. Values are the mean of 4 replicate cultures ± 1 SE.

DISCUSSION

The goal of these experiments was to test whether irradiance driven changes in carbohydrate content could affect sinking rate. Since carbohydrate is more dense than seawater, any increase in the carbohydrate content would increase the ballast of the cell. The increase in sinking rate seen in diatoms under high light (which runs contrary to the hypothesis of Waite *et al.* (1992c) that sinking rate control is energy dependent), supports the possibility that increased carbohydrate content could increase cell ballast and hence increase sinking rate. In order to compare the physiologically-determined sinking rate to what the sinking rate would be if there were no physiological control, sinking rates were measured on live and heat-killed cells.

I. *Thalassiosira weissflogii*

A) WHITE LIGHT: DIEL CYCLES UNDER HIGH AND LOW LIGHT

The lack of a positive correlation between sinking rate and carbohydrate content in low or high light grown cells shows that carbohydrate ballasting does not make a significant contribution to sinking rate. In cells grown under low light, there was actually a negative correlation between sinking rate and carbohydrate content.

The similar and low carbohydrate contents of heat-killed cells (carbohydrate probably leaked out of the cells) made it difficult to determine whether there was a relationship between carbohydrate content and sinking rate in heat-killed cells, which would have shown whether changes in carbohydrate content could affect cell density. Since there was a range of sinking rates in heat-killed cells despite their similar carbohydrate contents, it can be concluded that there are factors other than carbohydrate content that affect cell density in heat-killed cells.

The general lack of large increases in sinking rate after cells were heat-killed suggests that *T. weissflogii* does not rely on physiological sinking rate control. This is also shown by the similarity in the relationship between sinking rate and cell volume in live and heat-killed

cells. This is also supported by the data of Waite *et al.* (submitted) who found that treatment with a respiratory inhibitor (cyanide) did not increase the sinking rate of *T. weissflogii*.

Heat-killing did result in a large increase in sinking rate in cells grown under low light at the end of the light period, and a small increase in sinking rate in cells grown under high light at the end of the light period. This suggests that sinking rate was physiologically controlled at the end of the light period, but not at the end of the dark period. For physiological sinking rate control to occur at the end of the light period but not the end of the dark period, shows that cells at the end of the dark period are either less dense, making sinking rate control unnecessary, or cells are incapable of physiological sinking rate control, possibly because photosynthetic energy is unavailable. Since there was no large difference in the sinking rates of live cells from the end of the light period and the end of the dark period, it is more likely that physiological sinking rate control was unnecessary, presumably because cells were less dense. A loss of sinking rate control would have resulted in a larger sinking rate increase.

The larger sinking rates of heat-killed low light grown cells at the end of the light period compared to the end of the dark period suggest that the cells were less dense at the end of the dark period. Such a decrease in density would allow sinking rates to remain low despite the absence of physiological sinking rate control. While the decrease in carbohydrate content in these cells from the end of the light period to the end of the dark period was small, it could have contributed to the decrease in density.

Since most of the carbohydrate of high light grown cells at the end of the light period was lost during the heat-killing process, it cannot be determined from comparing heat-killed sinking rates whether there was a decrease in density in the high light grown cells similar to the low light grown cells. The heat-killed sinking rate of high light grown cells at the end of the light period may have been much higher if the carbohydrate had not leaked out. If the small difference in carbohydrate content in cells grown under low light produced the large difference in heat-killed sinking rate, then considering the much larger diel variation in

carbohydrate content of high light grown cells, the heat-killed sinking rates of high light grown cells at the end of the light period would be expected to be much higher than they were; dead *T. weissflogii* generally do not sink faster than about 0.12 m d^{-1} (Waite et al. submitted). This makes it doubtful that carbohydrate content is the main contributor to the density changes seen in low light grown cells.

In addition to changes in carbohydrate content, *T. weissflogii* showed decreases in protein, chlorophyll *a*, nitrogen and carbon contents from the end of the light period to the end of the dark period in cells grown under high and low light. Since there was no change in cell volume over this time, these compositional changes could have contributed to the decrease in density from the end of the light period than the end of the dark period.

T. weissflogii may rely less on physiological control of its sinking rate because there are chitan fibres on the cell that increase its form resistance (Walsby and Xypolyta, 1977). These chitan fibres are composed of glucosamine and have been known to account for 31-38% of the cellular material (including silica) on a dry weight basis (McLachlan et al. 1965). Despite the higher density of the fibres relative to the rest of the cell, they decrease the sinking rate by 1.7 times (Walsby and Xypolyta, 1977). *T. weissflogii* has also been known to secrete extracellular mucous and therefore decrease its sinking rate by increasing the viscosity of the culture medium. Although neither chitan fibres nor extracellular mucus were measured in this study (although they may have contributed to the overall carbohydrate content of the cells) both could keep sinking rates low even when cells were physiologically incapacitated.

B) BLUE LIGHT: DIEL CYCLES UNDER HIGH AND LOW LIGHT

In the first experiment where sinking rates were measured over a diel cycle (data in Appendix 1), the sinking rates of *T. weissflogii* under high light were not higher than under low light. This result did not agree with the results of Bienfang *et al.* (1983) who found higher sinking rates in *T. weissflogii* grown under high light (above 60 μmol m^{-2} s^{-1})

compared to those grown under low light (below 30 μmol m^{-2} s^{-1}). One difference between the experiments was that Bienfang *et al.* (1983) grew their cultures under blue light. In order to determine whether the difference between the experiments was due to the blue light, the experiment was repeated with cultures grown under high and low blue light.

The most striking difference between the experiments done under white light and those done under blue light is the strong correlation between sinking rate and carbohydrate content in cells grown under blue light, which was not observed in cells growing under white light. There were no large differences in sinking rate under white or blue light for the same treatment (high or low light, end of the light period or end of the dark period). Sinking rates under white light tended to increase from the end of the light period to the end of the dark period, while sinking rates under blue light tended to decrease.

It is unlikely that differences in carbohydrate metabolism are responsible for the correlation between sinking rate and carbohydrate content under blue light. While carbohydrate content tended to be higher and more variable under high blue light than high white light, changes in carbohydrate content over a diel cycle followed a similar pattern under both light treatments.

The greater changes in protein content observed under white light compared to blue light would, if anything, have contributed to a decrease in sinking rate from the end of the light period to the end of the dark period rather than the observed increase. The differences in cell volume between cells grown under blue and white light may have been due to long-term variation in cell size (e.g. Armbrust and Chisholm, 1992) rather than a light quality effect.

II. *Ditylum brightwellii*

A) WHITE LIGHT: DIEL CYCLES UNDER HIGH AND LOW LIGHT

The low sinking rates of all live *D. brightwellii* and the many-fold increase in sinking rates seen after heat killing in all treatments shows that sinking rate was under physiological

control. There was no evidence that carbohydrate content affected sinking rate in *D. brightwellii*, as shown by the lack of a difference in sinking rate between cells grown under high and low light, despite big differences in carbohydrate content, and the increase in sinking rate in cells grown under high light from the end of the light period to the end of the dark period, despite the approximate 50% decrease in carbohydrate content.

The lack of an effect of carbohydrate content on sinking rate is further illustrated by the absence of a relationship between sinking rate and carbohydrate content in heat-killed cells. Heat-killing did not remove as much carbohydrate from *D. brightwellii* as it did from *T. weissflogii*, so *D. brightwellii* grown under high light contained much more carbohdyrate after heat-killing than *D. brightwellii* grown under low light. The lack of a difference in heat-killed sinking rate under high and low light shows that carbohydrate does not affect density in these cells.

The negative relationship between sinking rate and carbohydrate content in live high light grown cells is more likely due to the higher energy level of cells grown under high light which allows them to maintain lower sinking rates, rather than an effect of carbohydrate content.

At the end of the light period, the sinking rate of cells grown under high light was negative (i.e. they had an ascent rate). This positive buoyancy may have been possible due to the light energy these cells were receiving, which would explain why cells were no longer positively buoyant at the end of the dark period. The possibility of cells being less dense at the end of the light period is supported by the higher sinking rates of heat-killed high light grown cells at the end of the dark period compared to the end of the light period. *D. brightwellii* grown under high, continuous light was not positively buoyant (data from 0 h of the dark exposure experiment) but the sinking rate measurement was made in the dark rather than in the light. Anderson and Sweeney (1978) found that changes in buoyancy in *D. brightwellii* grown under diel cycles did not occur under continuous light.

The diel changes in buoyancy observed in this study, with sinking rates lower in the light period than in the dark period, are similar to those found by Eppley *et al.* (1967), but opposite to those of Anderson and Sweeney (1977). Neither of those studies observed positive buoyancy in *D. brightwellii*, but sinking rate measurements were not made using the SETCOL method, so direct comparisons may not be valid. Positive buoyancy (up to 3 m d^{-1}) has been observed in *D. brightwellii* in the post-auxospore stage of the sexual cycle (Waite and Harrison 1992).

B) EFFECT OF PROLONGED DARKNESS ON SINKING RATE

Changes in carbohydrate content could have two opposing effects on the sinking rate of cells exposed to darkness. Initially, decreases in carbohydrate content could possibly reduce sinking rates by decreasing cell density. Once carbohydrate reserves were exhausted however, the cell would have lost an energy source that may have been used for physiological sinking rate control.

The loss of sinking rate control in live cells is shown by the way the relationship between sinking rate and cell volume changed from negative in live cells at 0 h, to flat at 100 h, to matching that of heat-killed cells by 244 h. The lack of a relationship between sinking rate and carbohydrate content in live or heat-killed cells suggests that changes in carbohydrate content had no detectable effect on sinking rate through changes in density.

The rapid decrease in carbohydrate content in the dark during the 3 h of the first sinking rate trial suggests that cells grown under continuous light quickly use up carbohydrate reserves in the dark. While no measurements of carbohydrate content were made between 0 and 100 h, it is estimated that carbohydrate reserves were exhausted by 24 h at the latest, based on the rate of consumption of carbohydrate reserves over the first 3 h in the dark. Since sinking rates had only increased slightly by 100 h, when carbohydrate reserves had been long exhausted, it seems unlikely that energy for sinking rate control was derived solely from carbohydrate reserves. The increase in sinking rate may have been delayed due to the almost

50% decrease in cell volume from 0 to 100 h, which could have decreased sinking rates by as much as 40%, according to Stokes' Law. However, since this decrease in size did not decrease sinking rates in heat-killed cells, it is unlikely that it decreased sinking rates in live cells.

While protein, carbon and nitrogen per cell decreased with time in the dark, they did not decrease per unit cell volume. For carbon content per unit cell volume not to change when carbohydrate content per unit cell volume decreased suggests that the carbohydrate reserves were being transformed into other compounds rather than being respired. The lack of a change in carbohydrate content from 100 to 244 h suggests that the carbohydrate remaining in these cells was probably structural and not available for consumption, similar to what was found for *Skeletonema costatum* (Handa 1969).

It is not clear why the sinking rate of live cells was higher than that of heat-killed cells after 244 h in the dark. The cells cannot be actively increasing their density, since they have no energy available to do so. It is unlikely that cells were clumping because there was no increase in average cell volume (as determined by the Coulter Counter). One possible explanation is that the heat-killing process makes the cells leak and they become less dense than they were when alive and senescent.

CONCLUSIONS

There appear to be differences in the sinking rate control mechanisms of *D. brightwellii* and *T. weissflogii*, similar to the results of Waite *et al.* (submitted). As a larger cell, *D. brightwellii* always has physiological control of its sinking rate. Physiological sinking rate control in *T. weissflogii* is intermittent, probably only occurring when increases in cell density make it necessary. Sinking rate was not positively correlated with carbohydrate content in either *T. weissflogii* or *D. brightwellii* grown under high or low white light. Sinking rate was positively correlated with carbohydrate content in *T. weissflogii* grown under

low or high blue light. Sinking rate control in *D. brightwellii* does respond to diel variations in light, as shown by the positive buoyancy observed in cells grown under high light at the end of the light period, but the positive buoyancy was lost only 8 h later at the end of the dark period.

CHAPTER II

INTRODUCTION

The previous chapter looked at how sinking rate and carbohydrate content were affected by diel cycles, differences in light intensity and prolonged exposure to darkness, all of which a phytoplankter might encounter in the ocean. Another characteristic of oceanic light fields is the selective absorption of longer wavelengths, leading to a preponderance of blue light at depth. Phytoplankton are known to respond to changes in light quality through changes in pigment composition and photosynthetic rate (Vesk and Jeffrey 1977), as well as changes in chemical composition and patterns and rates of carbon uptake and polymer synthesis (Rivkin 1989). Light quality is an important variable to consider when studying the biology of the ocean; Laws *et al.* (1990) found that primary production can be underestimated by as much as two times when samples taken from depth are incubated under white rather than blue light. Light quality could affect sinking rate through changes in cell composition, changes in the availability of energy for sinking rate control, and also through a direct effect on ion transport. Despite the changes in light quality that occur as light passes down through the water column and the known effects of light quality on phytoplankton physiology, no studies have been done on whether light quality affects sinking rate.

LIGHT IN THE OCEAN

Water selectively absorbs light of longer wavelengths, and by a depth of 10 m, red light has disappeared from the water column (Kirk 1992). The color of light that predominates at depth varies depending on the turbidity and the particulate matter in the water column, with the wavelength of penetrating light generally increasing with turbidity. The dominant light at depth changes from blue in the oligotrophic open ocean, to blue-green in highly productive oceanic waters, to green in coastal waters, to yellow-orange in turbid coastal waters (Dring 1987). The light field in the ocean is also affected by light scattering. While particles scatter light mostly in the forward direction, pure water scatters light equally

backward and forward. Particles scatter all wavelengths equally, but scattering by water increases exponentially with decreasing wavelength, further enriching the ocean in blue light (Kirk 1992).

The study of how these changes in light quality affect phytoplankton is complicated by the co-variation of light quantity and quality in the field. For most light quality responses, an organism responds to the number of photons absorbed (up to a saturating level), independent of other wavelengths (Dring 1987). While the light becomes more exclusively blue with increasing depth, there are fewer blue photons. Phytoplankton are not usually abundant right at the surface, and therefore they would rarely see the full spectrum of sunlight. They would never be exposed to red light exclusively, and when they would receive red wavelengths, it would always be at a relatively high irradiance. The light climate of a phytoplankter varies depending on its position in the water column and the particulate content of the water column (Dring 1987). This large variability in light climate may be responsible for the inconsistent response of phytoplankton to light quality, as well as the wide differences in spectral responses among taxa (Rivkin 1989).

PHYSIOLOGY OF LIGHT ABSORPTION

Phytoplankton cannot use light of every wavelength equally. While the extra energy of a blue photon contributes nothing to photosynthesis, diatoms are able to absorb more blue light than red light due to their pigment content. To understand the physiological effects of light quality, it is important to distinguish between three sets of terminology that are used to express the quantity of light: photosynthetically active radiation (PAR), photosynthetically usable radiation (PUR) and photosynthetically stored radiation (PSR) (Morel 1978).

Irradiance is commonly considered in terms of photosynthetically active radiation (PAR), which refers to the amount of radiant energy available at a certain depth, within the visible (400-700 nm) spectrum. Since algae cannot absorb photons at every wavelength, and not every photon of PAR is available for photosynthesis, it becomes necessary to consider

another quantity - photosynthetically usable radiation (PUR), which is the radiant energy available that can be absorbed by algae. The PUR is determined by both the pigment composition of the algae and the spectral composition of the light. Finally, since photosynthesis is not 100% efficient, the third quantity, photosynthetically stored radiation (PSR) refers to the amount of radiant energy that is converted into and stored as chemical energy (Morel 1978).

A comparison of PAR and PSR reveals how efficiently the algae are using the radiant energy entering the water, and is a function of both the water mass (coastal, oceanic, etc.) and the amount and kind of algae. The ratio of PSR to PUR in part determines the quantum yield (the number of CO_2 molecules formed per quantum absorbed) and is a function of the cells' physiology rather than a characteristic of the water column.

EFFECTS OF LIGHT QUALITY

Pigments

Changes in pigment content would have no direct effect on sinking rate, but could indirectly affect sinking rate by changing photosynthetic efficiency and thus affecting the availability of energy for sinking rate control. Cells grown under blue or blue-green light tend to have increased chlorophyll content compared to those grown under red light, perhaps to make them more efficient at depth (Wallen and Geen 1971, Jeffrey and Vesk 1977, Vesk and Jeffrey 1977, Jeffrey 1984, Rivkin 1989). Many species, especially diatoms, also show increases in the number of thylakoids per chloroplast when grown under blue light (Vesk and Jeffrey 1977, Jeffrey 1984, Senger and Bauer 1987). Complementary chromatic adaptation (a change in pigment ratios in response to a changing light environment) generally does not occur (Vesk and Jeffrey 1977), except in algal taxa that contain phycobilins (Cyanophyta, Cryptophyta, and Rhodophyta) (Klein 1992) although red light adapted *Chlorella kessleri* showed a 20% increase in total carotenoids per chlorophyll (Grotjohann *et al.* 1991).

Photosynthesis

Some evidence shows that maintenance of a low sinking rate is dependent on the availability of photosynthetic energy (Anderson and Sweeney 1978). Therefore, any effects of light quality on photosynthesis might translate into effects on sinking rate. Blue light has been reported to increase algal photosynthetic carbon fixation and oxygen evolution, and increase photosynthetic efficiency (Vesk and Jeffrey 1977). Increased oxygen evolution in blue light grown *Chlorella* was due to a higher number of reaction centres per total chlorophyll in photosystem II; red light cells had relatively more reaction centres and higher electron flow capacity in photosystem I, perhaps for increased ATP production by cyclic photophosphorylation (Grotjohann et al. 1991). In contrast to those results, Cunningham *et al.* (1992) found that when a red alga was transferred from green light to red light, PS I centres decreased and PS II centres increased. Presumably this occurred because green light is absorbed primarily by the phycobilisome antenna of PS II, while red light is absorbed mostly by the chlorophyll antenna of PS I.

Senger and Bauer (1987) have pointed out that while photosynthesis is generally reported as higher under blue light than red light, the light intensity curve of photosynthesis based on an equal amount of chlorophyll gives the inverse result, meaning that on a chlorophyll basis, photosynthesis is less efficient under blue light.

Enzymes and Cell Composition

Any change in cell composition that changes cell density has the potential to affect sinking rate. Since light quality usually affects carbohydrate and protein content by first affecting the enzymes involved in their production, the two areas will be discussed together.

Light can affect enzymes either directly, when the enzyme itself contains a light absorbing component (such as flavin in nitrate reductase), or indirectly by alterations of substrate and/or cofactor distribution in different cell components, and by de novo synthesis

via substrate induction (Ruyters 1987). Light control can be coarse, taking hours to days for effects to be seen, or fine, when enzyme activity changes within minutes.

Cells grown under blue light are often reported to have higher protein and lower carbohydrate content than cells grown under red light (Wallen and Geen 1971, Kowallik 1987). This is thought to occur due to an enhancement of carbohydrate degradation under blue light, which makes more intermediates available for amino acid and protein production. The blue light enhancement is due in part to a stimulatory effect on two bottleneck enzymes of glycolysis: phosphofructokinase and pyrvate kinase. While the capacity of these enzymes increases by no more than 100% and the change is slow (12-15 h), blue light also causes an increase of up to 10-fold in respiration (shown by increased oxygen uptake) that occurs within 5-10 minutes and decreases immediately when the cells are put in the dark (Kowallik 1987). The cause of this increased respiration under blue light is not known. Rivkin (1989) found increased rates of protein synthesis, carbon uptake and respiration under red light as well as blue light.

Ion Transport

It has been proven for at least one diatom that changes in sinking rate are due to changes in the vacuolar ion concentrations (Anderson and Sweeney 1978), a process which must involve ion transport. The amount of energy needed to change the concentration of a specific ion will vary depending on the membrane potential, which in turn depends on the concentrations and charges of ions on either side of the membrane. Therefore, any redistribution of ions inside or outside the vacuole could affect the ability of a cell to control its sinking rate.

There are sketchy reports of light quality affecting ion transport in algae. In early work, it was shown that light-stimulated ion uptake in *Cladophora* was more efficiently supported by equal quanta of red light than blue light (Iaglova 1958), and that net KCl uptake in *Nitella flexilis* was stimulated by red or blue light more so than green light (Nagai and

Tazawa 1962). Raven (1969) found that the action spectra for transport of Cl^-, K^+ and Na^+ were similar to the action spectrum for photosynthesis. More recently it was reported that far red light (>700 nm) increased potassium ion efflux in a colorless mutant of *Chlorella*, and that this efflux was probably accompanied by hyperpolarization of the plasmalemma (Kamiya 1994). Blue light has been shown to cause an extrusion of protons in the brown alga *Ectocarpus*, with the resultant acidification at the surface of the alga increasing the availability of CO_2 for uptake and enhancing photosynthesis (Schmid and Dring 1993). This effect did not occur in the diatom *Phaeodactylum tricornutum* or in any green or red alga tested (Forster and Dring 1994).

Growth

While sinking rates generally increase when cells are in stationary phase, cells with a low growth rate do not necessarily have a high sinking rate. It is unclear whether the aforementioned effects of light quality add up to a difference in growth rate. Part of the uncertainty stems from a lack of rigorous testing, as most of the previous studies were not designed to test for the separate effects of irradiance (the quantity of light) and spectral quality, and did not account for the difference in PAR and PUR (Rivkin 1989). At 50 μmol photons m^{-2} s^{-1}, growth rates of *Thalassiosira pseudonana* and *Dunaliella tertiolecta* were highest under blue light, intermediate under white light, and lowest under green light (Wallen and Geen 1971). At 80 μmol photons m^{-2} s^{-1}, growth rates of *Amphidinium* and *Biddulphia* were lower under red light than green, white or violet light (Humphrey 1983). Both of those studies were done under equal PAR rather than equal PUR. In two studies done under equal PUR, there was no chromatic effect on growth rate (Morel *et al.* 1987, Rivkin 1989).

GOALS OF THIS CHAPTER

Light quality has been shown to affect several parameters including pigment composition, photosynthetic rate, enzyme activites, cell composition and ion transport. Any

of these parameters could affect sinking rate either directly by affecting ion transport or changing cell composition, or indirectly by affecting the availability of energy for sinking rate control. Despite the potential for light quality to affect sinking rate, no studies have been done comparing the sinking rates of diatoms grown under different spectral regimes. To ensure the ecological validity of sinking rate work done under white light, it is important to establish that sinking rates under blue light are not different. As well, any differences in sinking rate due to changes in light quality could provide clues about the mechanisms of sinking rate control. The purpose of this work was to determine whether light quality (red, blue or white light) affects sinking rate by changing the carbohydrate content of the cells.

MATERIALS AND METHODS

GENERAL METHODS

The marine diatoms *Thalassiosira weissflogii* and *Ditylum brightwellii* were obtained from the Northeast Pacific Culture Collection and grown under continuous light in semi-continuous batch cultures on enriched artificial seawater (ESAW) as described in Chapter 1.

Illumination was provided by Vitalite fluorescent tubes. Red light was obtained with red-colored filters wrapped around the flasks (Roscolux #19), blue light with blue-colored filters (Roscolux #69) or blue Plexiglas (see Appendix 3 for transmittance spectra of filters). Irradiance was adjusted using neutral density screening. For the first experiment on *T. weissflogii*, all cultures received 35 μmol photons m^{-2} s^{-1}, and for the second experiment on *T. weissflogii*, all cultures received 85 μmol photons m^{-2} s^{-1}. For experiments on *D. brightwellii*, light levels were adjusted so all cultures would have similar growth rates: red light grown cells received 116 μmol photons m^{-2} s^{-1}, blue light grown cells received 65 μmol photons m^{-2} s^{-1}, and white light grown cells received 70 μmol photons m^{-2} s^{-1}.

Cells were acclimated for a minimum of 10 generations. Growth rates were measured both by *in vivo* fluoresence and cell counts as described in Chapter 1. Measurements of cell carbon, nitrogen, chlorophyll *a*, carbohydrate, protein and sinking rate were made on cells in mid-log phase as described in Chapter 1.

EXPERIMENTAL PROCEDURE

Sinking rate measurements on all treatments were made simultaneously, either in the dark or under the light regime that the cells were grown under. In the first set of sinking rate experiments on *T. weissflogii*, no cell composition measurements were made. For the second set of experiments with *T. weissflogii* and for *D. brightwellii*, carbohydrate, carbon, nitrogen, chlorophyll *a* and protein samples were taken from the remaining subsample of the culture during the sinking rate trial.

RESULTS

I. Thalassiosira weissflogii

GROWTH RATE

For the first set of experiments, all cultures received 35 μmol photons m^{-2} s^{-1} of light, which produced growth rates of 0.32 ± 0.07 d^{-1} for blue light-grown cells, 0.19 ± 0.01 d^{-1} for red light-grown cells and 0.45 ± 0.04 d^{-1} for white light-grown cells (Fig. 2.1A). These cultures were all light-limited, growing at about 30, 20 and 50% of their maximal growth rate, respectively.

The second set of experiments was done under higher irradiance to compare the effect of light quality on sinking rate when light was saturating. All cultures received 85 μmol photons m^{-2} s^{-1} of light, producing growth rates of 0.79 ± 0.03 d^{-1} for blue light-grown cells, 0.93 ± 0.02 d^{-1} for red light-grown cells and 0.84 ± 0.02 d^{-1} for white light-grown

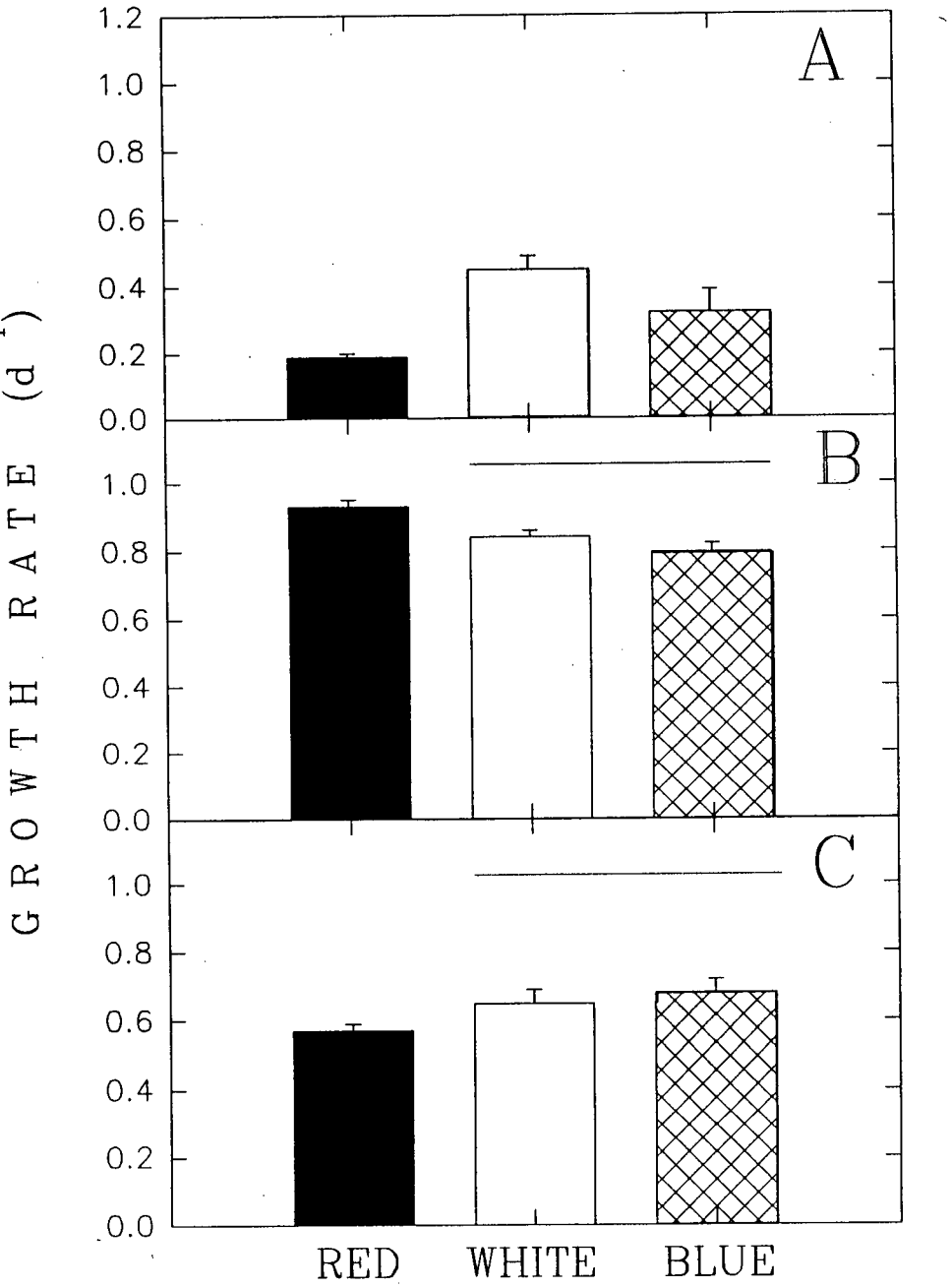

Figure 2.1 Growth rates under red, white or blue light for (A) *Thalassiosira weissflogii* at 35 μmol photons m^{-2} s^{-1} (B) *Thalassiosira weissflogii* at 85 μmol photons m^{-2} s^{-1}, and (C) *Ditylum brightwellii* at 115 μmol photons m^{-2} s^{-1} of red light, 70 μmol photons m^{-2} s^{-1} of white light and 65 μmol photons m^{-2} s^{-1} of blue light. Each bar represents the mean of 3 replicate cultures ± 1 SE. Treatments not significantly different from one another at $P = 0.05$ are joined by lines.

cells (Fig. 2.1B). These cultures were light-saturated (for μ vs irradiance curve, see Appendix 4).

SINKING RATE AND CARBOHYDRATE CONTENT

At both 35 and 85 μmol photons m^{-2} s^{-1}, sinking rates of cells grown under red light were significantly higher by about 2-fold (since sinking rates at 35 and 85 μmol photons m^{-2} s^{-1} were not significantly different, data were pooled in Fig. 2.2A). There was no difference in sinking rate for cells grown under white or blue light at 35 μmol photons m^{-2} s^{-1}. Sinking rates of cells grown under blue light at 85 μmol photons m^{-2} s^{-1} were not measured, but it is assumed they would be similar to those found at 35 μmol photons m^{-2} s^{-1} since that was the case for cells grown under red or white light.

Sinking rates of cells grown under white and blue light were similar to those found in the previous chapter; sinking rates of cells grown under red light were about double those of heat-killed cells from the previous chapter. Illumination with blue light during a sinking rate trial on cells grown under red light produced the same results as trials run under red light (data not shown).

The relationship between sinking rate and cell volume was similar for cells grown under red, white or blue light, except that sinking rates of cells grown under red light were consistently higher (Fig. 2.3A). Under red light, the largest size class (750 μm^3) sank significantly faster than the next largest size class (400 μm^3). Under blue or white light there were no differences in sinking rate among size classes.

There was no difference in carbohydrate content between cells grown under red, white or blue light at 85 μmol photons m^{-2} s^{-1} (Fig. 2.2B). Carbohydrate content of cells grown under 35 μmol photons m^{-2} s^{-1} was not measured. There was no relationship between sinking rate and carbohydrate content (Fig. 2.4A).

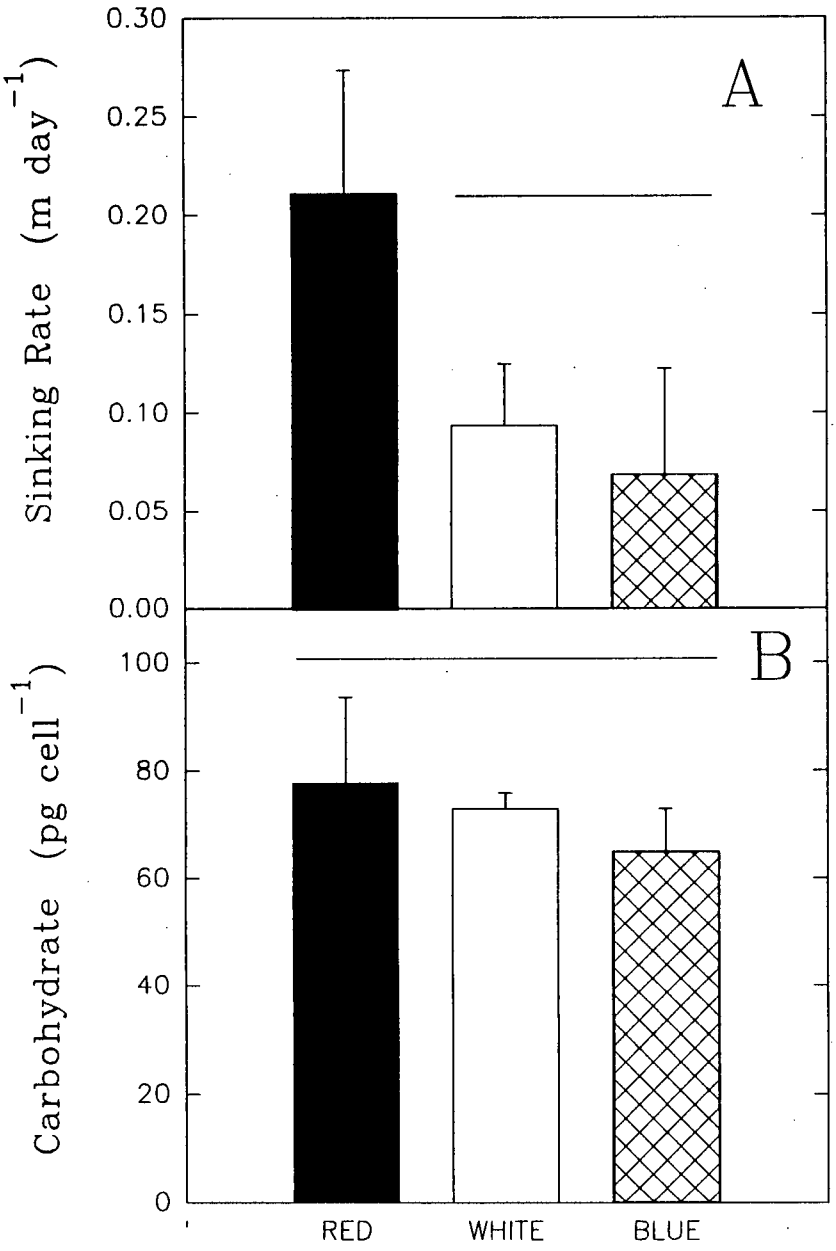

Figure 2.2 (A) Sinking rate (3 h SETCOL) and (B) carbohydrate content of *Thalassiosira weissflogii* grown under continuous red, white or blue light. Each bar represents the mean of three replicate cultures $\pm$ 1 SE. Treatments not significantly different from one another at P = 0.05 are joined by lines.

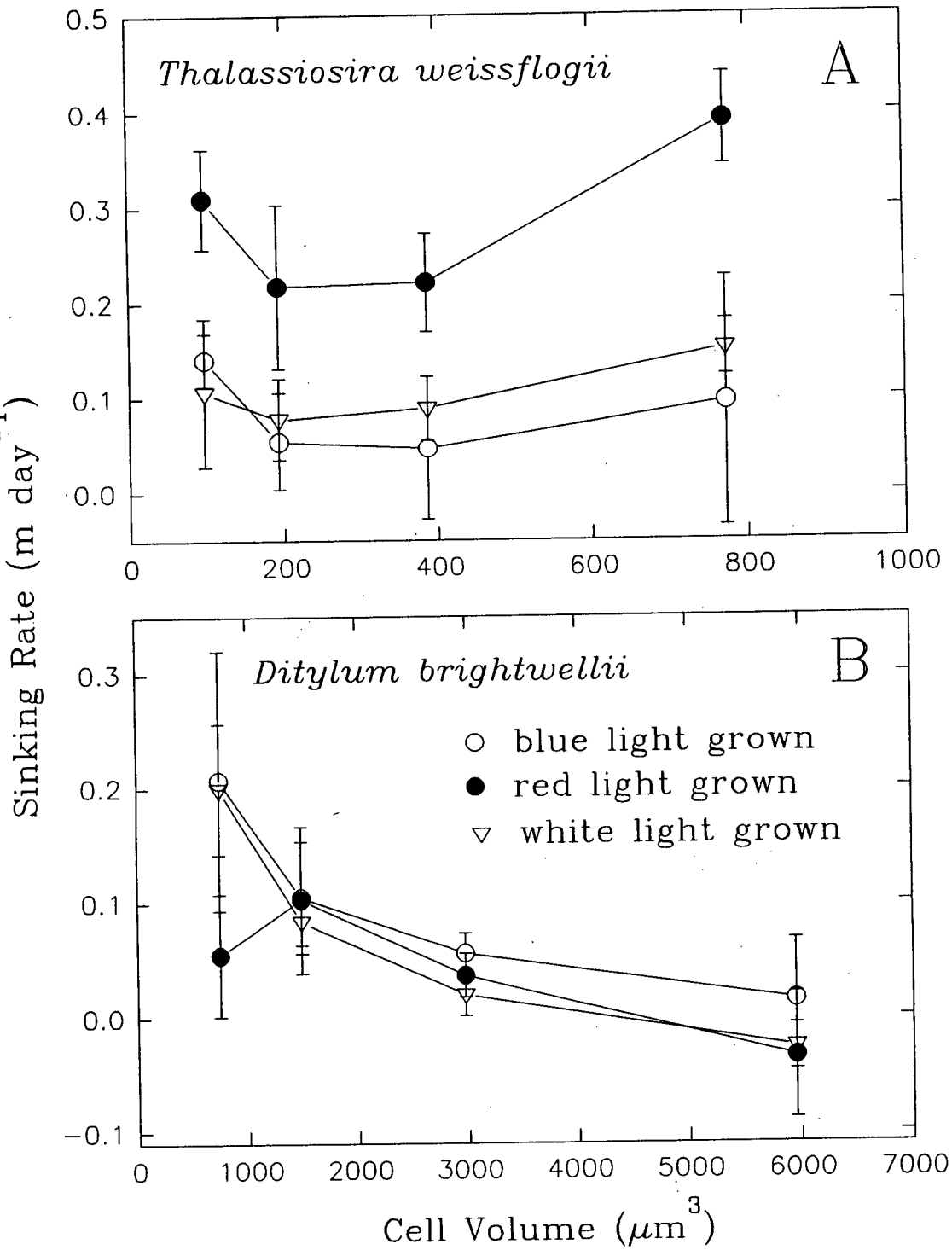

Figure 2.3 The relationship between sinking rate and cell volume for (A) *Thalassiosira weissflogii* (at 85 µmol photons m^{-2} s^{-1}) and (B) *Ditylum brightwellii* grown under red (115 µmol photons m^{-2} s^{-1}), white (70 µmol photons m^{-2} s^{-1}) or blue (65 µmol photons m^{-2} s^{-1}) light. Each point represents the mean of three replicate cultures ± 1 SE.

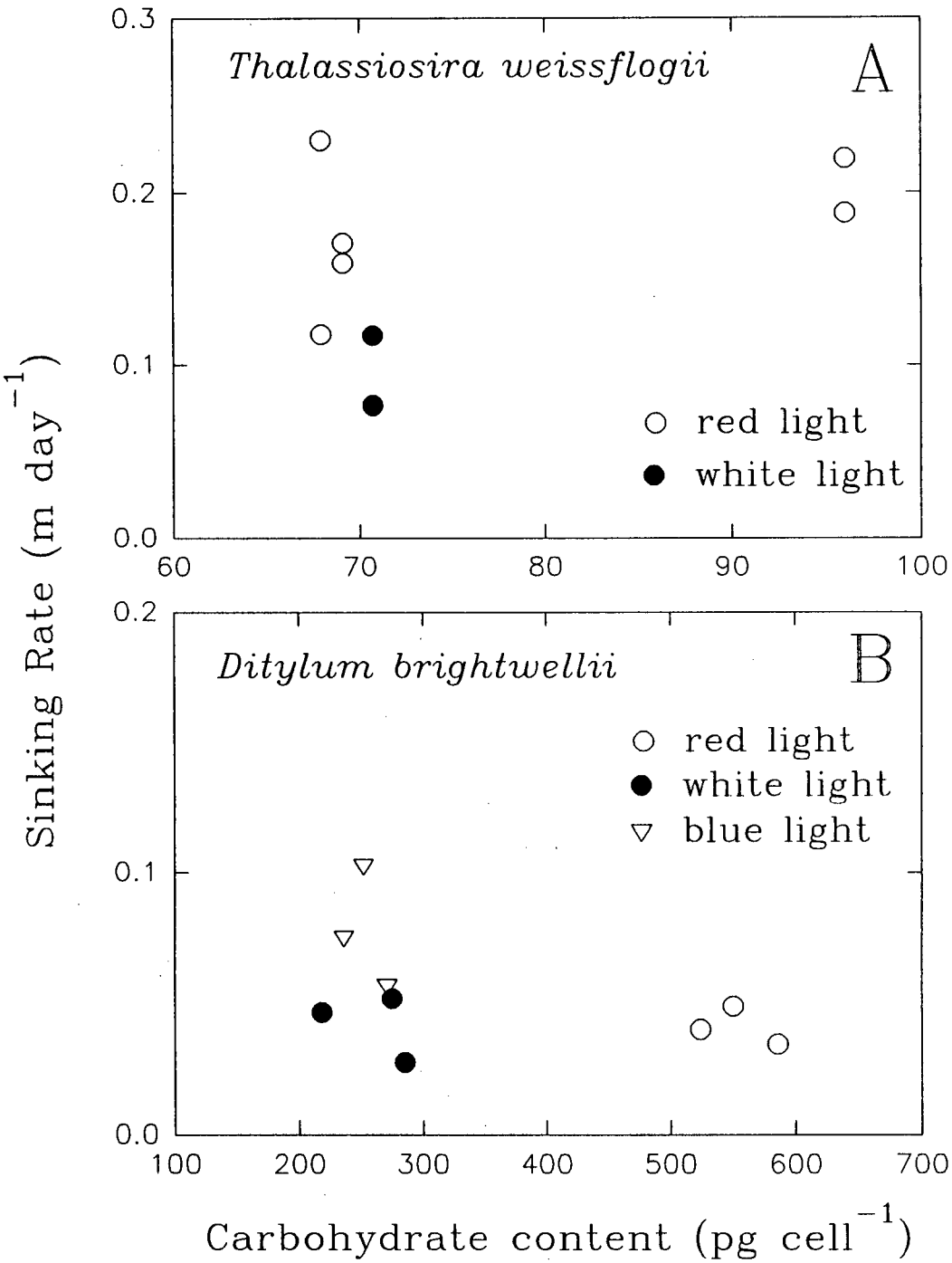

Figure 2.4 The relationship between sinking rate and carbohydrate content for (A) *Thalassiosira weissflogii* (at 85 µmol photons m^{-2} s^{-1}) and (B) *Ditylum brightwellii* grown under red (115 µmol photons m^{-2} s^{-1}), white (70 µmol photons m^{-2} s^{-1}) or blue (65 µmol photons m^{-2} s^{-1}) light.

CELL COMPOSITION

All the cell composition data are for cells growing under 85 μmol photons m^{-2} s^{-1}. Cell volume, carbon content and nitrogen content were significantly higher in cells grown under blue light than in cells grown under red light (Table 2.1). The differences in carbon and nitrogen content were smaller but still significant on a cell volume basis. There were no significant differences in chlorophyll *a* content, protein content or molar C:N ratio. Because cell counts were not available for these samples, cell number was estimated from a previously established relationship between cell number and chlorophyll *a* content under identical conditions.

II. *Ditylum brightwellii*

GROWTH RATE

Growth irradiances were adjusted so all cultures would have similar growth rates: 0.57 ± 0.02 d^{-1} for red light-grown cells (received 115 μmol photons m^{-2} s^{-1}), 0.68 ± 0.04 d^{-1} for blue light-grown cells (received 65 μmol photons m^{-2} s^{-1}), and 0.65 ± 0.04 d^{-1} for white light-grown cells (received 70 μmol photons m^{-2} s^{-1}) (Fig. 2.1C). These cells were light-limited, growing at about 65% of the maximal growth rate.

SINKING RATE AND CARBOHYDRATE CONTENT

There were no significant differences in sinking rate between *D. brightwellii* grown under red, white or blue light (Fig. 2.5A). The carbohydrate content of cells grown under red light was significantly higher (~ 2x) than that of cells grown under white or blue light (Fig. 2.5B). There were no differences in the relationship between sinking rate and cell volume for cells grown under red, white or blue light. In all cases, sinking rate decreased as cell volume increased (Fig. 2.3B). No relationship was found between sinking rate and carbohydrate content (Fig. 2.4B).

Table 2.1 Cell volume, chlorophyll *a*, protein, carbon and nitrogen content and molar C:N ratio of *Thalassiosira weissflogii* grown under red, white or blue light at 85 μmol photons m^{-2} s^{-1}. Values are mean of 3 replicate cultures with ± 1 SE (except for protein, carbon and nitrogen content under white light, for which only 1 sample was analyzed).

Light Quality	Cell Volume (μm^3)	Chlorophyll *a* (pg $cell^{-1}$)	Protein (pg $cell^{-1}$)	Carbon (pg $cell^{-1}$)	Nitrogen (pg $cell^{-1}$)	C:N Ratio (molar)
Red	626 ± 37	2.80 ± 0.27	41.3 ± 6.9	191 ± 16	32.6 ± 0.7	6.84 ± .055
White	675 ± 36	2.96 ± 0.28	68.4	217	38.6	6.58
Blue	689 ± 21	3.16 ± 0.34	51.9 ± 13	234 ± 12	41.6 ± 3.2	6.59 ± 0.19

Table 2.2 Cell volume, chlorophyll *a*, protein, carbon and nitrogen content of *Ditylum brightwellii* grown under red, white or blue light (irradiance was varied to produce nearly equal growth rate). Values are mean of 3 replicate cultures with ± 1 SE.

Light Quality	Cell Volume (μm^3)	Chlorophyll *a* (pg $cell^{-1}$)	Protein (pg $cell^{-1}$)	Carbon (pg $cell^{-1}$)	Nitrogen (pg $cell^{-1}$)	C:N Ratio (molar)
Red	4180 ± 130	11.5 ± 0.9	130 ± 3	450 ± 35	77.6 ± 6.7	6.78 ± 0.16
White	3470 ± 330	9.39 ± 0.79	135 ± 17	286 ± 65	53.8 ± 10.2	6.18 ± 0.28
Blue	3160 ± 50	8.16 ± 1.10	140 ± 13	299 ± 54	54.9 ± 8.3	6.34 ± 0.23

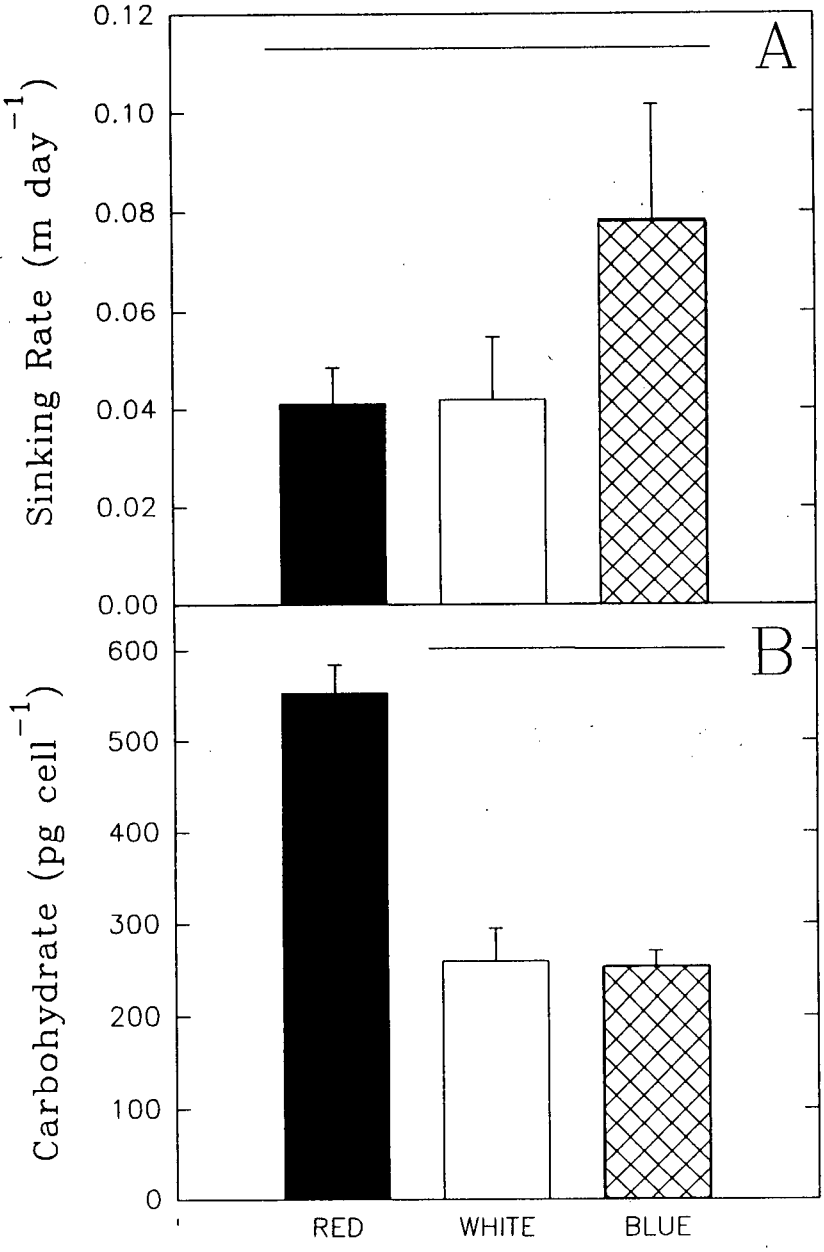

Figure 2.5 (A) Sinking rate (3 h SETCOL) and (B) carbohydrate content of *Ditylum brightwellii* grown under continuous red (115 µmol photons m^{-2} s^{-1}), white (70 µmol photons m^{-2} s^{-1}) or blue (65 µmol photons m^{-2} s^{-1}) light, with light levels adjusted to obtain growth rates of approximately 0.65 d^{-1}. Each bar represents the mean of three replicate cultures ± 1 SE. Treatments not significantly different from one another at P = 0.05 are joined by lines.

CELL COMPOSITION

Cell volume and chlorophyll *a* content of cells grown under red light were significantly higher than those of cells grown under white or blue light (Table 2.2). There were no significant differences in protein content per cell, but cells grown under red light contained less protein per cubic micron than cells grown under white or blue light. Cells grown under white or blue light had the same carbon and nitrogen content; cells grown under red light contained significantly more carbon (~ 50% higher) and about 40% more nitrogen than cells grown under white or blue light (Table 2.2), although there were no differences in carbon and nitrogen content per cubic micron. The C:N ratio was significantly higher in cells grown under red light than under blue or white light.

DISCUSSION

EFFECTS OF LIGHT QUALITY ON SINKING RATE

COMPOSITION

Carbohydrate and Protein

The lack of any differences in carbohydrate or protein content of *T. weissflogii* grown under red, white or blue light shows that the increased sinking rate in cells grown under red light was not due to an increase in cell density caused by higher protein or carbohydrate content.

The increased carbohydrate content of *D. brightwellii* grown under red light compared to cells grown under white or blue light did not result in a higher sinking rate. The compositional data for *D. brightwellii* is complicated by the larger cell volume of cells grown under red light. It cannot be determined whether this was a result of the red light or the higher irradiance received by cells growing under red light. On a cubic micron basis, *D. brightwellii* grown under red light contained more carbohydrate and less protein than cells

grown under white or blue light. This compositional difference is also shown by the higher C:N ratio for cells grown under red light. The increase in carbohydrate content under red light was greater than the decrease in protein content, resulting in a net increase in cell density. Because sinking rate did not increase, this suggests that the cells might compensate for the density increase by increased energy expenditure.

The increased carbohydrate content, increased C:N ratio and possibly lower protein content of *D. brightwellii* grown under red light is consistent with the literature. When *Chlorella* was grown under red or blue light where the irradiance was adjusted to give equal dry weight production, cells grown under blue light contained 15% carbohydrate and 60% protein, while cells grown under red light contained 39% carbohydrate and 29% protein on a dry weight basis. Similar results were found with *Euglena*, *Chlorogonium*, *Chlamydomonas*, *Cyclotella*, *Acetabularia*, *Scenedesmus* and *Spirulina* (Kowallik, 1987).

The lack of a spectral effect on the carbohydrate or protein content of *T. weissflogii* was unexpected, but not wholly unsupported. In *Chaetoceros protuberans*, protein production was more efficient under blue light than white light, but there was no difference in carbohydrate production (Gostan *et al.* 1986). Aidar *et al.* (1994) found that light quality did not affect protein content in *Cyclotella caspia* or *Tetraselmis gracilis*.

Chlorophyll

The lack of a difference in chlorophyll *a* content for cells grown under red, white or blue light for either species does not agree with the literature, which shows an increase in chlorophyll in diatoms grown under blue or blue-green light (Jeffrey and Vesk 1977; Vesk and Jeffrey 1977). The increase in chlorophyll *a* found by Vesk and Jeffrey (1977) occurred in diatoms growing at a relatively low irradiance (25 μmol photons m^{-2} s^{-1}) compared to those used for this study (85 μmol photons m^{-2} s^{-1} for *T. weissflogii*; 116, 70 and 65 μmol photons m^{-2} s^{-1} for red, white and blue light *D. brightwellii* respectively). It is possible that the blue light increase in chlorophyll *a* content only occurs at low irradiances and is more

closely related to low light (light quantity) than blue light (light quality) as found by Dring (1981). When the dinoflagellate *Amphidinium* and the diatom *Biddulphia* were grown under 80 μmol photons m^{-2} s^{-1} of white, green, red or violet light, chlorophyll *a* content was highest in red light grown cells (Humphrey 1983). However, Rivkin (1989) found higher chlorophyll content in a number of phytoplankton species grown under white or blue light compared to red light across a range of irradiances (12 - 200 μmol photons m^{-2} s^{-1}).

It is unlikely that the small differences in chlorophyll *a* content found in this study had any affect on sinking rate.

CELL VOLUME

Sinking rate increases exponentially with an increase in cell radius (as shown in Stoke's Law, Appendix 2). The increase in sinking rate expected with an increase in cell size is usually only apparent when cells are unable to physiologically control their sinking rate. Experiments from the previous chapter show that physiologically active *T. weissflogii* and *D. brightwellii* have similar sinking rates despite the almost 10-fold larger cell volume of *D. brightwellii*.

The greater cell volume of *T. weissflogii* grown under blue light and of *D. brightwellii* grown under red light did not result in a higher sinking rate. Using Stokes' Law and assuming the cells are spheres, the increased volume of *T. weissflogii* grown under blue light would account for a 7% higher sinking rate compared to red light. The larger cell volume of *D. brightwellii* grown under red light would increase sinking rate by 12 or 15% compared to cells grown under white or blue light, respectively. The assumption that the cells are spherical will not affect the relative differences in sinking rate, providing that light quality does not affect cell shape.

The relationship between size and sinking rate is more apparent within a treatment because there is less variation in other factors that affect sinking rate such as cell shape and density. In *D. brightwellii*, there was no spectral effect on the relationship between sinking

rate and cell volume, which fits with the lack of a spectral effect on sinking rate. The downward slope of this relationship is consistent with the theory of Villareal (1988) that larger cells are able to maintain lower sinking rates due to their lower surface area to volume ratio.

In *T. weissflogii*, sinking rates of cells grown under red light were higher across all size classes compared to white or blue light. Because any inability to control sinking rate would show up first in the largest cells, the increased sinking rate of the largest size class of red light grown cells compared to the one below it suggests that these red light grown cells are either denser than their white and blue light counterparts, or their sinking rate control mechanisms are less functional.

ENERGY AVAILABILITY

Growth

Since sinking rate control in diatoms is physiologically based, it could be affected by growth rate. Slow-growing (light-limited) cells would presumably have fewer reserves and less photosynthetic energy available for density control through selective ion accumulation and would also have a slower response time if synthesis of transporters was necessary.

The lower growth rate of *T. weissflogii* grown under red light at 35 μmol photons m^{-2} s^{-1} probably occurred due to the unequal PUR (photosynthetically usable radiation) of red and blue light, as diatoms are better able to absorb blue light than red light due to their accessory pigment composition. At 85 μmol photons m^{-2} s^{-1}, light is saturating and the lower efficiency of red light absorption would be compensated for by the increased photon flux density. In *D. brightwellii*, light levels were adjusted so growth rates would be similar (i.e. equal PUR). While cells grown under blue and white light received almost the same irradiance, about 50% more photons were necessary to obtain a similar growth rate under red light.

The results of this study are consistent with the literature. When cells are grown under equal PAR, growth rates are usually higher under blue light and lower under red light. When

T. pseudonana and *Dunaliella tertiolecta* were grown at 50 μmol photons m^{-2} s^{-1}, growth rates were highest in blue light, moderate in white light and lowest in green light (Wallen and Geen, 1971). Growth rates of *Amphidinium* and *Biddulphia* were slower under red light than green, white or violet light at 80 μmol photons m^{-2} s^{-1}. The diatom *Cyclotella caspia* grew faster under blue-green light than red light at 25 μmol photons m^{-2} s^{-1} (Aidar *et al.* 1994).

When PUR is made equal, there is no spectral effect on growth rate. Gostan *et al.* (1986) grew *Chaetoceros protuberans* under 3 different irradiances of blue and white light at equal PUR and found no differences in growth rate. A study designed to distinguish between the effects of light quality and light quantity found that under equal PUR, light quality did not affect growth rate in *Thalassiosira rotula* or *Dunaliella tertiolecta* (Rivkin 1989).

If sinking rate control requires energy, cells with a low growth rate (light-limited) would be expected to have higher sinking rates. The point at which slowed growth would affect a cell's ability to maintain a low sinking rate would depend on how much energy was needed for sinking rate control. Sinking rates often increase during senescence and when growth is limited by nutrients, but the results of the previous chapter show that low sinking rates can be maintained when growth is limited by light. It is unlikely that the relatively small differences in growth rate in *T. weissflogii* caused by the unequal PUR of red, white and blue light could account for the differences in sinking rate, especially since the sinking rate increase under red light was observed at both 35 and 85 μmol photons m^{-2} s^{-1}, when growth rates were different.

Photosynthesis

If photosynthetic energy is used in sinking rate maintenance, any change in photosynthetic rate could affect sinking rate. Photosynthetic rates were not measured in this study, but there is evidence in the literature of light quality affecting photosynthetic rate. Phytoplankton grown under blue light have higher photosynthetic rates compared to those grown under red light (Wallen and Geen 1971, Vesk and Jeffrey 1977). There is evidence

that the photosystems respond differently to light quality, with blue light enhancing photosystem II, and red light enhancing photosystem I (Grotjohann *et al.* 1991).

Photosynthetic energy may not necessarily be involved in sinking rate control in *T. weissflogii*, as shown in the previous chapter by higher sinking rates in *T. weissflogii* grown under high light compared to low light and no increase in sinking rate in *T. weissflogii* during the dark period. Sinking rates of *D. brightwellii* seem to be more responsive to light. In the dark period, cells were no longer positively buoyant as they had been during the light period. Anderson and Sweeney (1978) showed that the sinking rate of *D. brightwellii* increased after treatment with dichloromethyl urea, a photosystem II inhibitor. There seems to be a range in the response of diatom sinking rates to energy deprivation (Waite *et al.* submitted), with the sinking rates of larger cells, such as *D. brightwellii*, being more sensitive to energy deprivation than are the sinking rates of smaller cells, such as *T. weissflogii*. For light quality to affect sinking rate in the species whose sinking rate is less responsive to changes in irradiance suggests that red light is not increasing sinking rates in *T. weissflogii* by affecting photosynthesis.

Ion Transport

Sinking rate control in some diatoms is known to occur through selective ion accumulation and there are many reports of light quality affecting ion transport in algae. A red light effect on ion transport in *T. weissflogii* could explain the increase in sinking rate. While no measurements were made in this study of internal ion concentrations, ion fluxes or membrane potentials, there are some inferences that can be made from the data.

Anderson and Sweeney (1978) found that it took several hours for changes in internal ion concentrations to affect sinking rate in *D. brightwellii*. When *T. weissflogii* grown under red light was exposed to blue light for the duration of a sinking rate trial (3 h), sinking rates did not decrease. This may have been too brief a time for significant changes in sinking rate to occur, particularly if there is a blue light requirement for the synthesis of specific ion

transporters. On the other hand, if changes in ion transport can produce rapid changes in sinking rate, the lack of a change in 3 h would be evidence against red light affecting ion transport.

For red light to affect sinking rate in *T. weissflogii* through changes in ion transport, sinking rate must be controlled by selective ion accumulation. If this is true, *T. weissflogii* should lose its ability to control its sinking rate when it is physiologically incapacitated. In the previous chapter, *T. weissflogii* grown under high light did not sink faster after being heat-killed, suggesting that sinking rate is not always physiologically controlled in these cells.

While ion transporters in *T. weissflogii* and *D. brightwellii* would not necessarily respond to red light in the same way, the lack of a red light effect on sinking rate in *D. brightwellii*, a species known to control sinking rate through selective ion accumulation, argues against red light affecting ion transport.

HOW DOES RED LIGHT AFFECT SINKING RATE?

Of the parameters measured in this study, the only differences between *T. weissflogii* grown under red light compared to white or blue light were in sinking rate, cell volume, and growth rate. The differences in cell volume were far too small to cause the observed increase in sinking rate. The differences in growth rate can probably be ruled out because differences in light-limited growth rate do not seem to affect sinking rate, and the increased sinking rate under red light was observed at two different growth rates.

The sinking rates of *T. weissflogii* grown under red light were twice those of heat-killed white light cells from the previous chapter. Heat-killed cells are unable to physiologically adjust their sinking rate, and so presumably sink at maximal rates. For *T. weissflogii* grown under red light to sink faster than dead cells shows that red light must be causing an increase in cell density, since a loss of physiological sinking rate control would not make sinking rates higher than those of heat-killed cells.

An examination of the red light effect on sinking rate would not be complete without considering why it did not occur in *D. brightwellii*. The different sinking rate responses of the two species to red light could have occurred because they control sinking rate in different ways. The selective ion accumulation hypothesis has never been tested for *T. weissflogii*, and the lack of an increase in sinking rate of heat-killed high light cells from the previous chapter suggests that *T. weissflogii* may not always need to use physiological sinking rate control. Since *T. weissflogii* is a smaller cell than *D. brightwellii*, it has a smaller surface area to volume ratio, and would be less able to change its sinking rate, therefore it would have less to gain from selective ion accumulation.

Red light may affect *D. brightwellii* and *T. weissflogii* in the same way, but *D. brightwellii* is able to compensate for the red light effect and still maintain a low sinking rate. An increase in density would be less of a disadvantage to a large cell like *D. brightwellii* than a small cell like *T. weissflogii*, since the larger vacuole of *D. brightwellii* provides the cell with more potential lift. Since the ability of *D. brightwellii* to compensate for increased density relies on intact ion transport mechanisms, red light could not have affected ion transport in this species.

The observed results could be explained by increased silicification under red light. The resultant increase in cell density could be too much for a small cell like *T. weissflogii* to compensate for, whereas *D. brightwellii* with its larger vacuole and relatively less frustule could maintain low sinking rates. Increased silicification under red light would also explain why *T. weissflogii* grown under red light has a higher sinking rate than heat-killed *T. weissflogii* that was grown under white light.

ECOLOGICAL APPLICATIONS

In the introduction to this chapter it was stated that phytoplankton cells would never be exposed to pure red light, the results of this experiment showing increased sinking rate in *T. weissflogii* grown under red light may seem no more than a physiological curiosity. However, if instead of a red light effect, it is thought of as a lack of a blue light effect, the results become more ecologically meaningful, as they may suggest that blue light is necessary for the maintenance of low sinking rates in *T. weissflogii*.

While light quality changes with depth in the water column, and sinking rate responds to changes in light quality, it is unlikely that diatoms control their position in the water column by responding to changes in light quality. Since cells would only see the red component of white light near the surface where irrandiance is high, any acclimation of sinking rate under red light should also be observed under high white light.

CONCLUSIONS

Carbohydrate is not affecting sinking rates in either *T. weissflogii* or *D. brightwellii*. High sinking rates in *T. weissflogii* grown under red light were not accompanied by high carbohydrate content, and high carbohydrate content in *D. brightwellii* grown under red light was not accompanied by high sinking rates.

It is not clear why sinking rates are higher in *T. weissflogii* grown under red light, although there is evidence against it being through an affecting on growth rate, photosynthesis or ion transport. If red light is increasing cell ballast, the sinking rate increase seen in *T. weissflogii* would not necessarily occur in *D. brightwellii*, since the larger vacuole of *D. brightwellii* gives it greater scope to decrease its overall density, providing ion transport mechanisms are functioning.

The similarity in sinking rate of cells grown under white light compared to those grown under blue light supports the ecological validity of sinking rate studies done under white light.

SUMMARY

1. Sinking rate was not positively correlated with carbohydrate content in *T. weissflogii* or *D. brightwellii* grown under low or high white light, but there was a positive corelation between sinking rate and carbohydrate content in *T. weissflogii* grown under low or high blue light.

2. Sinking rate in *D. brightwellii* was under physiological control in all experiments, but in *T. weissflogii* physiological sinking rate control was intermittent, occurring at the end of the light period, but not at the end of the dark period.

3. *D. brightwellii* showed diel changes in buoyancy, with higher sinking rates at the end of the dark period than at the end of the light period, when cells were positively buoyant.

4. *T. weissflogii* grown under red light had higher sinking rates than cells grown under white or blue light, but there were no differences in carbohydrate content.

5. *D. brightwellii* grown under red light had twice as much carbohydrate as cells grown under white or blue light, but there were no differences in sinking rate.

FUTURE RESEARCH

For the most part, there was no positive relationship between sinking rate and carbohydrate content, showing that carbohydrate does not have a ballasting effect in either *T. weissflogii* or *D. brightwellii*. However, the positive relationship between sinking rate and carbohydrate content in *T. weissflogii* grown under blue light is unexplained. The question of whether carbohydrate is acting as ballast in *T. weissflogii* grown under blue light could be addressed by measuring the sinking rate of physiologically incapacitated cells (using an alternative to heat-killing). A positive relationship between sinking rate and carbohydrate when cells did not have physiological control would further support the possibility that carbohydrate is acting as ballast.

The increased sinking rate seen in *T. weissflogii* grown under red light is unexplained. The possibility of red light increasing the density of the cells by increasing the size of the frustule could be tested by growing the cultures as before and measuring the decrease of silicate in the medium.

Measuring the sinking rates of heat-killed *D. brightwellii* grown under red, white or blue light would show whether *D. brightwellii* is physiologically compensating for an increased density under red light, or whether red light does not increase the density of *D. brightwellii* as it does in *T. weissflogii*.

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APPENDIX 1. SINKING RATE AND CARBOHYDRATE CONTENT

This appendix contains the results of the first experiments done on *T. weissflogii* and *D. brightwellii* grown on diel cycles under high and low light which did not have a heat-killing component. The experiments were repeated with more replicate cultures and a heat-killing component and the results are shown in the results section of Chapter 1 (*T. weissflogii* in Figure 1.2, *D. brightwellii* in Figure 1.10). For *T. weissflogii*, there were some slight but significant differences between sinking rates of live cells in the first experiment (Figure A1.1) and the experiment with the heat-killing component (Figure 1.2). These differences are reported in the text of the results section for Chapter 1. For *D. brightwellii*, there were no siginificant differences between sinking rates of live cells in the two experiments.

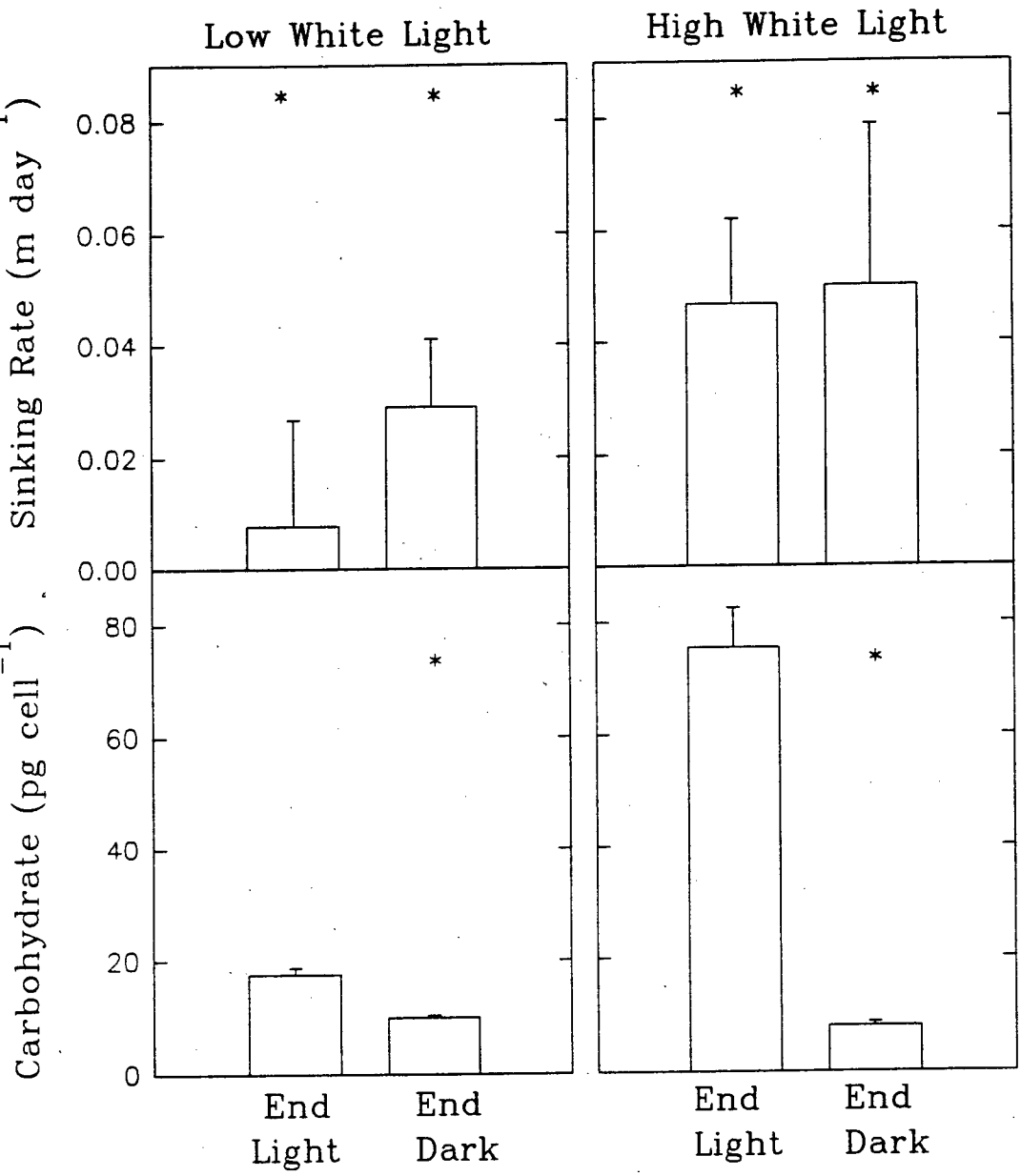


Figure A1.1 Sinking rate (3 h SETCOL) and carbohydrate content of *Thalassiosira weissflogii* grown on a 14:10 light:dark cycle under low white light (18 µmol photons m^{-2} s^{-1}) or high white light (200 µmol photons m^{-2} s^{-1}) and measured at the end of the light period (end light) or the end of the dark period (end dark). Each bar represents the mean of two replicate cultures ± 1 SE. Matching symbols are above bars that are not significantly different, comparison applies to adjacent graphs (i.e. high or low light).

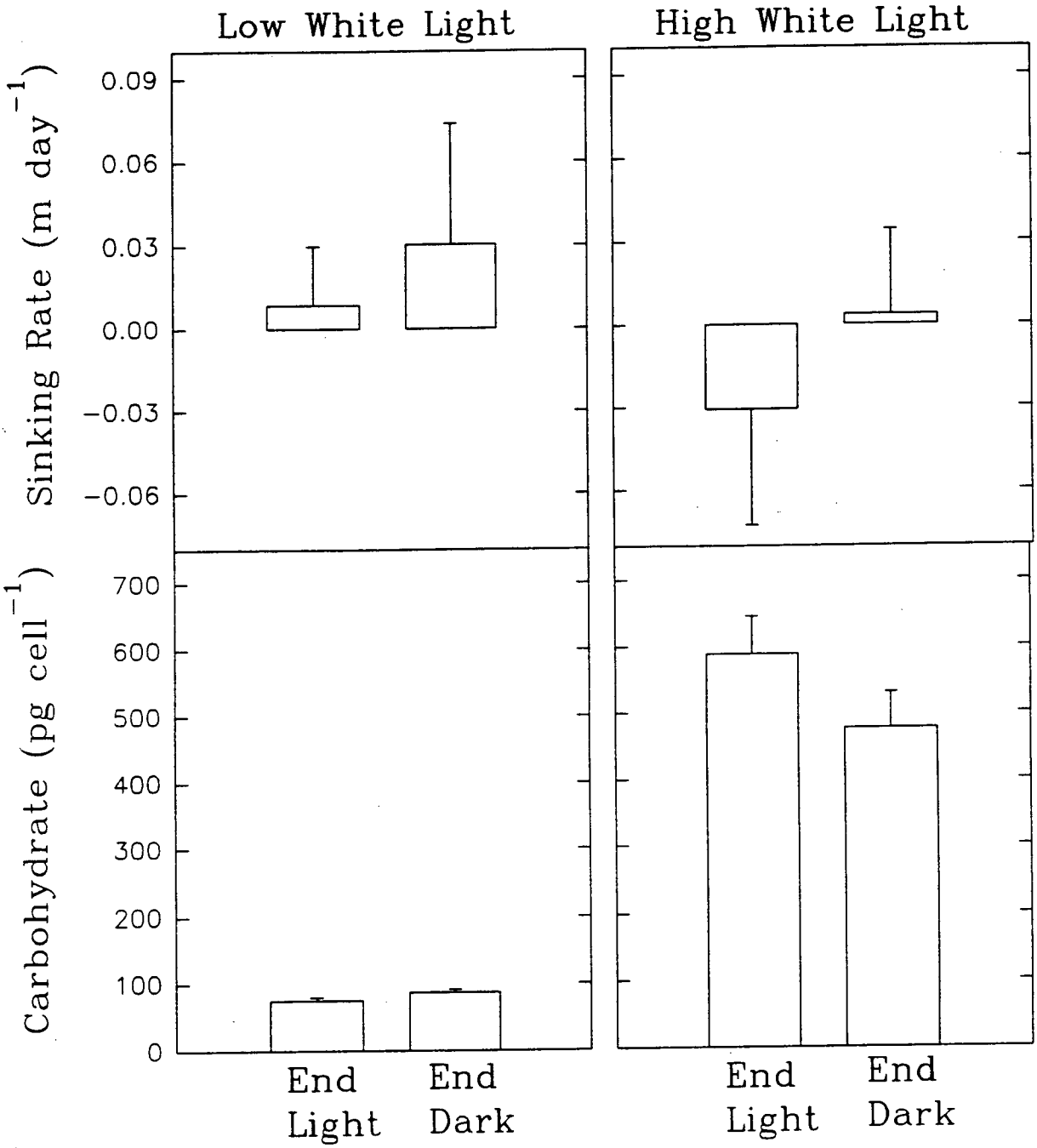


Figure A1.2 Sinking rate (3 h SETCOL) and carbohydrate content of *Ditylum brightwellii* grown on a 14:10 light:dark cycle under low white light (18 μmol photons m^{-2} s^{-1}) or high white light (200 μmol photons m^{-2} s^{-1}) and measured at the end of the light period (end light) or the end of the dark period (end dark). Each bar represents the mean of three replicate cultures ± 1 SE.

APPENDIX 2. ACCURACY OF THE SETCOL METHOD

INTRODUCTION

The purpose of these experiments was to ensure the accuracy of both the sinking rate calculation and the SETCOL apparatus. Since inert particles in the SETCOL should sink according to Stoke's Law (see below), one can compare the calculated Stoke's Law sinking rate for particles of known size and density and compare it to actual measured sinking rates of these particles. A sinking rate measurement can be made on a suspension of the particles in the same way that algal sinking rates are measured. A comparison of the calculated and measured sinking rates of the particles will show the accuracy of the method.

STOKE'S LAW

$$v = (2/9)gr^2(\rho' - \rho)\eta^{-1}\phi^{-1}$$

where v = terminal settling velocity of the particle

g = acceleration due to gravity

r = the radius of the particle

ρ' = the density of the particle

ρ = the density of the medium

η = the viscosity of the medium

ϕ = the coefficient of form resistance

METHODS

Two types of beads were used in these experiments: latex beads with diameters of 4.23, 9.05 and 21.1 μm and a density of 1.05 g ml^{-1} (Polysciences), and polymethylmethacrylate beads ranging in diameter from 1-10 μm with a density of 1.19 g ml^{-1} (Polysciences). Sinking rate experiments with these beads were done under the following conditions: in artificial seawater at 6, 16 and 24°C in a cold chamber, in distilled water at 16 °C in a cold chamber, and in artificial seawater at 16°C in water-jacketed setcols connected to

a cooling tank in the lab. This last setup is how algal sinking rates were measured for the experiments in this thesis; the cold chamber was used for most of bead experiments because its temperature could be changed.

Beads were dispersed in 350 mL of medium, to a final concentration of approximately 600 000 beads mL^{-1} for the 4.23 μm diameter latex, 170 000 beads mL^{-1} for the 9.048 μm diameter latex, 20 000 beads mL^{-1} of the 21.1 μm diameter latex, and 500 000 beads mL^{-1} of the PMMA beads. Before being added to the medium flasks the bead vials were sonicated for 30 s to break up any clumps. Duplicate samples were made of each bead size, and the same bead suspensions were used for the whole set of experiments. Immediately before a sinking rate trial, bead suspensions were mixed and sonicated for 30 s to ameliorate clumping. All sinking rate trial lasted for three hours. Sinking rates for the beads were determined in the same way as for algal samples as described previously in this thesis.

RESULTS

The theoretical relationship between sinking rate, particle size and density can be seen in Figure A2.1A. Sinking rate increases exponentially with particle radius, and is higher for denser particles (PMMA) than for less dense particles (latex). Sinking rates are highest at 24°C due to the decreased density and viscosity of the medium as compared to 6°C.

Figure A2.1B shows measured sinking rates for PMMA and latex beads at 6, 16 and 24°C. The pattern is similar to the top graph. In order to make direct comparisons of measured and calculated sinking rate, the values were plotted against one another, as shown in Figure A2.2. The 1:1 line is shown in each graph running from the lower left to upper right hand corner. Regression lines are shown through the data only, with the slope of the regression shown on the graph. For the measured values to be equal to the calculated values, the slope of the regression should not be different from one. In all cases but one (PMMA in seawater at 16°C), the slope is different than one, as determined using a t-test ($p < 0.05$).

DISCUSSION

The fact that the slopes of the regressions of calculated sinking rate versus measured sinking rate are different than one indicates that the SETCOL technique and/or sinking rate calculation are not wholly accurate. However, the biggest deviations occur at sinking rates greater than what is typically found for the diatoms studied in this work. It is rare for sinking rates to be higher than 0.1 m day^{-1} in healthy cells, or 0.5 m day^{-1} in heat-killed cells.

Sinking rates measured with water-jacketed SETCOLs at 16°C have a higher variation in sinking rate than for measurements made in the cold chamber at 16°C. It is not known what this variability is due to, but the fact that sinking rates in the cold chamber at 24°C have similarly high variability argues against its being solely due to some factor in the water-jacketed SETCOL setup.

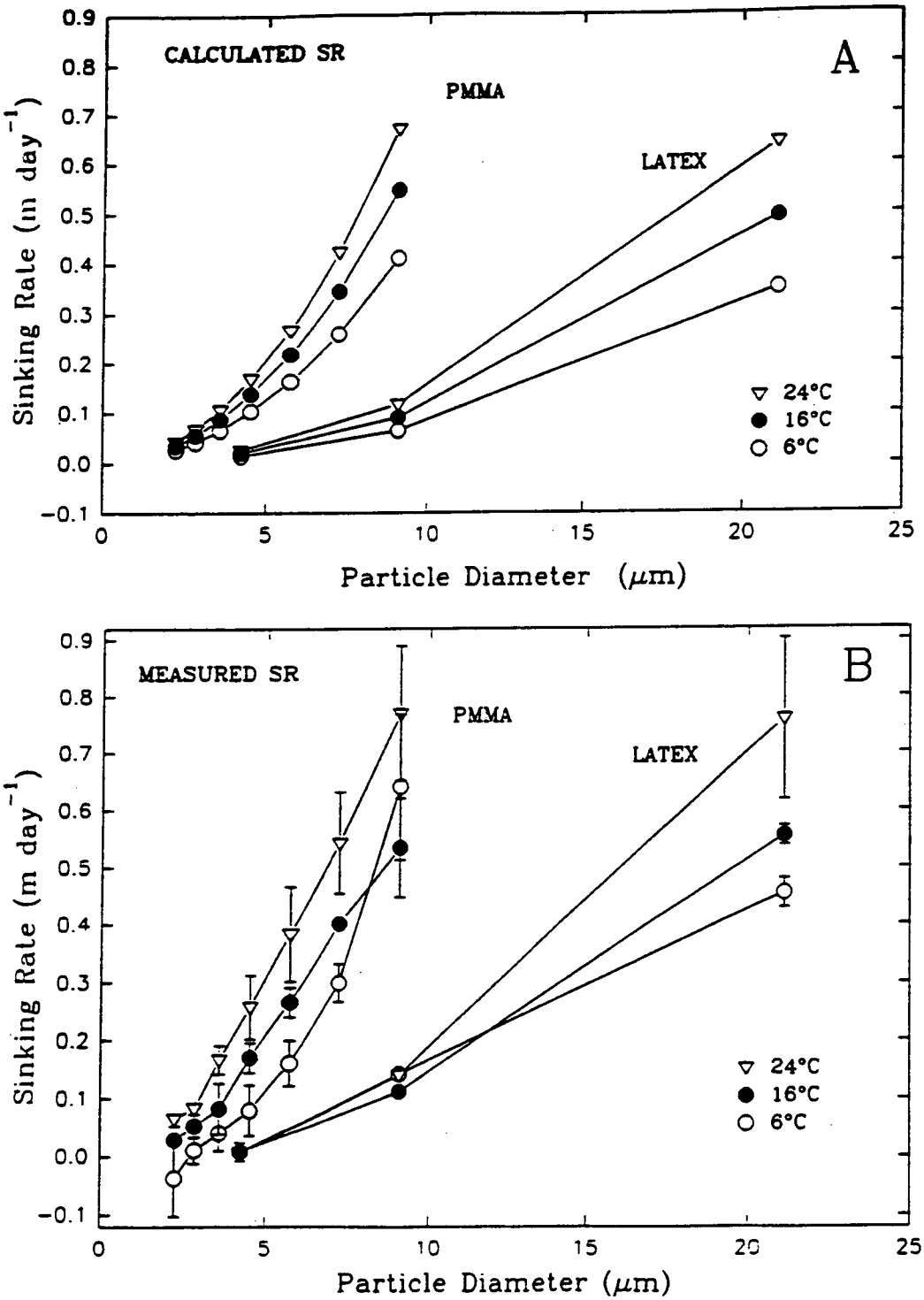


Figure A2.1 The A) theoretical and B) measured relationship between sinking rate (SR) and particle diameter for beads made of latex (density = 1.05 g ml^{-1}) or polymethylmethacrylate (PMMA) (density = 1.19 g ml^{-1}) at 6, 16 and 24°C.

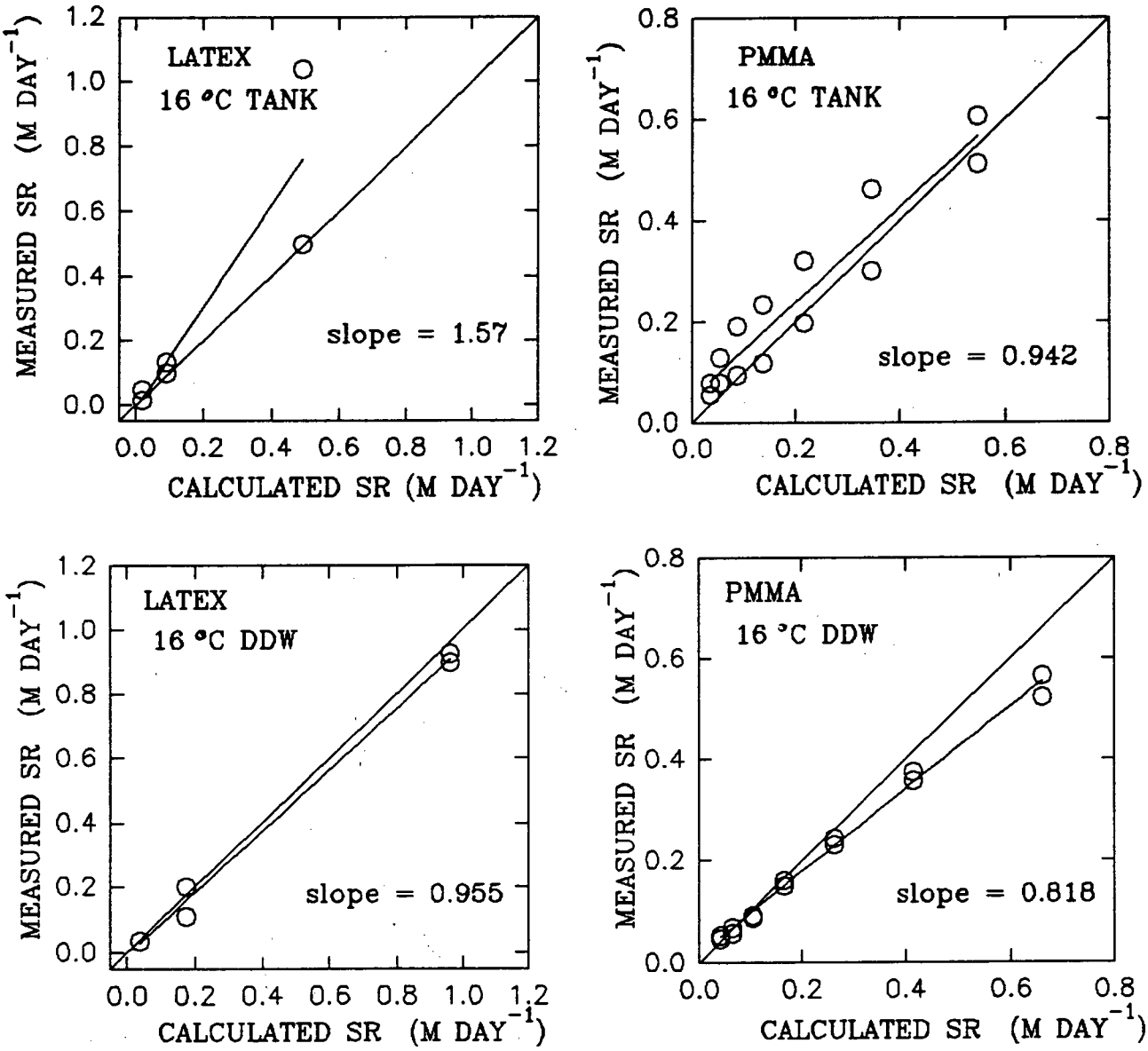
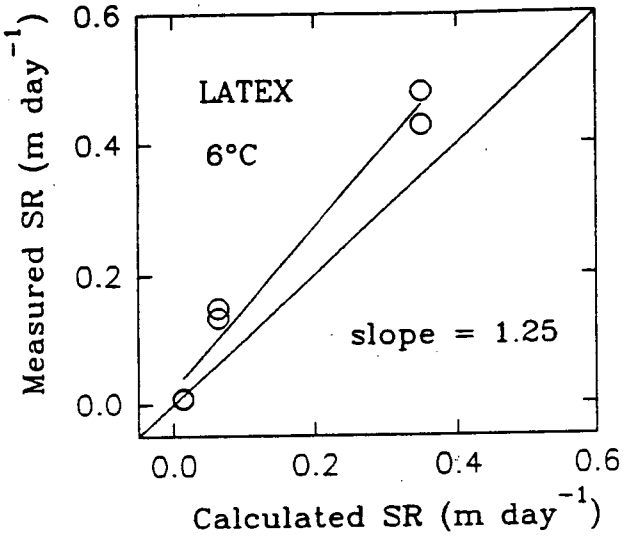
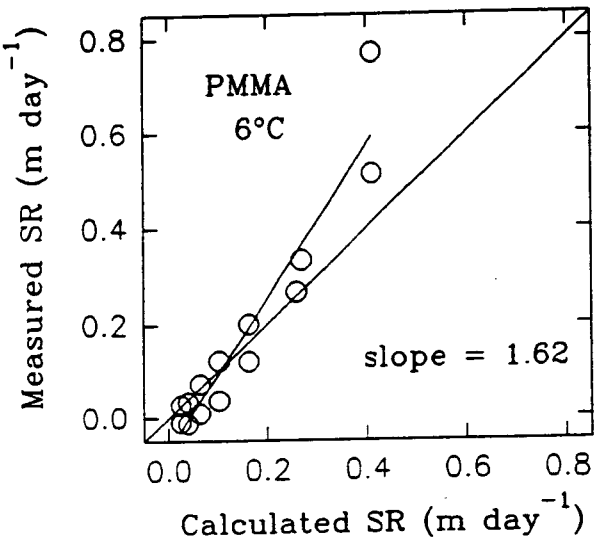
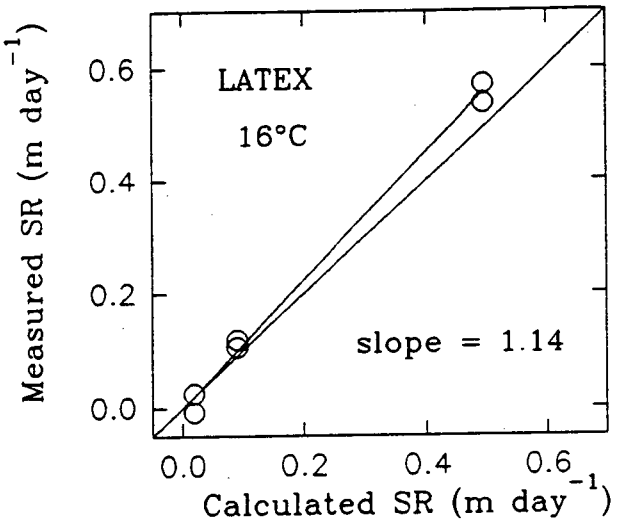
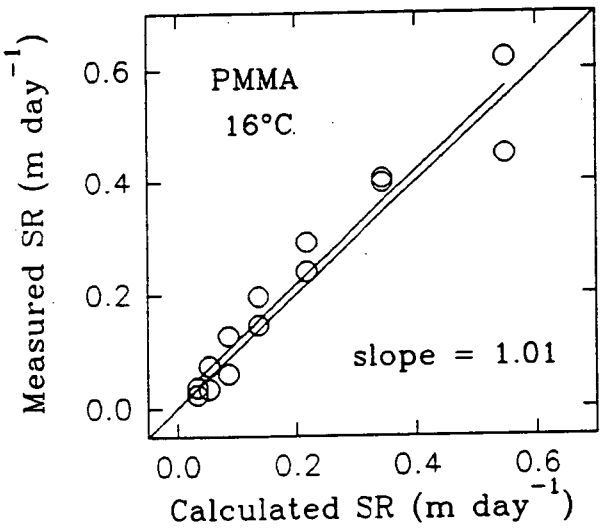


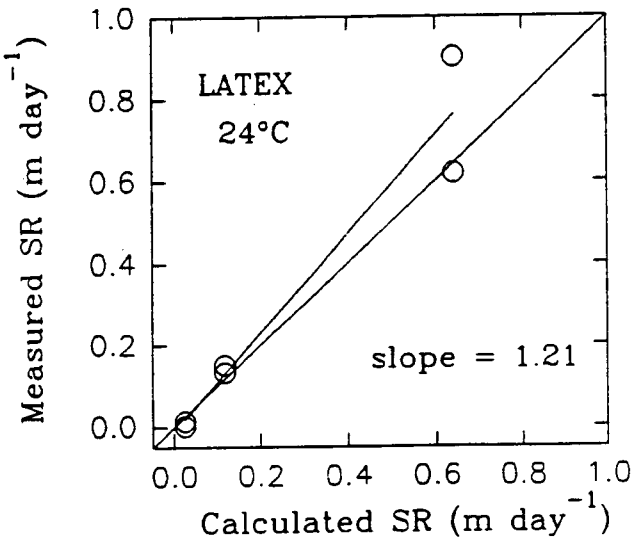
Figure A2.2 Comparison of calculated and measured sinking rates (SR) for beads made of latex (density = 1.05 g ml^{-1}) or polymethylmethacrylate (PMMA) (density = 1.19 g ml^{-1}) in distilled water at 16°C in a cold chamber (DDW), in seawater at 16°C in a water-jacketed SETCOL (TANK), or in seawater at 6, 16 or 24°C in a cold chamber (see following page).

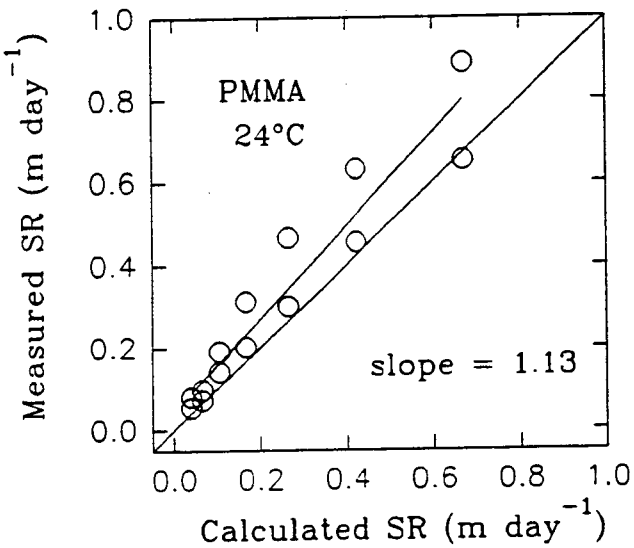

LATEX
6°C
slope = 1.25
Measured SR (m day^{-1})
Calculated SR (m day^{-1})
0.0
0.2
0.4
0.6


PMMA
6°C
slope = 1.62
Measured SR (m day^{-1})
Calculated SR (m day^{-1})
0.0
0.2
0.4
0.6
0.8


LATEX
16°C
slope = 1.14
Measured SR (m day^{-1})
Calculated SR (m day^{-1})
0.0
0.2
0.4
0.6


PMMA
16°C
slope = 1.01
Measured SR (m day^{-1})
Calculated SR (m day^{-1})
0.0
0.2
0.4
0.6


LATEX
24°C
slope = 1.21
Measured SR (m day^{-1})
Calculated SR (m day^{-1})
0.0
0.2
0.4
0.6
0.8
1.0


PMMA
24°C
slope = 1.13
Measured SR (m day^{-1})
Calculated SR (m day^{-1})
0.0
0.2
0.4
0.6
0.8
1.0

APPENDIX 3. SPECTRA OF RED AND BLUE LIGHT

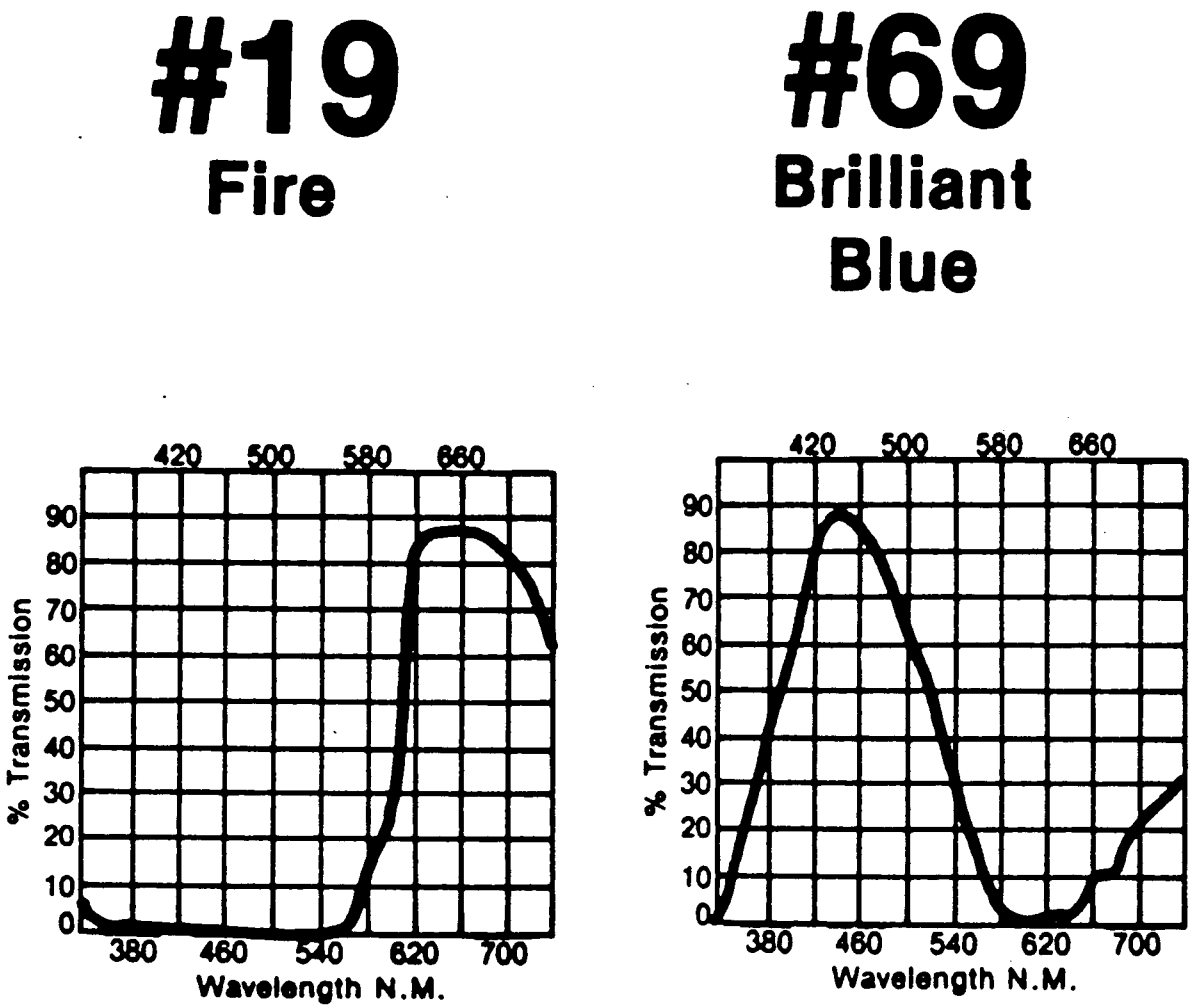


Figure A3.1 Transmittance spectra of Roscolux filters used to obtain red light (#19, Fire) and blue light (#69, Brilliant Blue).

APPENDIX 4. GROWTH IRRADIANCE RELATIONSHIP FOR *T. weissflogii*

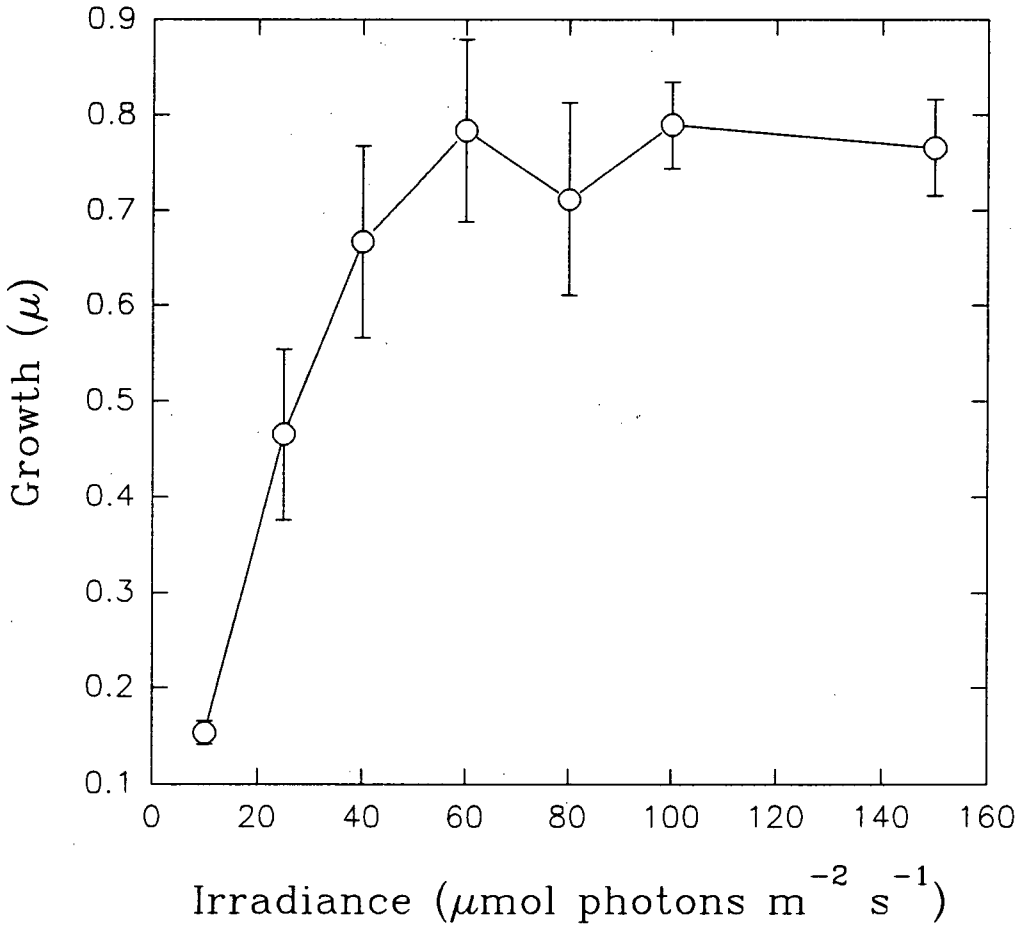


Figure A4.1 The relationship between growth rate and irradiance in *Thalassiosira weissflogii* grown on a light/dark cycle (14 L:10 D). Each symbol represents the mean of 3 grow-ups ± 1 SE.