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DOES LIGHT QUALITY AFFECT THE SINKING RATES OF MARINE DIATOMS?[1]

Anne E. Fisher

Department of Oceanography, 6270 University Boulevard, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

John A. Berges

School of Biology and Biochemistry, The Queen's University, Belfast, Northern Ireland BT7 1NN

and

Paul J. Harrison[2]

Department of Oceanography, 6270 University Boulevard, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

ABSTRACT

Although the spectral quality of light in the ocean varies considerably with depth, the effect of light quality on different physiological processes in marine phytoplankton remains largely unknown. In cases where experiments are performed under full spectral irradiance, the meaning of these experiments in situ *is thus unclear. In this study, we determined whether variations in spectral quality affected the sinking rates of marine diatoms. Semicontinuous batch cultures of* Thalassiosira weissflogii *(Gru.) Fryxell et Hasle and* Ditylum brightwellii *(t. West) Grunow in Van Huerk were grown under continuous red, white, or blue light. For* T. weissflogii, *sinking rates (SETCOL method) were twice as high ($\approx 0.2\ m \cdot d^{-1}$) for cells grown under red light as for cells grown under white or blue light ($\approx 0.08\ m \cdot d^{-1}$), but there were no significant differences in carbohydrate content ($\approx 105\ fg \cdot \mu m^{-3}$) or silica content ($\approx 17\ fg \cdot \mu m^{-3}$) to account for the difference in sinking rates.* Thalassiosira weissflogii *grown under blue light was significantly smaller (495 μm^3) than cells grown under red light (661 μm^3), which could contribute to its reduced sinking rate. However, cells grown under white light were similar in size to those grown under red light but had sinking rates not different from those of cells grown under blue light, indicating the involvement of factors other than size. There were no significant differences in sinking rate ($\approx 0.054\ m \cdot d^{-1}$) or silica content ($\approx 20\ fg \cdot \mu m^{-3}$) in* D. brightwellii *grown under red, white, or blue light, but cells grown under red light were significantly (20%) larger and contained significantly (20%) more carbohydrate per μm^3 than cells grown under white or blue light. Spectral quality had no consistent effect on sinking rate, biochemical composition (carbohydrate or silica content), or cell volume in the two diatoms studied. 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.*

Key index words: Bacillariophyceae; biochemical composition; carbohydrate; cell size; Ditylum brightwellii; *irradiance quality; silica; sinking rate;* Thalassiosira weissflogii

Since the discovery that the sinking rates of diatoms were not independent of physiology (Gross and Zeuthen 1948), many studies have been done to determine the nature of physiological sinking rate control. The sinking rate of a diatom is proportional to its size, shape, and the difference between the density of the diatom and its medium. The density of a diatom depends in part on its chemical composition. The major components of a diatom are carbohydrate, protein, and silica (all of which are more dense than seawater) and lipid (which is less dense than seawater) (Smayda 1970). Accumulations of any of the denser components could produce an increase in sinking rate. The density of a diatom may also vary due to the relative proportions of heavier and lighter ions in the vacuole (Gross and Zeuthen 1948, Anderson and Sweeney 1978). Because ion transport is often an energy-requiring process, the maintenance of a low sinking rate can depend on a diatom's energy status (Waite et al. 1992b). Given that the sinking rate of a cell depends on the sum interaction of many different factors, it is not surprising that sinking rate has been shown to vary, not always consistently, in response to environmental variables such as irradiance (Bienfang et al. 1983, Culver and Smith 1989, Granata 1991) and nutrients (Lannergren 1979, Bienfang et al. 1982).

Many of the factors that affect sinking rate are also affected by light quality. Light quality is known to affect many aspects of phytoplankton physiology such as pigment composition (Vesk and Jeffrey 1977, Rivkin 1989), photosynthesis (Grotjohann et al. 1991), chemical composition (Wallen and Geen 1971, Kowallik 1987), growth rate (Wallen and Geen 1971, Morel et al. 1987), and ion transport (Raven 1969, Schmid and Dring 1993). One example of a far-reaching effect of blue light on ion transport is the blue light–induced extrusion of protons at the sur-

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[2] Address for reprint requests.

face of *Ectocarpus*, with the resultant acidification increasing the availability of carbon dioxide for uptake and hence enhancing photosynthesis (Schmid and Dring 1993). The increase in protein content often seen in cells grown under blue light was found to be due to blue light stimulation of glycolytic enzymes that increased the rate of carbohydrate degradation (Kowallik 1987).

Given that sinking rate is in part determined by physiology, and given the wide-ranging physiological effects of light quality, there is potential for light quality to affect sinking rate. However, the effects of light quality on sinking rate have never been studied. Because many studies of sinking rate are conducted under white light for convenience, it is important to establish whether sinking rates are different under blue light (the predominant color a few meters below the water surface in the marine environment).

The study of light quality is complicated by the variable effects of growing cells under equal photosynthetically available radiation (PAR) or equal photosynthetically usable radiation (PUR) (Morel 1978). PAR refers to the amount of radiant energy available at a certain depth within the visible spectrum (400–700 nm). Because algae cannot absorb photons at every wavelength, and not every photon of PAR is available for photosynthesis, it becomes necessary to consider another parameter, PUR, which is the radiant energy available that can be absorbed by algae. PUR is determined by both the pigment composition of the algae and the spectral composition of the light and can be approximated as the irradiance providing equal growth rates.

The purpose of this study was to determine in two species of marine diatoms whether or not light quality (red, white, or blue light) affects sinking rate and to examine whether the effect is due to a change in cell volume, carbohydrate content, or silica content of the cells. The two species were chosen for the contrast in their size and extent of sinking rate control. *Ditylum brightwellii* is a large cell and shows a strong relationship between energy and sinking rate control, whereas *Thalassiosira weissflogii* is almost an order of magnitude smaller and shows only a weak relationship between energy and sinking rate control (Waite et al., unpubl.). One species (*T. weissflogii*) was grown at two irradiances (saturating and subsaturating) of equal PAR whereas the other (*D. brightwellii*) was grown under equal PUR.

MATERIALS AND 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). For all experiments except that measuring silica content, semicontinuous batch cultures were grown in enriched artificial seawater based on the recipe by Harrison et al. (1980) with sodium glycerophosphate replaced with an equimolar concentration of sodium phosphate, ferrous ammonium sulfate with an equimolar concentration of ferric chloride, and additions of selenite, nickel, and molybdate to achieve 1 nM final concentration. Cultures were grown in triplicate at 16° ± 1° C in a water bath, in 1- or 2-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. For the experiment measuring silica content, semicontinuous batch cultures were grown in triplicate in artificial seawater medium (Goldman and McCarthy 1978), with f/2 enrichment (Guillard and Ryther 1962) in 50-mL glass tubes in a controlled environment chamber at 22° ± 2° C.

In all experiments, cultures were grown under continuous light with illumination provided by Vitalite® fluorescent tubes. Red light was obtained using red-colored filters (Roscolux #19), and blue light was obtained using blue-colored filters (Roscolux #69). Irradiance was adjusted using neutral density screening. For the first experiment using *T. weissflogii* (measuring sinking rate only), all cultures received 35 μmol photons·m^{-2}·s^{-1}; for the second experiment (measuring sinking rate and carbohydrate content), all cultures received 85 μmol photons·m^{-2}·s^{-1}. For experiments using *D. brightwellii*, light levels were adjusted so all cultures had equal growth rates: red light–grown cells received 115 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}. For the experiment measuring silica content, all cultures (*T. weissflogii* and *D. brightwellii*) were grown at 85 μmol photons·m^{-2}·s^{-1}. These irradiances were measured in air inside empty culture vessels using a Biospherical Instruments QSL 100 light meter.

Growth rates were monitored by taking both *in vivo* fluoresence and cell counts daily. *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 midlogarithmic growth phase, after acclimation for a minimum of six generations.

Sinking rates were measured using the SETCOL method of Bienfang (1981) with modifications as in Waite et al. (1992b). The SETCOL was water-jacketed and maintained at 16° ± 0.5° C. Sinking rate measurements on all treatments were made simultaneously, either in the dark (for *D. brightwellii*) or under the light regime that the cells were grown under (for *T. weissflogii*). Cell counts measured with a Coulter Counter on samples preserved in Lugol's solution were used as the index of biomass in the sinking rate calculation. The population accessory on the Coulter Counter made it possible to measure the size distribution of the cells and to calculate a sinking rate for cells in each of a range of size classes in order to examine the relationship between sinking rate and cell volume. While the shrinkage caused by Lugol's solution 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. No correction was made for shrinkage, but the time interval between fixation and counting was the same for all samples to ensure shrinkage was consistent.

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-×-100-mm borosilicate glass test tubes with 2.5 mL of 3 N H_2SO_4 and 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; the extracts were pooled. The carbohydrate was assayed by the phenol-sulfuric acid method of Dubois et al. (1956) using 16-×-125-mm test tubes and directing the stream of acid onto the surface of the sample to ensure the sample was heated and mixed evenly.

Silica content was measured by filtering 25 mL of culture onto 25-mm, 0.8-μm Poretics Corp. polycarbonate filters, which were

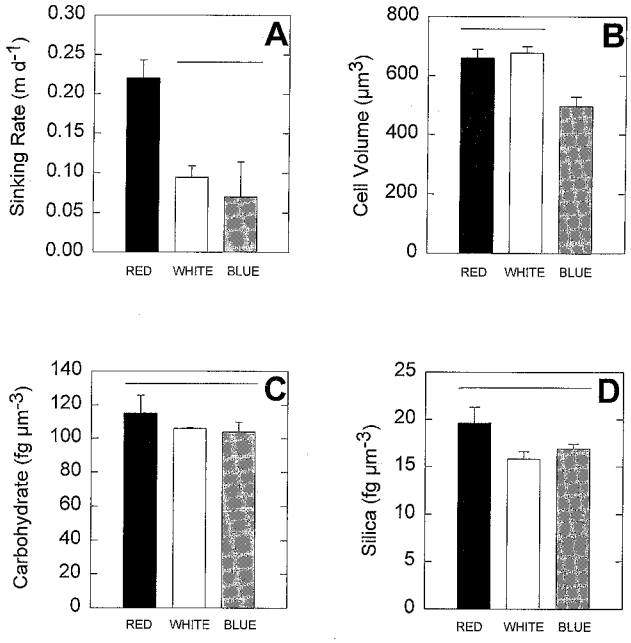

FIG. 1. *Thalassiosira weissflogii.* A) Sinking rate (3 h SETCOL) and B) cell volume when grown under continuous red, white, or blue light (data pooled from 35 and 85 µmol photons·m^{-2}·s^{-1}). Each bar represents the mean of n replicate cultures ± 1 SE (n = 5 for red light, n = 4 for white light, n = 2 for blue light). C) Carbohydrate content and D) silica content when grown under 85 µmol photons·m^{-2}·s^{-1} of continuous red, white, or 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.

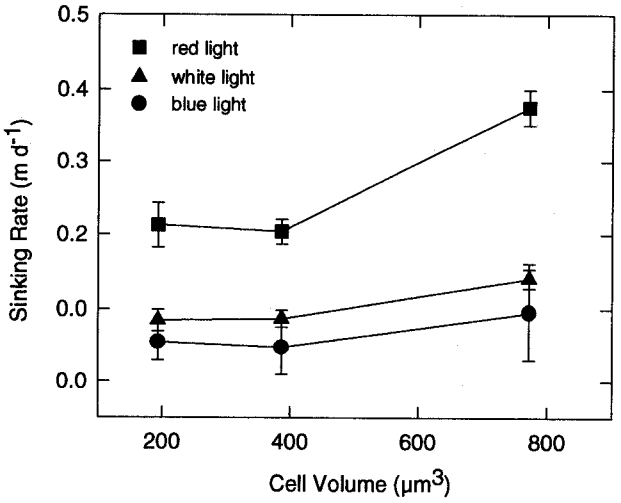

FIG. 2. The relationship between sinking rate and cell volume for *Thalassiosira weissflogii* (data pooled from 35 and 85 µmol photons·m^{-2}·s^{-1}). The distribution of cells in three size classes (cell volume) was measured using a Coulter Counter at the end of the sinking rate experiments. Each point represents the mean of n replicate cultures ± 1 SE (n = 5 for red light, n = 4 for white light, n = 2 for blue light).

frozen at −20° C until analysis. Silica was hydrolyzed by the method of Paasche (1980) and analyzed by the method of Koroleff (1983).

Protein content was measured 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 1 N NaOH. Protein was assayed using the method of Bradford (1976), using the microassay procedure of the Bio-Rad Protein Assay Kir (Bio-Rad Laboratories, 500-001) and bovine serum albumin (Sigma Chemical Co. A 7638) as a standard.

Statisical comparisons were made using one-way analyses of variance followed by Tukey multiple-comparison analyses looking for significant differences at the 95% confidence level.

RESULTS

Thalassiosira weissflogii. 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. 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 an average growth rate of 0.85 ± 0.07 d^{-1}. Because sinking rates at 35 and 85 µmol photons·m^{-2}·s^{-1} were not significantly different, data are shown pooled in Figures 1A and 2.

At both 35 and 85 µmol photons·m^{-2}·s^{-1}, sinking rates of cells grown under red light were about twice as high as the white and blue light treatments (Fig. 1A). There was no difference in sinking rate for cells grown under white or blue light at 35 µmol photons·m^{-2}·s^{-1}. The light spectrum under which sinking rate determinations were conducted made no difference, as 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).

Cells grown under blue light had a significantly lower average volume than those grown under white or red light; the volumes of cells grown under red and white light were not significantly different (Fig. 1B). Sinking rates of cells grown under red light were significantly higher than those of cells grown under white or blue light across a range of cell size classes (Fig. 2). Linear regression analysis showed no relationship between sinking rate and cell volume for cells grown under blue or white light, but there was a significant positive relationship between sinking rate and cell volume for cells grown under red light (Fig. 2).

There was no difference in carbohydrate content (Fig. 1C), silica content (Fig. 1D), or protein content (Fisher 1994) among cells grown under red, white, or blue light at 85 µmol photons·m^{-2}·s^{-1}.

Ditylum brightwellii. Growth irradiances were adjusted so all cultures would have similar growth rates: 0.57 ± 0.02 d^{-1} for red light–grown cells (which received 115 µmol photons·m^{-2}·s^{-1}), 0.68 ± 0.04

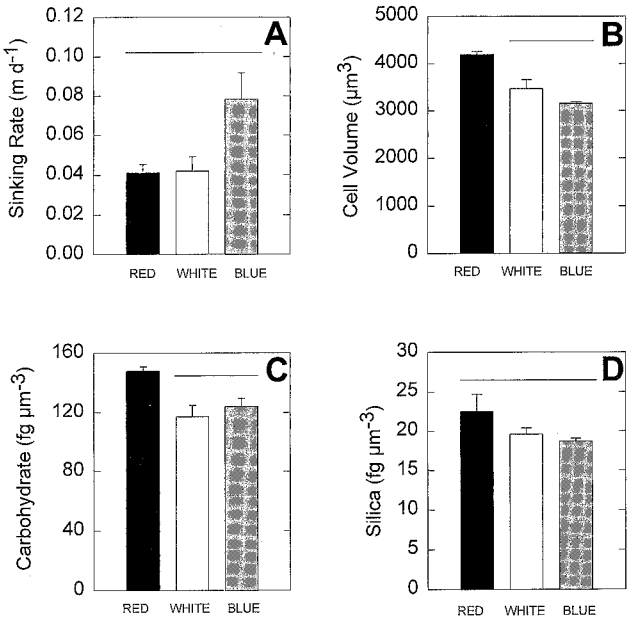

FIG. 3. *Ditylum brightwellii.* A) Sinking rate (3 h SETCOL), B) cell volume, and C) carbohydrate content when grown under continuous red (115 µmol photons·m^{-2}·s^{-1}), white (70 µmol photons·m^{-2}·s^{-1}), or blue (65 µmol photons·m^{-2}·s^{-1}) light, with light levels adjusted to obtain growth rates of approximately 0.65 d^{-1} (0.65 µmax). D) Silica content when grown under 85 µmol photons·m^{-2}·s^{-1} of continuous red, white, or blue light. 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.

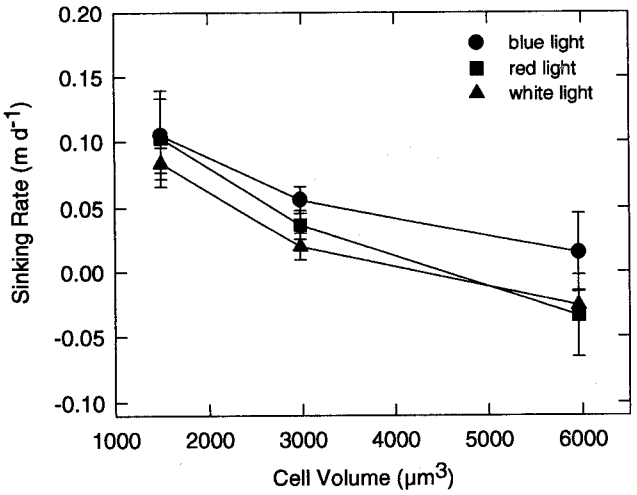

FIG. 4. The relationship between sinking rate and cell volume for *Ditylum brightwellii* grown under red (115 µmol photons·m^{-2}·s^{-1}), white (70 µmol photons·m^{-2}·s^{-1}), or blue (65 µmol photons·m^{-2}·s^{-1}) light. The distribution of cells in three size classes (cell volume) was measured using a Coulter Counter at the end of the sinking rate experiments. Each point represents the mean of three replicate cultures ± 1 SE.

d^{-1} for blue light–grown cells (which received 65 µmol photons·m^{-2}·s^{-1}), and 0.65 ± 0.04 d^{-1} for white light–grown cells (which received 70 µmol photons·m^{-2}·s^{-1}). These cells were all light-limited, growing at about 65% of their maximal growth rate.

There were no significant differences in sinking rate between *D. brightwellii* grown under red, white, or blue light (Fig. 3A). Cells grown under red light were significantly larger than those grown under white or blue light (Fig. 3B). There were no differences in the relationship between sinking rate and cell volume for cells grown under red, white, or blue light (Fig. 4). Linear regression analysis showed a significant negative relationship between sinking rate and cell volume for all treatments.

The carbohydrate content (per µm^3) of cells grown under red light was significantly higher (~20%) than that of cells grown under white or blue light (Fig. 3C). There were no differences in silica content (Fig. 3D) or protein content (Fisher 1994) in cells grown under red, white, or blue light.

DISCUSSION

The purpose of these experiments was to determine whether variations in spectral quality affected the sinking rates of marine diatoms. The two main factors affecting sinking rate are cell size (volume) and cell density (determined by cell composition and physiological sinking rate control mechanisms), as described mathematically by Stokes' Law:

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

where v is sinking rate, g is acceleration due to gravity, r is particle radius, ρ' is the density of the particle, ρ is the density of the medium, ϕ is the coefficient of form resistance, and η is the viscosity of the medium (Smayda 1970).

Cell size. It can be seen from Stokes' Law that the sinking rate of a diatom should be proportional to the square of its radius, meaning that larger cells would sink faster than smaller cells. In *T. weissflogii,* the volume of cells grown under red light was about 30% larger than that of cells grown under blue light. Using Stokes' Law and assuming all variables other than size to be equal, the preceding difference in cell volume would account for only a 20% difference in sinking rate, not the twofold difference in sinking rate observed. Cells grown under red light had a similar cell volume to those grown under white light, yet sinking rates of red light–grown cells were twice as high.

In *D. brightwellii,* cells grown under red light were significantly larger (25%) than cells grown under blue light, which would theoretically produce a 17% increase in sinking rate; however, sinking rates of cells grown under blue light were not significantly different from those of cells grown under red light. These data indicate that size is not the main determinant of sinking rate in either species, which is further supported by the fact that *D. brightwellii* is almost 10 times larger than *T. weissflogii* but sinks only half as fast. The difference in sinking rate between the two species may be due in part to differ-

ences in shape. *Ditylum brightwellii* is more elongated with spines sticking out at either end, and so would have higher form resistance. However, the lower sinking rate of *D. brightwellii* cannot be due solely to form resistance, because sinking rate in this species increases markedly (approximately 10-fold) when cells are treated with a physiological inhibitor such as cyanide (Waite et al. 1992b).

Composition. Carbohydrate and silica are both denser than seawater (about 1.6 and 2.6 $g \cdot cm^3$, respectively), so significant cellular accumulations of these substances will increase overall cell density. Carbohydrate content is known to affect the buoyancy of cyanobacteria as cells with high carbohydrate content sink and cells with low carbohydrate content are buoyant (Oliver 1994). In this study, the only case where light quality affected composition was in higher carbohydrate in *D. brightwellii* grown under red light. There was no corresponding increase in sinking rate although any increase in cell density caused by the higher carbohydrate content could have been compensated for by physiological sinking rate control mechanisms. The lack of a relationship between sinking rate and carbohydrate content was also shown in another study in which carbohydrate content was varied by growing diatoms under light/dark cycles at 20 or 100 μmol photons $\cdot m^{-2} \cdot s^{-1}$ (Fisher and Harrison 1996). Despite the differences in the sinking rate characteristics of these two species (i.e. the much larger *D. brightwellii* having a lower sinking rate than *T. weissflogii*), their silica and carbohydrate content per unit of cell volume are similar. This provides further evidence that these components are not the primary ones involved in changing sinking rate.

There is evidence in the literature to support light spectral effects on carbohydrate content. 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, whereas cells grown under red light contained 39% carbohydrate on a dry weight basis (Kowallik 1987). Similar results have been found for *Euglena, Chlorogonium, Chlamydomonas, Cyclotella, Acetabularia, Scenedesmus,* and *Spirulina* (Kowallik 1987). This phenomenon was also observed in *D. brightwellii* in the present study, but not in *T. weissflogii.* The lack of a spectral effect on the carbohydrate content of *T. weissflogii* was unexpected, but not wholly unsupported. Carbohydrate production in the diatom *Chaetoceros protuberans* did not differ under equal PUR of blue and white light (Gostan et al. 1986).

Protein and lipid can also be a significant proportion of diatom composition. Protein content was measured in this study and was found not to be significantly affected by light quality (Fisher 1994). Lipid content was not measured, but previous work (unpubl.) in our laboratory showed that the lipid content of actively growing cells was fairly constant, and Anderson and Sweeney (1977) showed that lipid content did not affect sinking rate.

It can be seen from Stokes' Law (equation 1), that small changes in density result in large differences in sinking rate. Using the sinking rates found for *T. weissflogii* of 0.08 $m \cdot d^{-1}$ (blue or white light–grown cells) and 0.2 $m \cdot d^{-1}$ (red light–grown cells), and substituting into equation 1 (we assumed constant viscosity of 1.1717, seawater density of 1.0255, and coefficient of form resistance of 1 and used the equivalent spherical radii calculated from Coulter Counter volumes), the densities of the cells would be 1.0258 $g \cdot cm^{-3}$ for cells sinking at 0.08 $m \cdot d^{-1}$ and 1.0263 $g \cdot cm^{-3}$ for cells sinking at 0.2 $m \cdot d^{-1}$. This is a density difference of only 0.0005 $g \cdot cm^{-3}$, or 0.05%. A coefficient of form resistance of 1 represents a spherical particle. In fact, most cells have values >1 due to shape differences (Smayda 1970); in such cases, the apparent difference in density becomes somewhat larger. Estimating a theoretical maximum coefficient of form resistance for *T. weissflogii* of 2 (see Hutchinson 1967:fig. 75), the density difference would at most double to 0.001 $g \cdot cm^{-3}$. Such small differences are beyond the resolution of even the best density centrifugation methods (cf. van Ierland and Peperzak 1984, Schwinghamer et al. 1991).

We wished to see whether or not changes in silica or carbohydrate could cause density differences sufficient to produce the observed changes in sinking rates. We assumed a constant cell composition for components other than carbohydrate and silica and assigned this component a density of 0.9 $g \cdot cm^{-3}$. For cell composition, we used values typical of cells in our study, with 105 $fg \cdot \mu m^{-3}$ of carbohydrate and 17 $fg \cdot \mu m^{-3}$ of Si, with respective densities of 1.6 and 2.6 $g \cdot cm^{-3}$. Using Stokes' Law and knowing the sinking rate, we then calculated the overall density of the cell. We estimated how much of the cell was something other than carbohydrate or silica using the following equation: (silica content)(2.6) + (carbohydrate content)(1.6) + (other content)(0.9) = total cell density. From this we estimated the cell contents to be 2% silica, 15% carbohydrate, and 83% "other," and we used these values to calculate how changes in silica or carbohydrate content would affect cell density. We found that an increase in carbohydrate from 105 to 106 $fg \cdot \mu m^{-3}$ (a smaller difference than we can resolve in our analytical method) would produce a density difference of 0.001 $g \cdot cm^{-3}$, whereas a change in Si from 17 to 18 $fg \cdot \mu m^{-3}$ would produce a density difference of 0.002 $g \cdot cm^{-3}$. We conclude that compositional differences that are too small to resolve using our methods could cause density differences and, hence, sinking rate differences, even larger than those we observed.

Growth rate. The lower growth rate of *T. weissflogii* grown under red light at 35 μmol photons $\cdot m^{-2} \cdot s^{-1}$ occurred due to the unequal PUR of red and blue light; the chlorophyll and accessory pigments of di-

atoms absorb blue light better than red light. Because light is saturating at 85 μmol photons·m^{-2}·s^{-1}, the lower efficiency of red light absorption is unimportant, resulting in the observed similarity in growth rates. In *D. brightwellii,* grown under equal PUR, 50% more photons were necessary to obtain a similar growth rate under red light.

The results for growth rate 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. 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} (Humphrey 1983). 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). 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).

Differences in light-limited growth rate do not affect sinking rate in these species (Fisher and Harrison 1996), so it is probable that growth rate is not a factor affecting sinking rate in this study. Furthermore, higher sinking rates under red light in *T. weissflogii* were observed at two different growth rates (produced by irradiances of 35 and 85 μmol photons·m^{-2}·s^{-1}).

Ion transport. The lack of a decrease in sinking rate when *T. weissflogii* grown under red light was exposed to blue light during a sinking rate trial (3 h) shows that red light is not affecting sinking rate through a fast-acting mechanism. Sinking rate can change significantly within several hours (Anderson and Sweeney 1977). Presumably if red light affected sinking rate through a physiological process (i.e. ion transport), one might expect sinking rate to decrease relatively rapidly once the cells were no longer exposed to red light; this did not occur. However, if blue light is required for the synthesis of ion transporters, it could take longer than 3 h before any decrease in sinking rate occurs.

The difference in the relationship between sinking rate and cell volume for the two species suggests that sinking rate in *D. brightwellii* is affected more by physiological control than in *T. weissflogii* (also shown in Waite et al., unpubl.). This is also clear from absolute rates: despite the fact that *D. brightwellii* has almost 10 times the cell volume of *T. weissflogii,* its sinking rates are slower. For *D. brightwellii,* there were significant negative relationships between sinking rate and cell volume in all light treatments, showing that sinking rates are slower in larger cells, as postulated by Villareal (1988). The negative sinking rates of the largest size class of cells shows that these cells were less dense than seawater. In contrast, sinking rate in *T. weissflogii* grown under red light increased with cell volume; the faster sinking rates of larger cells indicate that sinking rate is determined by physical (rather than physiological) factors. There was no such relationship for *T. weissflogii* grown under white or blue light. The higher sinking rates of *T. weissflogii* grown under red light compared to those grown under blue or white light shows that these cells are denser than their white or blue light counterparts and/or are less able to decrease sinking rate through physiological means.

The lack of a sinking rate increase in *D. brightwellii* grown under red light indicates that physiological sinking rate control in this species is not affected by red light. Sinking rate in *D. brightwellii* is known to be sensitive to physiological changes, as shown by the large increase in sinking rate that occurs when cells are heat-killed (Fisher 1994) or treated with respiratory inhibitors (Anderson and Sweeney 1977, Waite et al. 1992a). The much smaller increases in sinking rate that occur when *T. weissflogii* is physiologically inactivated shows that sinking rate in this species does not respond to physiological changes to the same extent as in *D. brightwellii* (Waite et al., unpubl.). If red light were affecting sinking rate through an effect on ion transport, one would expect the effect to be more extreme in *D. brightwellii* than *T. weissflogii,* which was not the case.

Ecological significance. At the ocean surface, diatoms are exposed to the highest irradiances as well as the most red light and ultraviolet (UV) radiation, making it tempting to speculate that increased sinking rates under red light are an adaptation to prevent photoinhibition or UV damage. If this were true, however, the effect should also be observed in cells growing under white light (due to its red light component), which was not the case. Because diatoms in nature are never exposed to exclusively red light, there is no reason to expect that they have made any physiological adaptations to it. Rather than considering the increased sinking rate as something that occurs in response to red light, it may be more useful to consider it as something that occurs due to a lack of blue light, implying that blue light is perhaps required in certain species for the maintenance of low sinking rates.

While the importance of single-cell sinking rates to vertical carbon flux is thought to be much less than that due to aggregation, single-cell sinking rates are important in relation to aggregation. Single-cell sinking rates determine the distribution of phytoplankton in the ocean before sedimentation occurs, and studies have shown that results from sinking rate studies done with the SETCOL apparatus are comparable to those done with sediment traps (Riebesell 1989). In coastal areas where production is high and distance to the sediments is short, diatoms have been observed to reach the bottom without large-scale formation of aggregates (Waite et al. 1992a). Single-cell sinking rates are also relevant to large diatoms such as *Ethmodiscus* that live in oligotrophic seas and can vertically migrate through the nutricline (Villareal and Carpenter 1994). Be-

cause aggregation depends on cell stickiness, which varies with species and physiological state, single-cell processes will also affect aggregation. Some species, such as *T. weissflogii,* never become sticky and will only aggregate in the presence of transparent exopolymeric particles (Crocker and Passow 1995). Aggregation rate also depends on the frequency of collisions between particles, a factor that is directly influenced by cell sinking rate (Crocker and Passow 1995). Single-cell sinking rates may also become important when aggregation is prevented by bacteria (Smith et al. 1995).

In summary, the sinking rates of *T. weissflogii* grown under red light were over twice those of cells grown under white or blue light. This difference could not be explained by changes in cell volume, carbohydrate content, or silica content, and there is evidence against it being due to red light effects on physiological sinking rate control through ion transport. There were no spectral effects of sinking rate in *D. brightwellii,* although cells grown under red light were larger and contained more carbohydrate. There was no consistent relationship between sinking rate and light quality effects mediated through growth rates, cell volume, or carbohydrate or silica content.

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DOES CARBOHYDRATE CONTENT AFFECT THE SINKING RATES OF MARINE DIATOMS?[1]

Anne E. Fisher and Paul J. Harrison[2]

Department of Oceanography, 6270 University Boulevard, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1Z4

ABSTRACT

We tested the hypothesis that positive relationships between sinking rate and irradiance were due to increases in cell density caused by accumulations of carbohydrate. In semicontinuous batch cultures of Thalassiosira weissflogii *(Gru.) Fryxell et Hasle and* Ditylum brightwellii *(t. West) Grunow in Van Huerk, carbohydrate content was varied by growing cells under diel cycles of high or low light. Sinking rate was measured at the end of the light period and the end of the dark period, on live and heat-killed cells. No positive correlations were found between sinking rate (which varied from* -0.060 *to* $0.13\ m \cdot d^{-1}$*) and carbohydrate content (which varied from 10 to 950* $pg \cdot cell^{-1}$*), indicating that accumulations of carbohydrate did not significantly affect sinking rate. There were no large diel variations in the sinking rate of* T. weissflogii, *but sinking rates of* D. brightwellii *grown under high light ranged from being negative (i.e. cells were floating) at the end of the light period to positive at the end of the dark period. This is the first report of positive buoyancy in vegetative* D. brightwellii, *a phenomenon that may only occur in* D. brightwellii *grown under diel cycles.*

Key index words: Bacillariophyceae; carbohydrate; diel periodicity; Ditylum brightwellii; *irradiance; sinking rate;* Thalassiosira weissflogii

The possibility that sinking rate in diatoms can be physiologically controlled arose following observations that sinking rate varied with growth stage and that sinking rates were higher in preserved cells than in live cells (Gross and Zeuthen 1948). The means of this control was found to be through the selective exchange of heavier ions for lighter ions in the vacuole, which is an energy-requiring process (Anderson and Sweeney 1978). Physiological sinking rate control in diatoms has been shown to vary with the energy status of the cells, with sinking rates increasing when energy is decreased by nutrient limitation, prolonged darkness, or metabolic inhibitors (Waite et al. 1992). The response of sinking rate to variations in light energy shows the opposite trend, with cells exposed to high light having higher sinking rates. A positive correlation has been found between sinking rate and irradiance for *Chaetoceros gracilis, C. flexosum* (Culver and Smith 1989), *T. weissflogii* (Bienfang et al. 1983), and *Coscinodiscus concinnus* (Granata 1991). Some studies done under diel cycles have shown sinking rates to be maximal near the end of the light period and minimal near the end of the dark period (Anderson and Sweeney 1977, Granata 1991). Mats of *Rhizosolenia* were found to sink faster in the afternoon (Villareal et al. 1993). In all these examples, sinking rates were higher when the cell was receiving higher light energy, which contradicts the hypothesis that sinking rate control is solely energy-dependent.

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[2] Author for reprint requests.