PARTICLE DYNAMICS AND SINKING RATES OF THE MARINE DIATOM

Skeletonema costatum

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ABSTRACT

The distribution of marine phytoplankton, and marine diatoms in particular, is of major importance to global climate and primary production. The changes in the distributions of these diatoms is strongly controlled by their sinking rates (Smayda, 1970). Therefore, sinking rates of diatoms have been measured in both the laboratory and in the field by many methods.

The relationship between particle size and particle sinking rate is important for our understanding of the biological and physical controls of this vertical flux. This relationship is also used by modelers to predict particle export from surface waters. A new method for determining the sinking rates of marine diatoms has been developed by A. Waite and K. O’Brien at the Centre for Water Research (CWR) at the University of Western Australia (UWA). This method uses the concept of non-disruptive observation of sinking particles as developed by Waite et al. (1997). Past methods for determining sinking rates of marine diatoms include the SETCOL as developed by Bienfang (1981).

We investigated the effect of nutrient limitation on the size versus sinking rate relationship using both methods, on the marine diatom Skeletonema costatum, and the aggregates formed of this species. Aggregation was induced by a shear on nitrogen depleted cultures.

Stokes’ Law has commonly been applied to the sinking of marine phytoplankton. The applicability of Stokes’ Law to a range of single chain and aggregates of Skeletonema costatum was tested. The results showed that sinking rate was proportional to the equivalent diameter of a given chain or aggregate ($w_s \propto d$). This did not did not confirm earlier work that suggested that sinking rate is proportional to the square of the diameter ($w_s \propto d^2$).

Hutchinson (1967) had introduced a coefficient for the form drag of a particle or aggregate into Stokes’ Law, the effect of this coefficient was clearly applicable to the data obtained. Reynolds (1984) and Waite et al. (1992a) also found that sinking rate of diatoms is proportional to the diameter. Skeletonema costatum is known to be affected by drag via its cell-cell linkage. We suggest that changes in drag can occur as the result of changes in physiological state, the shape of individual cells, cells in aggregates, as well as the shape and porosity of these aggregates.
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I would like to dedicate this thesis to the memory of my father, Guy Meunier, who was inspirational in making me the person I am today.

Daniel Guy Meunier
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1 INTRODUCTION

The distribution of marine phytoplankton and in particular marine diatoms is of major importance to global climate. The changes in distribution of these diatoms are controlled strongly by their sinking rate to depths where they can no longer be active in the process termed the ‘biological pump’. Marine diatoms will sink out of the photic zone of the ocean for several reasons. One of these reasons is nutrient depletion. This removal of photoautotrophic biomass is a major loss to the primary production resource of the oceans, it is also a potential loss of atmospheric CO$_2$ due to the action of the ‘biological pump’.

The ‘biological pump’ describes the removal of CO$_2$ from the atmosphere by means of photosynthesis by marine phytoplankton (including diatoms). The determination of the rates at which these diatoms sink is of great importance in determining the rate of loss of these phytoplankton and their effect on the global climate.

Several laboratory experiments have been developed, in time, to determine the rates of sinking of these microscopic particles, as well as the sinking rates of the aggregates that they form. Methods developed include the SETCOL (Bienfang et al., 1981) and the MARS instrument (Bienfang et al., 1978).

The ‘stickiness’ or rate of aggregation of marine diatoms had been a topic of much research. Several methods have been developed to elucidate this effect. The most effective and least intrusive method of determining the ‘stickiness’ of the particles is by the video analysis of flocculator images (Waite et al., 1997a).

The aim of this thesis was to determine the sinking rates of aggregates formed by nitrogen limited cultures of the marine diatom _Skeletonema costatum_. The sinking rates of individual particles of this species of marine diatom have been conducted previously (e.g. Waite et al., 1997b) but few studies have been done on the larger aggregates formed from laboratory culturing.
Several outcomes are expected of this thesis:

- To determine the applicability of Stokes’ Law of sinking to the aggregates of the marine diatom *Skeletonema costatum*.
- To determine the accuracy and relevance of the new Video Observation method for sinking rate determination.
- To develop a methodology that can be applied to other marine diatoms.

The process by which these aims are achieved and the outcomes of experimentation are outlined in the following sections of this thesis.
2 LITERATURE REVIEW

2.1 Marine Phytoplankton: Diatoms

Diatoms are unicellular plants belonging to the plant class Bacillariophyceae of the phylum Chromophyta (Tomas, 1996). Diatoms dominate the phytoplankton of cold nutrient rich waters, such as upwelling areas of the oceans, and recently circulated lake waters (Graham & Wilcox, 2000). They are ubiquitous, occurring in marine and fresh waters, where they may be planktonic, benthic, periphytic (growing on plant or seaweed surfaces) or epizoic (on animals). Some species are capable of active movement but others depend on currents for transport. Individual diatoms range in size from 2 μm to several millimetres, although there are very few species that are larger than 200 μm. The actual number of extinct and extant diatom species may well be over 50,000 (Tomas, 1996).

2.1.1 Cell Structure

Diatoms are characterised by a skeleton, or capsule, the frustule, which is composed of the epitheca and the hypotheca, that fit together (McConnaughey, 1970) (Figure 2.1.1). These valves are impregnated with silica, which gives them a glasslike character (McConnaughey, 1970). The two theca are held together by what is termed the girdle. The valves are transparent and usually beautifully and symmetrically ornamented with a variety of markings. There are two common shapes; centric and pennate (Figure 2.1.2). The centric diatoms are typically discoid or cylindrical cells that have radial symmetry in face or ‘valve’ view. In contrast, valves of pennate diatoms have more or less bilateral symmetry. Some pennate diatoms possess slits in the frustule – the raphe system – that are associated with the ability to accomplish rapid gliding motion (Graham & Wilcox, 2000).
Figure 2.1.1: Section showing epitheca and hypotheca.

Figure 2.1.2: *Coscinodiscus sp.* an example of a centric diatom. Scale bar = 40 µm. *Achnanthes taeniata* an example of a pennate diatom. Scale bar = 20 µm.

Most diatoms occur singly, but many species form characteristic chains or aggregates of individuals that are held together by protoplasmic junctions, mucilage, or interlocked spiny or hairlike projections from the frustules (McConnaughey, 1970). The shapes are characteristic of different genera and are often quite complex (McConnaughey, 1970). Some shapes such as that of *Skeletonema costatum*, look superficially like filamentous algae (Figure 2.1.3).

Figure 2.1.3: *Skeletonema costatum* a chain forming marine diatom. Scale bar = 4 µm. Cells can be seen as marked, with hairlike protrusions joining them.

**External Cell Structure**

Pores of various sizes and type (areolae) penetrate both valves. These pores are thought to function as passageways for entry into or exit from cells of gases, nutrients and other materials
The pores are usually complex and often have a silicified layer (known as a velum) penetrated by smaller pores or slits stretched over them. Many centric diatoms (and many pennate diatoms without raphe systems) possess a rimoportula, a tubular structure that passes through the valve, ending on the inside in a slit, as if the tube were laterally compressed (Graham & Wilcox, 2000). This structure is known as a labiate process and is associated with polysaccharide mucilage secretion (Medlin et al., 1986). The other type of mucilage secretion structure seen in centric diatoms is the ocellus. This is an elevated plate of silica that is perforated by pores and surrounded by a rim.

**Internal Cell Structure**

The cytoplasm forms a relatively thin lining along the inside walls of the valves, surrounding a vacuole filled with cell sap (McConnaughey, 1970). Within this cytoplasm is the protoplast which contains several other components of the diatom. The nucleus has cytoplasmic strands extending from it to other parts of the cell (McConnaughey, 1970). The cytoplasm, which contains chloroplasts, the sites of photosynthesis (McConnaughey, 1970). The brownish colour characteristic of most diatoms is caused by the pigment diatomin which is present in the chloroplasts (McConnaughey, 1970).

**2.1.2 Photosynthesis and Photorespiration**

Photosynthesis is the process by which algae (and other photoautotrophs) convert the energy of light into the chemical energy of organic molecules (Lawlor, 2001). One of the major factors in controlling atmospheric CO$_2$ and O$_2$ is photosynthetic CO$_2$ fixation and photorespiratory CO$_2$ release by algae (Tolbert & Preiss, 1994). Most organisms now living on our globe depend on the organic matter produced by photoautotrophs (Nielsen, 1975). In the sea, at least in all oceans, phytoplankton are by far the most important vegetation (Nielsen, 1975).

Photosynthesis occurs in the presence of light in a region known as the photic zone. This zone is strictly defined as the deepest depth in which the rate of photosynthesis is greater than the rate of respiration over a daily cycle.

Diatoms have accessory photosynthetic pigments known as carotenoids. This makes it possible for them to carry out photosynthesis over a broad range of wavelengths from 350 to 700 nm.
The large surface area to volume ratio of phytoplankton enables efficient uptake of nutrients (Nielsen, 1975). Free CO₂ is the carbon source for most phytoplankton and as the concentration of this free CO₂ is minute but of major importance in photosynthesis, the small size of phytoplankton confers important advantages (Nielsen, 1975).

Diatoms use ribulose biphosphate carboxylase/oxygenase, or Ribisco for photosynthetic carbon metabolism (Tolbert & Preiss, 1994). The carboxylase reaction initiates gross CO₂ fixation by the C₃ reductive photosynthetic carbon cycle, and the oxygenase reaction initiates the C₂ oxidative photosynthetic carbon cycle for energy and CO₂ loss by respiration (Figure 2.1.5) (Tolbert & Preiss, 1994). These reactions can coexist, but it has been hypothesised that they depend on the concentrations of CO₂ and O₂ (see Tolbert & Preiss, 1994, Graham & Wilcox, 2000).

2.1.3 Reproduction

Diatoms reproduce vegetatively by binary fission, with two new individuals formed within the parent cell frustule (Tomas, 1996). Each daughter cell receives one parent cell theca as epitheca, and the cell division is terminated by the formation of a new hypotheca for each of the daughter cells (Tomas, 1996). This type of division, with the formation of new siliceous components inside the parent cell, leads to size reduction of the offspring (Tomas, 1996).

All diatoms are diploid, with meiosis at the end of the gametogenesis (Tomas, 1996). The zygote develops into an auxospore (Tomas, 1996). In the centric diatoms, sexual reproduction is by oogamy with flagellated male gametes, while most pennate diatoms are morphologically
isogamous and lack a flagellated stage (Tomas, 1996). Relatively little is known regarding the environmental cues that induce diatom sexual reproduction (Graham & Wilcox, 2000).

2.1.4 Diatom Spores and Resting Cells
Spores and resting cells allow diatoms to survive periods that are not suitable for growth, and then germinate when conditions are suitable (Graham and Wilcox, 2000). Unsuitable conditions for growth include periods of nutrient limitation. Both resting cells and spores are characteristically rich in storage materials that supply the metabolic needs for germination (Graham & Wilcox, 2000). Resting cells remain morphologically similar to vegetative cells, whereas the frustules of spores become very thick and exhibit less elaborate ornamentation than vegetative cells. Spores are believed to be capable of surviving for decades in benthic sediments. The spores of marine diatoms often occur in mucoid aggregations (marine snow), and are important in transporting organic carbon and silica to the sediments (Graham and Wilcox, 2000).

2.2 The Biological Pump and the Global Carbon Cycle

The increasing levels of atmospheric CO₂ are of global concern to many research disciplines from geology and ecology to the social sciences (Tolbert & Preiss, 1994). A major factor in controlling atmospheric CO₂ and O₂ includes photosynthetic CO₂ fixation and photorespiratory CO₂ release by algae (Tolbert & Preiss, 1994). This process of removal of free CO₂ from the atmosphere and the ocean is commonly called the ‘biological pump’ (Figure 2.2.1). Second only to water vapour in its importance as a heat trapping or ‘greenhouse’ gas, CO₂ in the atmosphere is largely maintained by exchanges with the much larger oceanic reservoir (Figure 2.2.2) (Norse, 1993, Tolbert & Preiss, 2000, Lawlor, 2001).
CHAPTER 2  LITERATURE REVIEW

Figure 2.2.1: Schematic diagram of the biological pump in the ocean.

Figure 2.2.2: Simplified scheme of the main pools and fluxes of carbon between the atmosphere, biosphere and geosphere. The size of each is given in gigatonnes of carbon (GtC). The fluxes are also in GtC. The CO2 concentration in the atmosphere is shown as volumes per volume (equivalent to volume parts per million (vpm)) or as partial pressure (Pa) (Lawlor, 2001).
CHAPTER 2  LITERATURE REVIEW

The surface waters of the world’s oceans contain less dissolved carbon than deep waters. This vertical gradient is produced by phytoplankton in the photic zone (Norse, 1993), which take dissolved carbon out of solution by photosynthesis (Norse, 1993, Lawlor, 2001). As a result of several different factors (Section 2.3.2) the organic tissue of these diatoms sinks into deeper waters or to the ocean floor, where it decomposes (Norse, 1993). The process of photosynthesis followed by photoautotrophic sinking therefore pumps carbon from the surface to the deep ocean.

The total flow of fixed carbon downward from the surface waters of the global ocean is still poorly known (Norse, 1993). The global carbon cycle is a good representation of the significance of this ‘biological pump’ (Figure 2.2.2). Some of the most productive regions of the sea, where the pump is working ‘hardest’, include the upwelling areas of continental shelves and slopes, and the upwelling areas in the open ocean associated with wind driven divergences (Norse, 1993). Although the oceans’ primary producers are very unlikely to die en masse, the Greenhouse Ocean of the future is likely to be less productive than today’s, just as today’s ocean is known to be less productive than the ocean during the glacial periods (Norse, 1993).

2.3 Phytoplankton Sinking Rate

As has been previously discussed the vertical flux of particulate carbon in the ocean is an important parameter in the global carbon cycle and of potential significance to long-term changes in atmospheric CO₂ (e.g. Tolbert & Preiss, 1994).

This vertical transport is a sink of atmospheric CO₂ (e.g. Tolbert & Preiss, 1994, Graham & Wilcox, 2000). A better understanding of the extent of this sink, in terms of volume of carbon removed from the atmosphere, and the mechanisms and processes that control it, would be of great benefit to current and future attempts to avert global warming (Tolbert & Preiss, 1994). One process associated with this sink that has been studied for some time now is the sinking rate of the phytoplankton and in particular marine diatoms (e.g. Beinfang et al., 1982, 1983 & 1984). The sinking rates of many phytoplankton species and natural assemblages have been measured in previous studies (Smayda, 1970, Walsby & Reynolds, 1980, Beinfang, 1981, Bienfang et al., 1982, Bienfang & Harrison, 1984). These studies reveal a wide range of sinking rates, from positive buoyancy in the dinoflagellate Noctiluca to 30m/day for the large diatom Coscioidiscus wailesii (Smayda, 1970). However, Smayda (1970) suggests, that under certain circumstances,
much higher sinking rates are possible. The study of the sinking rates of different species of phytoplankton \textit{in situ} and in laboratory experiments, has resulted in more precise estimates of the effectiveness of this carbon sink and the factors that control it.

The sinking velocity of a falling sphere was originally described by Stokes’ Law and is applicable for Reynolds numbers less than one (John & Habermann, 1980). The Reynolds number is the ratio of inertial forces to viscous forces (Streeter, 1971), and is described by the equation:

\[
\text{Re} = \frac{\rho_w Ud}{\mu}
\]

\textit{Equation 2.3.1}

Stokes’ Law defines the terminal sinking velocity of a sphere through a fluid that is otherwise at rest (Streeter, 1971). Stokes’ Law is described by the following equation:

\[
w_s = \frac{d^2 (\rho_p - \rho_w)g}{18\mu}
\]

\textit{Equation 2.3.2}

Since phytoplankton are living and are often irregularly shaped, relative to a sphere, Hutchinson (1967) modified Stokes’ Law to adjust sinking rates for the proportionality or shape factor of nonspherical phytoplankton cells (Jassby, 1975). This modified form of Stokes’ law is:

\[
w_s = \frac{d^2 (\rho_p - \rho_w)g}{18\mu \phi}
\]

\textit{Equation 2.3.3}

Where:

- \(w_s\) is the sinking velocity in m s\(^{-1}\).
- \(g\) is the gravitational acceleration of the earth (9.8 m s\(^{-1}\)).
- \(r_s\) is the radius (m) of a sphere of volume equivalent to that of the algal cell.
- \(\rho'\) is the density of the algal cell (kg m\(^{-3}\)).
- \(\rho\) is the density of the fluid medium (kg m\(^{-3}\)).
- \(\mu\) is the viscosity of water (kg m\(^{-1}\) s\(^{-1}\)).
\( \phi \) stands for the proportionality or shape factor and is dimensionless.

Phytoplankton that sink rapidly require more rapid vertical mixing to remain in the water column than do cells that sink slowly (Graham & Wilcox, 2000). Phytoplankton have a number of adaptations to reduce sinking rates (Graham & Wilcox, 2000):

- Small cell size, to allow for easier transport by currents.
- Larger diatoms have a reduced surface-to-volume ratio compared to smaller diatoms. This means that they have proportionally less siliceous frustule material and are thus less dense than small diatoms. Some marine diatoms such as species of *Rhizosolenia* and *Ethmodiscus* can be positively buoyant (Graham & Wilcox, 2000).

### 2.3.1 Sinking Rate Measurement

Several methods had been employed in the past to measure the sinking rates of marine diatoms. These methods can be broadly classified into two categories, depending on the initial distribution of the sample in the settling chamber (Bienfang & Laws, 1977):

1. The discrete sample layer (DSL) method (e.g. Eppley et al., 1967).
2. The homogeneous sampling (HS) method (e.g. Titman 1975).

An analysis of technical and theoretical problems associated with the measurement of phytoplankton sinking rates by these past methods indicates that they are both inaccurate and imprecise (Bienfang & Laws, 1977).

More recently several new instruments have been developed that have improved the accuracy of measuring phytoplankton sinking rates. These include the MARS instrument, the SETCOL method, and the newly developed videography method. All of these methods have shown a marked improvement in accuracy in comparison to the older methods.

**The MARS instrument**

The MARS instrument was developed as an improvement on the past methods described above. The procedure involves detecting the transit time of radioactive \( ^{14}\mathrm{C} \) labeled cells through a settling column (Bienfang & Rothwell, 1978). A multichannel assembly for radio-plankton sinking (MARS) instrument is used to detect the beta radiation emitted by the cells. The MARS
The SETCOL method
Bienfang (1981) also developed the SETCOL method. The SETCOL method was developed further by Waite et al (1992a) (Figure 2.3.1). The method developed by Waite et al (1992a) utilises solely chlorophyll a concentrations instead of a combination of cell counts and chlorophyll a concentrations, as was used by Beinfang (1981). This development measured final concentrations of all SETCOL fractions (top, middle, and bottom fractions of the column) and used these fractions to determine a mean sinking rate for a given culture (Waite et al., 1992a).

The equation used to determine sinking rate by this method is:

\[
ws \, (\text{m/day}) = \frac{[(Vol_{\text{bottom}} \times Chl[a]_{\text{bottom}}) - (Vol_{\text{bottom}} \times AveChl[a])] \times Length_{\text{cell}}}{TotalVol \times AveChl[a] \times Time_{\text{settle}}} \]

Equation 2.3.4

Where:
\(Vol_{\text{bottom}}\) is the volume drawn from the bottom outlet of a given SETCOL tube.
\(Chl[a]_{\text{bottom}}\) is the chlorophyll a concentration of the volume drawn from the bottom outlet of a given SETCOL column.
\(AveChl[a]\) is the average chlorophyll a concentration over the length of a given SETCOL tube. This average is determined by summing the chlorophyll a concentrations for the top, middle and bottom volumes drawn from a given tube and dividing this value by three.
\(TotalVol\) is the total volume drawn from a given SETCOL tube, i.e. the sum of the top, middle and bottom volumes drawn.
\(Length_{\text{cell}}\) is the length of the given SETCOL tube.
\(Time_{\text{settle}}\) is the time over which the SETCOL is left to settle before the three volumes are drawn off.

The columns of the SETCOL instrument are filled with the sample, stirred and then left in the dark for a given time to allow settling. After the settling time volumes are drawn off at the top middle and bottom of each column. The volumes drawn off are then processed to determine
chlorophyll $a$ concentrations. The values obtained are then substituted into Equation 2.3.4 giving a bulk sinking or ascent rate for the sample.

**Figure 2.3.1:** SETCOL instrument as developed by Waite et al (1997). Consisting of four symmetric columns (1, 2, 3 & 4) immersed in a water bath, with outlets for the columns at the top, middle and bottom.

**Video Observation**

This method is a new development that employs video images to determine the sinking rate and size of phytoplankton. The method uses the same concept of non-disruptive observation of a simulation of phytoplankton dynamics as used by Waite et al (1997).

The system used here is slightly different to that seen in the flocculation observation method (Section 2.4.3). The camera still uses the same objective of 20x but due to the lower speeds of the moving particles the shutter speed is reduced (Figure 2.3.2).
2.3.2 Factors Affecting Sinking Rate

Phytoplankton sinking rates are dependent upon both physical and physiological phenomena (Munk and Riley, 1952, Smayda & Boleyn, 1966a, b, Hutchinson, 1967, Eppley et al., 1967, Smayda, 1970, and others). Both decreasing turbulence levels and nutrient deficiency are known to greatly increase sinking rates of diatom cells (Bodungen et al., 1981). In the ocean nitrogen limitation commonly occurs at the end of a phytoplankton spring bloom (Waite et al., 1992b). Limitation may also occur from deficiency of phosphorus, silica, iron or other micronutrients.


2.4 Aggregation

The existence of particle aggregates centimetres in size in the oceans, known as ‘marine snow’, has been acknowledged for several decades (Kiørboe et al., 1990). These aggregates typically consist of inorganic particles, detrital organic particles, biological particles as well as microorganisms (Alldredge et al., 1988). Diatom aggregation can affect the sedimentation rate of the intense pulses of carbon formed during diatom blooms (Waite et al., 1997). Flocculation of phytoplankton cells into large ‘marine snow’ aggregates with enhanced settling velocities has been invoked as a mechanism to explain mass sedimentation events following diatom blooms in the ocean (Kiørboe et al., 1993). The association of phytoplankton with marine snow is also
considered to be a mechanism for their rapid removal from a nutrient depleted environment (Smetacek, 1985).

This process can terminate a bloom before nutrients are fully depleted at the surface, and enhance the ‘biological pump’ process. If the depths to which these aggregates sink are large, the particulate carbon in such aggregates will not be returned to the atmosphere for long periods of time, as shown by Tolbert and Preiss (1994). Aggregation also changes the availability of food to grazers, especially those with size preferences for their prey as shown by (Rubenstein & Koel, 1977). Therefore, the dynamics of aggregates associated with marine diatoms are of major importance to the fate of diatom carbon in coastal ecosystems (Waite et al., 1997) and to primary productivity.

2.4.1 Causes of Aggregation: Observations

Aggregation has been monitored both in the laboratory and in the field. In the field aggregates are commonly referred to as marine snow, which can consist of material other than a specific phytoplankton species. Observations in the field and laboratory have led to the formulation of several different hypotheses on the conditions required to cause such aggregation. The main premise of these theories is that the rate of formation of these aggregates depends on the rate at which single cells collide (Kiørboe et al., 1990).

2.4.2 Formation of Aggregates

The rate of formation of phytoplankton aggregates depends on the rate at which single cells collide (Kiørboe et al., 1990). This process is considered to be mainly physically controlled, and depends on the probability of adhesion upon collision (Kiørboe et al., 1990). This probability is in turn dependent upon physio-chemical and biological properties of the cells (Kiørboe et al., 1990). In nature these factors can occur as the result of different processes.

Diatom Blooms

Seasonally high nutrient concentrations often result in algal blooms with high biomass and particle concentrations (Jackson et al., 1992). After such blooms flocs have been observed to form (e.g. Alldredge et al., 1988). This is due to the resulting low nutrient levels at the bloom site and the induction of resting states of the phytoplankton or mucilage excretion, which may cause the cells to ‘stick’ together and sink out of the water column (Graham & Wilcox, 2000).
Settling of Particles and other particulate matter

Aggregates have also been observed as they sink in the water column, increasing their average size with increasing depth (Kranck & Milligan, 1988).

Nutritional state

Laboratory studies with algal cultures have shown that algal cells do indeed, have a finite chance of sticking when they collide, and that this probability can change with nutritional state of the algae and the algal species (Kiørboe et al., 1990).

2.4.3 Aggregation Quantification Methods

Several methods have been developed to determine the rate of aggregation of phytoplankton. Past methods have been inaccurate in their ability to estimate ‘stickiness’ and aggregate size. New methods, however, have shown increased accuracy of the measurement of cell stickiness and aggregate size as a result of minimizing, and even preventing completely, the disturbance of aggregates formed.

Past Methods

Typically, aggregation rates are quantified by observing phytoplankton cultures or field samples in a cylindrical device known from the sanitary engineering literature as a flocculator (Waite et al., 1997). This device generates a known laminar shear, and thus a known number of particle collisions per unit time, for a given cell diameter and density. The collision and attachment of cells in the flocculator results in changes in particle size distribution, which are quantified by regularly sub sampling the spinning flocculator over short periods of time (< 1hour). From the data acquired, one can quantify important aspects of aggregation such as the probability of attachment between cells (stickiness, or $\alpha$).

Disruption of aggregates has been observed to occur as a result of sub sampling and electronic particle counting submitting suspensions to the high shear in the flocculator. This disruption is difficult to predict because it is likely to be highly variable and species specific (Waite et al., 1997). Cultures forming tight aggregates may be less susceptible to disruption than species
forming loose aggregates. The accuracy of past aggregation measurements is potentially in doubt (Waite et al. 1997).

Other methods of measuring stickiness have been attempted, but they involve either manual quantification of individual aggregates or adhesion to artificial surfaces like glass or glass beads.

**Recent Methods**

Most recently video has been employed to study plankton populations in the ocean. This is a non-invasive technique, as it uses optical sampling. This technique of non-invasive optical sampling led to the creation of a modified flocculator with a video analysis system fitted to it, as developed by Waite et al. (1997) (Figure 2.4.2). Quantitative videography allows for measurement of particle size and number, non-disruptively, for a wide range of particle sizes and shapes (Waite et al., 1997). This method also increases possible resolution of aggregate measurements, and yields images of the actual particles from which we can observe aggregate shape and porosity.

![Figure 2.4.1: Schematic of flocculation device and video set up.](image)

**Analysis of Aggregation**

There are several calculations applicable to the analysis of aggregation. In the case of the new video analysis method the determination of cell stickiness (α) and cell size are most important.

**Calculation of Cell Size**
The shape of cells has commonly been assumed to be spherical. This has been done in order to gain a particle diameter measurement from a cross sectional area (Waite et al. 1997). The particle diameter \( d \) can therefore be calculated from the following equation:

\[
d = 2(X/\pi)^{1/2}
\]

*Equation 2.4.1*

Where \( X \) is the cross sectional area.

The cross sectional area of the cells is determined by video analysis.

**Calculation of Stickiness**

The video analysis of aggregation uses the method of determining stickiness developed by Kiørboe et al (1990). This method depends on the increase in mean particle size over time as cells collide and adhere to each other within the flocculator, according to the formula:

\[
\alpha = \left\{m \cdot \pi \cdot \exp\left(s^2 / d^2\right)\right\}/\left(7.824 \cdot V \cdot S\right)
\]

*Equation 2.4.2*

Where \( \alpha \) is stickiness, \( m \) is the slope of the natural log of particle diameter (\( \mu m \)) versus time (s), \( s^2 \) is the variance in the size distribution of the particles (\( \mu m \)) at \( t=0 \), \( d \) is the mean particle diameter (\( \mu m \)), \( V \) is the volume fraction of particles at \( t=0 \) (ppm), and \( S \) is the mean shear rate (s\(^{-1}\)). This simplification is based on the assumption that any increase in mean particle size is caused only by the collision and adhesion of single cells, forming doublets (Kiørboe et al., 1990).

**Applicability of the Kiørboe et al (1990) Method**

Waite et al (1997) tested the assumption of Kiørboe et al (1990) that any increase in mean particle size is caused only by the collision and adhesion of single cells, forming doublets using the video method they developed and two simple calculations. The distribution of nearest neighbour distances (NNDs) within a culture allowed Waite et al (1997) to estimate the number of cells packed in aggregates. NNDs were calculated by video analysis. They were then able to compare the mean cell size in an experiment with one expected if all aggregates were doublets, as assumed by the method of Kiørboe et al (1990).
The method of Kiørboe et al (1990) was proven to be applicable in determining stickiness for the initial stages of flocculation. Stickiness does not increase with increasing aggregate size, but only with increased shear or increased physiological stressing of the diatom. Therefore, for a given culture, the stickiness value determined from the early stages of flocculation is valid for all time.
3 METHODOLOGY

The starting point of experimentation was the culturing of *Skeletonema costatum* for several generations\(^1\) to obtain a consistent growth rate (determined by fluorescence readings). Once this had been achieved, and whilst this ‘healthy’ culturing was being continued, a range of volumes of inoculum required to cause nitrogen limitation\(^2\) in a following generation were determined. The volumes established allowed for the creation of two replicate 20 litre nitrogen limited cultures, limited to the same extent. The growth of the replicate cultures were monitored by daily fluorescence, cell count, and chlorophyll \(a\) concentration measurements, this was done to ascertain a growth rate. Sinking rates were determined for both aggregated and non-aggregated volumes of each replicate by means of videography and SETCOL (Beinfang, 1981). These sinking rate measurements were performed on specific days along the growth curve of the cultures, once during the log phase of growth, at the end of log phase/commencement of stationary phase, and deep into stationary phase (Figure 3.1.1). The determination of TEP, as an aggregation parameter, was also performed on these days.

![Figure 3.1.1: Points along the growth rate curve where major sinking rate and aggregation experiments were performed.](image)

\(^1\) A new generation is created when sterile media is inoculated with a volume of the previous culture, this is done during the log phase of growth of the culture used for inoculation.

\(^2\) Nitrogen limitation is the result of a lack of nitrogen in a new generation.
3.1 Culturing

The preparation of media, its constituents and its disinfection are of major importance so as to maintain the validity of testing done on replicate cultures. The conditions under which a culture is grown are of great importance to its health and consistency of growth, and extreme changes in temperature and light conditions can have severe physiological effects on a culture (Bienfang et al., 1982b, Bienfang et al., 1983). Several generations of *Skeletonema costatum* where grown until consistency was achieved in the growth curves (determined by fluorescence readings) of these cultures. The cultures were grown in G2 media (APPENDIX II). The culture generated for aggregate sinking rate testing was limited in nitrogen so as to stress the diatoms. This stress was expected to result an increase mucilage secretion by the cells, which in turn would produce aggregates when a shear was introduced on the roller table (Section 3.2.1). Both aggregated and non-aggregated volumes were analysed for individual (Video) and bulk (SETCOL) sinking rates.

3.1.1 Inoculation

Inoculation of all generations was done in a Laminar Flow Hood. The prepared media and the previous cell generation were placed in the hood and treated with UV light for approximately five minutes to kill off any introduced bacteria. Items coming into contact with one another were either flamed (such as test tubes and conical flasks) or had previously been autoclaved and stored to avoid contamination. The inoculation of new generations was done during the log phase of growth to ensure the health of the *Skeletonema costatum* cultures.

3.1.2 Culturing Conditions

The conditions, such as light and temperature, under which the cultures were grown, were controlled so that the growth of the cultures remained constant. Due to the different sizes of the culture vessels the generations grown prior to the inoculation of the 20 litre culture vessels were treated to slightly different conditions, with respect to light and temperature. The ‘healthy’ generations were growth in small 50 mL test tubes as well as three litre conical flasks. The light and temperature conditions of the different culturing vessels could be assumed to be similar so that the effect of the change in these conditions on the cultures would be negligible. The two larger vessels were both stirred with magnetic stirrer bars, the 50 mL vessel, however, was not. Comparison of the three liter and 50 mL cultures fluorescence levels was used to quantify any discrepancies in growth rates due to turbulence, the effect of this was seen to be minimal.
Culture Media
The cultures were grown in a G₂ media prepared as described in Appendix II. Prior to inoculation of a new generation, the media was autoclaved to ensure disinfection. The source of phosphorus was autoclaved separately and added prior to the inoculation of a new culture as it would have precipitated if autoclaved with the other constituents. The addition of the phosphorus was done in a Laminar Air Flow Hood with flaming of all contact surfaces. This was done to avoid infection of cultures and the introduction of unwanted material.

Light Regime
The light regime for culturing consisted of sixteen hours of daylight (culture lighting on) followed by 8 hours of darkness (culture lighting off). This was achieved by the use of a timing mechanism that turned the lights on and off when required (Figure 3.1.3). The lighting system for culturing of the ‘healthy’ generations and for the replicate 20 liter carboys consisted of four evenly spaced Thor 36 Watt white colour lights on racks either side of the vessels (Figure 3.1.4).

Figure 3.1.2: The timing device used to turn the lighting on and off.
**Figure 3.1.3:** The lighting system for culturing. The two replicate 20 litre carboys can be seen as well as the 50 mL test tubes. During culturing the second rack is positioned in front of the two carboys.

The light levels for the cultures were measured with a LI-COR Integrating Quantum/ Radiometer Photometer. By changing the sensor the light meter could be used in air or submerged under water. The light levels in and around the full 20 liter vessel were then measured. Several measurements at different positions were done, these positions were (Figure 3.1.5):

A: Submerged in the centre of the 20 liter vessel. The probe was pointing downwards during this test and the light source was perpendicular to this.

Outside the carboy, two points were taken for measurement.

B: Against the edge of the carboy, nearest to one of the light sources.

C: Against the edge of the carboy furthest from the two light sources.
Figure 3.1.4: Positions of light meters for light level measurement.

The external light levels were then measured with a DSE Q-1400 Digital Lux Meter to determine the accuracy of the LI-COR Photometer.

Table 3.1.1: The results of the LI-COR Photometer and the Digital Lux Meter. Using the conversion rate of 1 klux = 12 µE.

<table>
<thead>
<tr>
<th>Position</th>
<th>LI-COR</th>
<th>Lux Meter</th>
<th>Converted Lux Meter</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>150µE</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>B</td>
<td>380µE</td>
<td>30 klux</td>
<td>360µE</td>
</tr>
<tr>
<td>C</td>
<td>200µE</td>
<td>17 klux</td>
<td>204µE</td>
</tr>
</tbody>
</table>

The light levels experienced by the cultures were assumed to be accurately measured by the LI-COR Photometer.

The Effects of Changing Light Levels

Maintaining consistent light levels to a culture is one of the most important means of attaining consistency in growth rate. The effects of changing the irradiance under which a culture grows can severely affect the consistency of experiments performed on such cultures. This has been
shown by Bienfang (1983), who proved that growth at low irradiance results in physiological changes, which are manifested as changes in population buoyancy. Species within a culture adapt to a given light regime if it is consistent, and will show consistent growth.

**Reasons for Changing Light Levels**

There are several reasons why the light levels within a culture may change. Firstly, as a culture grows the biomass increases, and this increase may change the colour of the culture medium. Light levels within the culture can also be affected by reflection and shading. It is therefore vital to monitor the light levels within a large culture to ensure growth rate is not affected. Secondly, coagulation can have an effect on light levels. Coagulation can be thought of as the process by which small particles are converted into larger particles (Jackson et al., 1992). Aggregates cause shading, which affects light levels received by individual diatoms and the aggregates themselves. This effect would not be expected to occur in a 20 litre culture, due to the action of the magnetic stirrer rod. Thirdly, the simulation of day and night is a common practice in culturing techniques. Light levels within a culture room can be set by a timer that switches the lights on and off at given times. This diurnal change in light levels, if kept constant, will not affect a culture because of adaptation between light and dark. The temperature changes that occur as a result of this change in light level could, however, have an effect on the growth of a culture (Bienfang et al., 1982b).

**Temperature Conditions**

Due to the heating action of the light and the cooling action of the culture room temperature changes in the cultures can be expected. The temperature conditions within the cultures were monitored over a two-day period to determine any diurnal changes. To measure this the StowAway® TidbiT® water temperature logger was used. The result of this monitoring showed a diurnal temperature change of approximately 2°C. Due to the adaptive nature of *Skeletonema costatum* (Mortian-Bertrand et al., 1988) with successive generations this effect was considered negligible. These diurnal temperature changes are also a normal part of life in a water body, due to the heating action of the sun.

**Mixing Conditions**

Mixing conditions within a culture solution can have considerable effect on the growth of the culture. The two larger vessels were well mixed by magnetic stirrer rods. The result of stirring the
larger 20 litre vessel with a nitrogen limited culture could be the occurrence of unwanted aggregates forming in the vessel (Figure 3.1.5). Simply having the stirrer speed low enough was expected to avoid this effect.

![Fluid motion in large 20L carboy.](image)

**Figure 3.1.5:** Fluid motion in large 20L carboy.

### 3.1.3 Nitrogen Limitation

Stress was induced by nitrogen limitation, this was expected to cause the *Skeletonema costatum* to exude a mucus or other exopolymeric material, and when a shear was induced to create aggregates, which would then be observed for sinking rate and size.

**Determination of Nitrogen Limitation Levels**

To create a nitrogen limited generation the previous generation is added to G2 media, this new media has not had the limiting substrate (nitrogen) added. The only supply of nitrogen is what remains dissolved in the media of the previous generation. Several different volumes of a previous generation were used to determine a range of different levels of nitrogen limitation attainable. The raw fluorescence (fsu) of these cultures was monitored. A drop in the maximum fluorescence attained by these limited cultures in comparison to the previous ‘healthy’
generations value would indicate growth limitation of the culture, provided other conditions in which the cultures are grown do not drastically change.

The culture to be used had been grown for several generations and had shown a consistent growth rate of 1.3 day\(^{-1}\). Four different volumes of the ‘healthy’, non-limited, culture were added to 50mL of G\(_2\) medium that had no nitrogen added to it. This represented four different reduced concentrations of nitrogen. The volumes added were 0.5mL, 1mL, 2mL, 4mL. The fluorescence levels of all four cultures were then measured daily.

All four cultures showed an incremental level of growth limitation (Figure 3.1.6). Therefore the volume of the previous generation to be added to the 20 liters of G\(_2\) media (without nitrogen) could be determined depending on the level of limitation required.

**Figure 3.1.6:** Growth curves of limited cultures, as measured by raw fluorescence.

**Inoculation of Nitrogen Limited Culture**

The two 20 litre carboys containing the G\(_2\) media (without nitrogen) were inoculated with 800mL of ‘healthy’ culture. The ‘healthy’ culture was at the same stage of growth as in the nitrogen limitation determination experiment. The volume of 800mL was intended to simulate the addition of 2mL of ‘healthy’ culture to 50mL of G\(_2\) media (no nitrogen).
3.1.4 Replication and Inoculation of 20L cultures

The two 20 litre carboys were inoculated with 800mL each of the previous generation’s culture. This was to achieve a form of replication so that results obtained from experiments could be compared. Replication is the best means by which to validate results of such experiments.

3.1.5 Growth Rate Measurement

Several methods were used to monitor the growth rate of the two replicate cultures. These procedures and measurements were performed daily at similar times of the day over the course of the experiment. The equation typically used to define the growth rate curve of marine phytoplankton is, defined by Graham & Wilcox (2000) as:

\[
\frac{dN}{dt} = rN \left(\frac{K - N}{K}\right)
\]  

\textit{Equation 3.1.1}

where K is the carrying capacity of the environment (same units as N).
N is the number of cells, the raw fluorescence, or the chlorophyll a concentration.
r is the growth rate (day\(^{-1}\)).
t is time in days.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{growth_curve.png}
\caption{Graphical representation of Equation 3.1.1.}
\end{figure}

This equation includes a carrying capacity of the environment (K), which defines the maximum biomass obtainable as a result of limiting factors such as a nutrient deplete environment. The exponential section of this curve gives the growth rate according to the following equation:

\[
\ln \frac{N}{N_0} = rt
\]  

\textit{Equation 3.1.2}
The variables are the same as those in Equation 3.1.1 except \( N_0 \) is the number of cells, the raw fluorescence, or the chlorophyll \( a \) concentration at the start of the logarithmic section of the curve.

**Coulter Counter Cell Count**

The coulter counter gives an accurate measurement of the distribution of individual cell sizes. Other outputs from this device are mean cell size, standard deviation of size and median population statistics. The aperture used to measure the cultures was 200\( \mu \)m. Three size ranges were used every time the test was run in order to ensure that all particles were counted, or to determine if the population cell size distribution was changing. The size ranges used were 5 to 15\( \mu \)m, 4 to 12\( \mu \)m, and 2 to 6\( \mu \)m.

**Fluorescence**

The raw fluorescence (fsu) was measured with a Turner Designs TD-700 Fluorometer on a daily basis over the course of the experiment for both replicates. This was to certify the results of the other growth rate monitoring methods.

**Chlorophyll \( a \) Concentration**

50mL of each replicate culture was filtered through with a Whatman Glass Microfibre Filter (GF/C) daily. The filters were then wrapped in foil and frozen for subsequent processing to determine the chlorophyll \( a \) concentration by the method described in Appendix III.

**3.2 Aggregation**

Volumes of both replicates were aggregated on a roller table for approximately 120 minutes at 6 revolutions per minute. The aggregate volumes formed were tested for sinking rates using the SETCOL and video methods. TEP measurements were taken as a measure of the amount of exopolymeric material exuded by the *Skeletonema costatum*.

**3.2.1 The Roller Table**

2 litre SCHOTT bottles were placed on the roller table for approximately two hours at a time. The roller table spun the bottles on their axes at 6 revolutions per minute so as to create a shear within the fluid. This imposed shear was expected to cause the particles in solution to collide with one another and form aggregates (Figure 3.1.8).
Figure 3.1.8: Roller table with 2 litre SCHOTT bottle rotating on top. The creation of a shear within the bottle can be seen with maximum velocity at the sides of the bottle and minimum velocity in the centre.

3.2.2 TEP Measurements
TEP stands for transparent exopolymer particles, the occurrence of such particles has been postulated to occur in diatom cultures when they are ‘stressed’. Changes in the level of TEP were monitored daily for both replicates to determine its effect on sinking rate, if any. The semiquantitative colorimetric method has been employed in this case. The TEP is first stained with alcian blue. The dye complexed with TEP is then redissolved and measured spectrophotometrically. This process was performed on a daily basis.

3.3 Sinking Rate Measurement

As has been explained, two methods were employed to determine the sinking rates of aggregated and non-aggregated volumes of both replicate cultures. These were the SETCOL and the Video Observation method.

3.3.1 SETCOL
The SETCOL method described in section 2.3.1 provides a value for the bulk sinking rate of both aggregated and non-aggregated volumes of the replicates. For each non-aggregated and
aggregated volume, from each replicate, two SETCOL columns were used as a form of replication. All SETCOL experimentation was performed in the dark in a cooled room.

3.3.2 Video Observation

This method was introduced in section 2.3.1. This method was used to determine the sinking rates of both aggregated and non-aggregated volumes of the replicate 20L cultures.

The experiment is set up as described in Figure 2.3.2. Culture volumes are diffused into the square column (Figure 3.3.1). Once the column is full all other lighting is turned off and the light source is turned on. The camera, with microscopic objective, is focused on the illuminated plane within the culture media. The camera is not adjusted after this stage. Video recording is started and concludes after approximately 1.5 hours.

![Figure 3.3.1: Schematic view of column into which the culture is diffused and the light source. The camera would be positioned looking into the column from this position.](image)

After the recording of the illuminated sinking particles a micrometer is placed in the column so that the light beam illuminates it. This image is then recorded on video. This recording is used for the calibration of particle sizes and sinking rates.
Calibration
Before the sinking rate and size are determined a calibration factor must be determined. The calibration factor defines the number of pixels separating ten lines of the micrometer. This value is then used to determine particle sizes and sinking displacements. To determine this calibration factor a frame of the recording of the micrometer is ‘grabbed’, this ‘grabbed’ image is saved as a ‘tif’ file. To read the image from the ‘tif’ file to a MATLAB image a MATLAB m-file (a program file) is used (Appendix VI) (Figure 3.3.2). Once the image of the micrometer has been generated in MATLAB the calibration m-file can be run (Appendix V). However, several variables must be defined in MATLAB, first:

- The spacing between the lines of the micrometer are 0.1 mm. This is defined in MATLAB by the statement `unit=0.1`.
- The number of lines of equal separation to be used to determine the size of the screen are then defined in MATLAB, by the statement `[x,y]=ginput(n)`, where n is the number of lines to be used.

![Figure 3.3.2: Example of MATLAB image of micrometer, showing 15 lines to be considered in the calibration (lines 1 and 15 are labeled). The x and y lengths are shown.](image)

After these variables have been defined the ‘n’ number of lines are selected on the image by ‘clicking’ on them with the pointer. The calibration m-file program is then run.
The output of this program is two x and two y values. The average of the two y values can be assumed to define the number of pixels separating ten lines of the micrometer, i.e., 1mm. Two y values are given due to the slight inaccuracy of visually selecting the exact positions of the lines of the micrometer (Figure 3.3.2). The size of the image is considered to be square and so the number of pixels per millimeter in the horizontal direction is considered the same as that in the vertical direction (y value).

The calibration value is determined for each video due to any slight changes in the distance from which the camera observes the particles, as this will have changed the field of view. The use of this calibration factor is described below.

**Determination of Particle Velocity**

As with the micrometer footage, frame ‘grabs’ of the sinking chains and aggregates are taken, but in this case a series of images are ‘grabbed’ at equal time intervals. Once these images are grabbed the ‘trail’ image of a sinking particle can be created. This is done by converting the ‘tif’ images and then overlaying these images on one another, this can be done by the same m-file program that converted the micrometer image (Figure 3.3.3). The m-file for converting the images also makes the background black and the illuminated particles white.

**Figure 3.3.3:** An example of the image created when overlaying frames. The real image has been cropped from the sides.
The image of the trail of the particle can then be analysed to determine the size (mm$^2$) and the sinking rate (mm s$^{-1}$) of the given chain or aggregate. Both size and sinking rate are determined by running the same MATLAB m-file program (APPENDIX VI). To determine sinking rate several variables must be defined for the given trail in the MATLAB m-file program:

- The time step between ‘grabbed’ images must be defined (dt).
- The calibration factor must be defined (mmpx), as determined previously.

Once these variables have been adjusted for a given trail the number of particles in the trail must be defined. In MATLAB define \([x,y]=	ext{ginput}(n)\), where \(n\) is twice the number of particles in the trail. On the trail image the top left and bottom right of each particle in the trail are selected with the pointer, down through the series of particles (Figure 3.3.4). The m-file program for sinking velocity and size is then run and the velocity of the sinking particle is given (mm s$^{-1}$).

The velocity of the particle is determined by the change of the displacement of the particle from the top of the trail to the bottom over the given time period (dt) (Figure 3.3.4).

**Figure 3.3.4:** An example of the points used to determine sinking rate (green circles), and the total displacement. The edges of the green box surrounding the aggregate are the limits of the area within which the size of the aggregate is determined. This image total has been cropped.
3.4 Particle Sizes

The determination of the individual particle sizes was performed on the video images obtained with the use of a prepared MATLAB m-file program. Particle size distributions within the aggregated and non-aggregated volumes were determined by the Counter Counter instrument.

3.4.1 Coulter Counter
The Coulter Counter was used to determine the particle size distributions for both the SETCOL and Video Sinking Rate methods. All three size range tests were performed to allow for possible population size changes, as described in section 3.1.5.

3.4.2 MATLAB
The size estimation of specific *Skeletonema costatum* chains and aggregates was performed by the same MATLAB m-file program and process as used to determine sinking rate. When determining size the Central Limit Theorem is employed. There are slight differences in the size of a particle in a trail due to the application of light intensity cut-offs. As a result of these slight differences from particle to particle within a trail the range of sizes measured is assumed to be part of a normal distribution.

Initially an area ($x$) for each particle in a trail is determined, based on the assumption that each particle is defined by the area taken up by white pixels which have an intensity greater than that of the background (black).

A distribution of the particle sizes due to the selection of multiple particles in a trail is then analysed. This analysis is based on the central limit theorem\(^3\). Firstly a standard deviation ($\sigma$) of the approximated particle sizes ($x$) is determined. Then a standard error ($\sigma_x$) based on the standard deviation and the number of particles sampled ($n$) is determined, based on the relationship $\sigma_x = \sigma / \sqrt{n}$.

\(^3\) The central limit theorem states that if a random sample of $n$ observations is selected from a population, then when $n$ is sufficiently large, the sampling distribution of $x$ will be approximately a normal distribution.
The z-statistic gives the distance between the estimated particle mean size and the actual particle mean size, in units equal to the standard deviation:

\[ z = \frac{x - \mu}{\sigma_x} \]  

*Equation 3.4.1*

From this equation a range, or error, in estimating the actual size can be determined. Rearranging equation 3.4.1 gives:

\[ x = z \times \sigma_x \pm \mu \]  

*Equation 3.4.2*

The plus (+) and minus signs (-) allow for estimates of the upper and lower limits, respectively, of the particle size. The mean particle size is therefore determined as the average of these upper and lower limits. The z-statistic is dependent on the number of particles used to define a trail, generally the number of particles in the experiments was 5. Assuming a confidence interval of 95%, the relevant z-statistic is equal to 2.571.
4 RESULTS

4.1 Growth rate
As has been described, several ‘healthy’ generations showing consistent growth rate needed to be attained. This was to ensure the validity of any testing done. Only one method was used to determine the growth rates of the generations prior to the inoculation of the 20 litre cultures, this was raw fluorescence. The raw fluorescence curves for the three generations prior to the inoculation of the 20 litre culture show a distinct consistency in slope from generation to generation. A consistent growth rate of approximately 1.3 day⁻¹ was achieved for the ‘healthy’ generations (Figure 4.1.1), this relates to a doubling of population size in half a day. The results of the tests to monitor the growth of the major 20 litre replicate cultures show a distinct similarity between the two, they are not, however, exactly the same. The growth rates during the exponential phase of growth for the two replicate cultures were 0.71 day⁻¹ and 0.64 day⁻¹ respectively, using the raw fluorescence values obtained.

![Figure 4.1.1: The growth rate curves of the three previous ‘healthy’ generations of Skeletonema Costatum prior to inoculation of the 20 L replicates.](image)

4.1.1 Raw Fluorescence
The fluorescence levels of the replicates peaked on the same day (day 3) to approximately the same levels (Table 4.1.1). The level of fluorescence dropped after this day (Table 4.1.1).
Table 4.1.1: Daily raw fluorescence readings for both replicates, named Culture 1 and Culture 2, respectively.

<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>Culture 1</th>
<th>Culture 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/8/01</td>
<td>0</td>
<td>~</td>
<td>~</td>
</tr>
<tr>
<td>16/8/01</td>
<td>1</td>
<td>3.9</td>
<td>6.1</td>
</tr>
<tr>
<td>17/8/01</td>
<td>2</td>
<td>6.5</td>
<td>8.7</td>
</tr>
<tr>
<td>18/8/01</td>
<td>3</td>
<td>16.2</td>
<td>21.8</td>
</tr>
<tr>
<td>19/8/01</td>
<td>4</td>
<td>10.7</td>
<td>12.4</td>
</tr>
<tr>
<td>20/8/01</td>
<td>5</td>
<td>6.1</td>
<td>7.4</td>
</tr>
<tr>
<td>21/8/01</td>
<td>6</td>
<td>2.8</td>
<td>3.5</td>
</tr>
<tr>
<td>22/8/01</td>
<td>7</td>
<td>2.1</td>
<td>2.4</td>
</tr>
<tr>
<td>23/8/01</td>
<td>8</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td>24/8/01</td>
<td>9</td>
<td>1.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

4.1.2 Chlorophyll a

The chlorophyll a values over the course of the experiment closely matched those of the raw fluorescence. This comparison is best seen when plotting fluorescence on one y-axis and chlorophyll a concentration on a secondary y-axis (Figure 4.1.1).

Figure 4.1.2: Comparison of fluorescence and chlorophyll a concentration over the course of experimentation.
4.1.3 Coulter Counter Cell Count

The Coulter Counter was the final means by which growth of the culture was measured. Once again plotting raw fluorescence on one y-axis against cell count on a secondary y-axis shows the similarity between the two over the first three days (Figure 4.1.2). The cell count, however, does not seem to drop after the third day as chlorophyll \( \alpha \) and raw fluorescence do, this could be due to the fact that the dead cells that do not contribute to fluorescence and chlorophyll \( \alpha \), are still being counted by the Coulter Counter.

![Figure 4.1.3: Comparison of fluorescence and cell counts over the course of experimentation.](image)

4.2 Nitrogen Levels

The dissolved nitrogen levels were monitored over the course of the experiment. The filtrate of the chlorophyll \( \alpha \) measurements was frozen for this purpose and sent off for analysis. The levels of dissolved nitrogen in both cultures was exactly the same for day zero and day one (Table 4.2.1). From day two onwards the level of dissolved nitrogen was practically non existent for both replicates (approx. \(<7 \, \mu\text{gL}^{-1}\)).
Table 4.2.1: Dissolved nitrogen levels for both replicates over the course of experimentation. Values are in µg.N/L.

<table>
<thead>
<tr>
<th>Date</th>
<th>Day</th>
<th>Culture 1</th>
<th>Culture 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/8/01</td>
<td>0</td>
<td>8600</td>
<td>8600</td>
</tr>
<tr>
<td>16/8/01</td>
<td>1</td>
<td>390</td>
<td>390</td>
</tr>
<tr>
<td>17/8/01</td>
<td>2</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>18/8/01</td>
<td>3</td>
<td>4</td>
<td>&lt;2</td>
</tr>
<tr>
<td>19/8/01</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>20/8/01</td>
<td>5</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>21/8/01</td>
<td>6</td>
<td>3</td>
<td>&lt;2</td>
</tr>
<tr>
<td>22/8/01</td>
<td>7</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>23/8/01</td>
<td>8</td>
<td>&lt;2</td>
<td>3</td>
</tr>
<tr>
<td>24/8/01</td>
<td>9</td>
<td>~</td>
<td>~</td>
</tr>
</tbody>
</table>

This drop in the levels of dissolved nitrogen occurred the day before the peak in growth. Nitrogen limitation is therefore assumed to be the primary cause of limitation of growth of both replicates, as no other factors were changed before or after the inoculation of the replicates.

4.3 Aggregation

4.3.1 TEP measurements

The TEP measurements rose over the logarithmic phase of growth, and fell after day 3 (Figure 4.1.4).

Figure 4.1.4: TEP measurements over the course of experimentation for both replicate cultures.
4.4 SETCOL Size and Sinking Rates

The SETCOL and Video analysis methods were employed to determine the sinking rates of aggregated and non-aggregated volumes of both replicates. As has been explained the results of both methods differ in their final output as a result of their method of analysis. Each SETCOL test produces one sinking rate value for the whole volume, whilst the Video analysis method gives the sinking rates and sizes of individual particles or aggregates.

4.4.1 SETCOL Non-Aggregated Sinking Rates

The sinking rates as determined by the SETCOL method for the non-aggregated volumes of both replicates show a significant increase in the mean sinking rate from day 2 two to day 5. Both replicates show very low sinking rates on day 2 and for replicate culture 2 column 1 positive buoyancy occurs. The mean sinking rates then drop slightly from day 5 to day 7 (Tables 4.4.1 & 4.4.2).

Table 4.4.1: Sinking rates for replicate culture 1 for both columns of the SETCOL.

<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>Sinking rate for column 1 (m day(^{-1}))</th>
<th>Sinking rate for column 2 (m day(^{-1}))</th>
<th>Mean SR (m day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>17/8/01</td>
<td>0.1340</td>
<td>0.1104</td>
<td>0.1222</td>
</tr>
<tr>
<td>5</td>
<td>20/8/01</td>
<td>0.3180</td>
<td>0.1511</td>
<td>0.2345</td>
</tr>
<tr>
<td>7</td>
<td>22/8/01</td>
<td>0.1632</td>
<td>0.2897</td>
<td>0.2265</td>
</tr>
</tbody>
</table>

Table 4.4.2: Sinking rates for replicate culture 2 for both columns of the SETCOL.

<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>Sinking rate for column 1 (m day(^{-1}))</th>
<th>Sinking rate for column 2 (m day(^{-1}))</th>
<th>Mean SR (m day(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>17/8/01</td>
<td>-0.0378</td>
<td>0.1135</td>
<td>0.0378</td>
</tr>
<tr>
<td>5</td>
<td>20/8/01</td>
<td>0.4147</td>
<td>0.4128</td>
<td>0.4138</td>
</tr>
<tr>
<td>7</td>
<td>22/8/01</td>
<td>0.3139</td>
<td>0.3586</td>
<td>0.3362</td>
</tr>
</tbody>
</table>

4.4.2 SETCOL Aggregated Sinking Rates

Once again, as was seen in the non-aggregated results of the SETCOL there is a sharp increase in the mean sinking rates from day 2 to day 5 for both culture replicates (Tables 4.4.3 and 4.4.4).
The values for replicate culture 1 are significantly larger than replicate culture 2. The slight drop of the mean sinking rate from day 5 to day 7 seen in the non-aggregated SETCOL tests occurs in the aggregated equivalent of culture 2, but not in replicate culture 1. The accuracy of the results for both replicates is in doubt.

**Table 4.4.3:** Sinking rates for aggregated volumes of replicate culture 1 for both columns of the SETCOL.

<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>Sinking rate for column 1 (m day⁻¹)</th>
<th>Sinking rate for column 2 (m day⁻¹)</th>
<th>Mean SR (m day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>17/8/01</td>
<td>0.2372</td>
<td>0.3848</td>
<td>0.3110</td>
</tr>
<tr>
<td>5</td>
<td>20/8/01</td>
<td>0.2628</td>
<td>1.7571</td>
<td>1.0099</td>
</tr>
<tr>
<td>7</td>
<td>22/8/01</td>
<td>-0.1193</td>
<td>0.6671</td>
<td>0.2739</td>
</tr>
</tbody>
</table>

**Table 4.4.4:** Sinking rates for aggregated volumes of replicate culture 2 for both columns of the SETCOL.

<table>
<thead>
<tr>
<th>Day</th>
<th>Date</th>
<th>Sinking rate for column 1 (m day⁻¹)</th>
<th>Sinking rate for column 2 (m day⁻¹)</th>
<th>Mean SR (m day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>17/8/01</td>
<td>0.0654</td>
<td>0.0565</td>
<td>0.0609</td>
</tr>
<tr>
<td>5</td>
<td>20/8/01</td>
<td>0.3649</td>
<td>0.2883</td>
<td>0.3266</td>
</tr>
<tr>
<td>7</td>
<td>22/8/01</td>
<td>0.2460</td>
<td>0.2275</td>
<td>0.2368</td>
</tr>
</tbody>
</table>

**4.4.3 Coulter counter cell size distribution**

The output of the Coulter Counter device not only gives the mean, median and standard deviation of a given sample but it also gives a size distribution function. For the non-aggregated volumes of both replicate cultures the Coulter Counter is applicable to determine the size distribution (Table 4.4.5). However, the results of the aggregated volumes, when tested on the Coulter Counter, may be questionable. This question arises due to the possibility that the high velocity flow through the aperture of the Coulter Counter would tear the aggregates apart.
Table 4.4.5: Particle size statistics for both replicate cultures.

<table>
<thead>
<tr>
<th>Replicate Culture</th>
<th>Units of µm</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture 1</td>
<td>Mean</td>
<td>3.415</td>
<td>3.443</td>
<td>3.408</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.146</td>
<td>3.212</td>
<td>3.146</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>0.698</td>
<td>0.685</td>
<td>0.665</td>
</tr>
<tr>
<td>Culture 2</td>
<td>Mean</td>
<td>3.505</td>
<td>3.822</td>
<td>3.46</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.396</td>
<td>3.509</td>
<td>3.276</td>
</tr>
<tr>
<td></td>
<td>Std. Dev.</td>
<td>0.532</td>
<td>0.814</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Due to the chain forming feature of *Skeletonema Costatum* the size statistics given in Table 4.4.5 relate only to the diameter of a single cell in the chain length. The actual size is dependent upon the number of these cells contained in the chain length, this number can range from single cells to greater than ten cells per chain.

4.5 Video Observation Results

The results of video analysis of sinking rate showed unexpected size range measurements. The size ranges determined in both aggregated and non-aggregated volumes for both replicates were very similar (Tables 4.4.6 & 4.4.8). A combination of the results for both aggregated and non-aggregated for each replicate on each day seemed justified and gave a much larger data set.

Table 4.4.6: Non-aggregated volumes diameter ranges (µm).

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture 1</td>
<td>2.4 to 152</td>
<td>16 to 223</td>
<td>20 to 219</td>
</tr>
<tr>
<td>Culture 2</td>
<td>11.3 to 126</td>
<td>19 to 159</td>
<td>30 to 178</td>
</tr>
</tbody>
</table>

Table 4.4.7: Non-aggregated volumes sinking rate ranges (m day⁻¹).

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture 1</td>
<td>0.18 to 5.4</td>
<td>0.086 to 2.16</td>
<td>0.31 to 1.9</td>
</tr>
</tbody>
</table>
Table 4.4.8: Aggregated volumes diameter ranges (µm).

<table>
<thead>
<tr>
<th>Culture</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture 1</td>
<td>16 to 192</td>
<td>19 to 209</td>
<td>23 to 138</td>
</tr>
<tr>
<td>Culture 2</td>
<td>16 to 232</td>
<td>19 to 193</td>
<td>15 to 128</td>
</tr>
</tbody>
</table>

Table 4.4.9: Aggregated volumes sinking rate ranges (m day⁻¹).

<table>
<thead>
<tr>
<th>Culture</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture 1</td>
<td>0.06 to 4.23</td>
<td>0.14 to 2.1</td>
<td>0.27 to 1.66</td>
</tr>
<tr>
<td>Culture 2</td>
<td>0.095 to 4.99</td>
<td>0.1 to 1.18</td>
<td>0.19 to 1.3</td>
</tr>
</tbody>
</table>

The number of particles identified and analysed for size and sinking rate varied considerably between tests, the smallest being 18 and the largest being 83 (Table 4.4.10).

Table 4.4.10: The number of particles identified in each test. Values in brackets represent total particle numbers when combining the relative sets of data.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture 1 Non-Aggregated</td>
<td>49</td>
<td>75</td>
<td>33</td>
</tr>
<tr>
<td>Culture 1 Aggregated</td>
<td>18 (67)</td>
<td>24 (99)</td>
<td>24 (57)</td>
</tr>
<tr>
<td>Culture 2 Non-Aggregated</td>
<td>42</td>
<td>83</td>
<td>65</td>
</tr>
<tr>
<td>Culture 2 Aggregated</td>
<td>58 (100)</td>
<td>74 (157)</td>
<td>31 (96)</td>
</tr>
</tbody>
</table>
5 DISCUSSION

Analysis of the results of the Video Observation method lead to the relationship between the sinking rate and diameter of chains and aggregates of *Skeletonema costatum* that contradicts the relationship proposed by Stokes’. The sinking rates appeared to be proportional to the diameter \((w_s \propto d)\) as opposed to the diameter squared \((w_s \propto d^2)\). There was very little difference between the replicate cultures when tested by videography. The SETCOL method, however, provided unreliable results.

5.1 Video Observation

Stokes’ Law states that sinking rate is proportional to the diameter squared \((w_s \propto d^2)\). To test whether or not Stokes’ Law was applicable to the aggregates and single cells of *Skeletonema costatum*, the logarithm of sinking rate \((\log_{10}(w_s))\) versus the logarithm of equivalent particle diameter \((\log_{10}(d))\) was plotted. The trend lines of the plots for both replicates on each day of testing resulted in a gradient of approximately 1 to 0.7 over the days of experimentation for both replicate cultures (Figures 5.1.4 to 5.1.6 and Figures 5.1.10 to 5.1.12). This value for the gradient gives the power that the diameter should be raised in the relationship of sinking rate to size (Equation 5.1.3). The equation of the trend line when plotting the logarithm of sinking rate versus the logarithm of size will have the form: \(y = mx + b\). Where \(y\) is equivalent to \(\log_{10}\) (sinking rate), \(x\) is \(\log_{10}\) (size), and \(m\) and \(b\) are constants. So:

\[
\log_{10}(w_s) = m \log_{10}(d) + b \tag{Equation 5.1.1}
\]

Rearranging gives:

\[
\log_{10}\left(\frac{w_s}{d^m}\right) = b \tag{Equation 5.1.2}
\]

\[
\Rightarrow w_s = d^m \times 10^b \tag{Equation 5.1.3}
\]

Some of the particles analysed for size and sinking rate were discarded from the analysis that determined the relationship described above. Some particles were discarded because they were not completely illuminated and the particle size was questionable. The low correlation between the trend lines of the non-adjusted data series (Figures 5.1.1 to 5.1.3 & Figures 5.1.7 to 5.1.9) in comparison to that of the trend lines for the adjusted data series supports the removal of the inaccurate data.
Figure 5.1.1: The non-adjusted data for culture 1, day 2. 67 data points.

Figure 5.1.2: The non-adjusted data for culture 1, day 5. 99 data points.

Figure 5.1.3: The non-adjusted data for culture 1, day 7. 57 data points.

Figure 5.1.4: The adjusted data for culture 1, day 2. 57 data points.

Figure 5.1.5: The adjusted data for culture 1, day 5. 99 data points.
Figure 5.1.6: The adjusted data for culture 1, day 7. 55 data points.

Figure 5.1.7: The non-adjusted data for culture 2, day 2. 100 data points.

Figure 5.1.8: The non-adjusted data for culture 2, day 5. 157 data points.

Figure 5.1.9: The non-adjusted data for culture 2, day 7. 96 data points.

Figure 5.1.10: The adjusted data for culture 2, day 2. 98 data points.
The relationship of Stokes’ Law does not appear to be applicable to the marine diatom *Skeletonema Costatum* and its aggregates. The relationship between sinking rate and diameter derived from the laboratory data collected is:

$$w_s \propto d^1 \text{ to } w_s \propto d^{0.7}$$

To determine whether or not Stokes’ Law is applicable the assumption made in deriving the law must be examined, as well as the other contributing factors such as density and viscosity, which comprise the equation.
Stokes’ Law is considered applicable for low Reynolds number flow \((Re<1)\). The maximum Reynolds number achieved by the observed particles is:

\[
Re_{\text{max}} = \frac{\rho_w \times d \times w_s}{\mu} = \frac{1000 \times (250 \times 10^{-6}) \times (5 + (24 \times 60 \times 60))}{10^{-3}} = 0.01445 \quad \text{Equation 5.1.4}
\]

The maximum Reynolds number is clearly less than 1. Therefore, with respect to Reynolds number, Stokes’ Law holds true for the chains and aggregates of \textit{Skeletonema costatum}.

The first assumption made when deriving Stokes’ Law is that the particle is travelling at its terminal velocity. The largest time required to reach this terminal velocity would be for the largest diameter particle (assuming a density of 1300 kg m\(^{-3}\)), the longest time required is therefore:

\[
T_p = \frac{\rho_p d_p^2}{18\mu} = \frac{1300 \times (232 \times 10^{-6})^2}{18 \times 10^{-3}} = 3.9 \times 10^{-3} \text{ seconds} \quad \text{Equation 5.1.5}
\]

The particles were observed toward the bottom of the column and because of this short time required to reach terminal velocity, the chains and aggregates were considered to have reached this velocity. Stokes’ Law still seems applicable, having considered the time taken to achieve terminal velocity and the maximum size of the Reynolds number.

The equation for Stokes’ Law comprises a density factor as well as a viscosity factor. With respect to the viscosity factor the media through which the \textit{Skeletonema costatum} sink is assumed to have a viscosity similar to that of water \((10^{-3} \text{ kg m}^{-1} \text{ s}^{-1})\) and therefore is of no effect. The effect of mucilage secretion as a means of changing density, measured by TEP (Section 4.3.1), has been proposed as a means of reducing sinking rate. The effect of a mucilage ‘sheath’ in reducing the overall density of the cell is mitigated by the consequent increase in the cell size. Sinking rate would, however, be reduced if the mucilage was to comprise a larger volume than the cell alone.

From the video images the effect of this mucilage excretion in slowing the decent of the chains and aggregates does not seem to be applicable. The solid edges of the chains and aggregates are
visible and the amount of mucilage excreted would have to comprise a significant volume of the size of the cells.

Another factor that could affect the density of the chains and aggregates of *Skeletonema costatum* is the porosity of the aggregates and the hairlike protrusions that link the cells in chains. The density of these porous aggregates would be significantly less than the density of a nonporous particle of equivalent diameter. This density difference with respect to size may have an effect on the density component of the Stokes’ Law equation, by simply reducing the density of the aggregate relative to what the density would have been if the aggregate had been solid.

Finally, Hutchinson (1967), introduced the concept of form resistance ($\phi$), this provides a factor that determines the slowing effect that the shape of a chain or aggregate has on its sinking velocity. Reynolds (1984) defines this form resistance as a ratio of predicted Stokes’ velocity ($v_s$) to actual velocity ($w_s$).

$$\phi = \frac{v_s}{w_s}$$

*Equation 5.1.7*

Introducing this factor into Stokes’ Law gives:

$$w_s = \frac{d^2(\rho_p - \rho_w)g}{18\mu\phi}$$

*Equation 5.1.8*

The value of $v_s$ can be defined as the gradient of Stokes’ relationship of log$_{10}(v_s)$ versus the log$_{10}(d)$, which is 2. The value of $w_s$ is therefore the value of the gradient of the plot of log$_{10}(w_s)$ versus the log$_{10}(d)$, which ranges from approximately 1 to 0.7 (Table 5.1.1).

**Table 5.1.1:** The form resistance values over the lifecycle of *Skeletonema costatum*.

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture 1</strong></td>
<td>1.4</td>
<td>2.1</td>
<td>3.0</td>
</tr>
<tr>
<td><strong>Culture 2</strong></td>
<td>2.0</td>
<td>2.7</td>
<td>2.8</td>
</tr>
</tbody>
</table>

The application of this form resistance to the trendlines derived earlier would give the relationship of sinking rate to diameter squared as proposed by Stokes’.

**5.2 SETCOL**
The SETCOL method provides a bulk measurement of the sinking rate of both aggregated and non-aggregated volumes. Several errors are associated with this form of determining bulk sinking rate. These errors include changes in the diatom community during the course of experimentation, breaking and forming of aggregates during stirring of media prior to commencement of testing period, inaccurate particle size distribution determination and inconsistency between replicate columns of the SETCOL. These errors are best seen in the results of the aggregated volumes of the SETCOL. The non-aggregated volumes showed good consistency, but the identification of aggregates by the Video Observation method show that these tests were not performed on completely non-aggregated volumes. The results of the SETCOL for these experiments can be considered inconclusive.
6 CONCLUSIONS

The two methods used to determine sinking rates of the marine diatom *Skeletonema costatum* are vastly different in technique and output.

Analysis of the results of the Video Observation method used to determine sinking rate showed that there was a relationship between sinking rate and diameter \( (w_s \propto d) \) as opposed to Stokes’ relationship, that sinking rate is proportional to the diameter squared \( (w_s \propto d^2) \). The conditions for the application of Stokes’ Law to the chains and aggregates of *Skeletonema costatum* were, however, satisfied. As a result the concept of form resistance \( (\phi) \) was added to the Stokes’ Law equation, as proposed by Hutchinson (1967) and Reynolds (1984). This form resistance factor, when included in Stokes’ Law adjusted the relationship between sinking rate and diameter previously observed \( (w_s \propto d) \), to that of Stokes’ Law \( (w_s \propto d^2) \). This form resistance is the result of the non-spherical shape of the chains and aggregates of the marine diatom *Skeletonema costatum* as well as a factor of porosity.

The SETCOL method is generally considered to give a good approximation of non-aggregated settling rates of the marine diatom *Skeletonema costatum*. The SETCOL results for the non-aggregated volumes are in question because of the observation of aggregates, in these volumes, by the Video Observation method. These unwanted aggregates in the non-aggregated volumes may be the result of the motion of the media within the 20 litre carboys.

The conclusions made about what has affected the sinking rate of the chains and aggregates of the marine diatom *Skeletonema costatum* would be confirmed if different levels of nitrogen limitation were tested as well as the testing of different forms of limitation.
REFERENCES


REFERENCES


- 53 -


Smayda T.J., & Bolyen B. J. (1966a) Experimental observations on the floatation of marine diatoms. II. *Skeletonema costatum* and *Rhizosolenia setigera*. Limnol. Oceanogr. 11: 18-34


## Appendix I: CONSTITUENTS OF ARTIFICIAL SEAWATER

<table>
<thead>
<tr>
<th>HYDRATED SALTS</th>
<th>Grams/Litre</th>
<th>Grams/15 Litres</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgCl₂•H₂O</td>
<td>9.395</td>
<td>140.925</td>
</tr>
<tr>
<td>CaCl₂•2H₂O</td>
<td>1.316</td>
<td>19.74</td>
</tr>
<tr>
<td>SrCl₂•6H₂O</td>
<td>0.0214</td>
<td>0.321</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ANHYDROUS SALTS</th>
<th>Grams/Litre</th>
<th>Grams/15 Litres</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>20.758</td>
<td>311.37</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>3.477</td>
<td>52.155</td>
</tr>
<tr>
<td>KCl</td>
<td>0.587</td>
<td>8.805</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.17</td>
<td>2.55</td>
</tr>
<tr>
<td>KBr</td>
<td>0.0845</td>
<td>1.2675</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.0225</td>
<td>0.3375</td>
</tr>
<tr>
<td>NaF</td>
<td>0.0027</td>
<td>0.0405</td>
</tr>
</tbody>
</table>
**Appendix II: CONSTITUENTS OF G₂ MEDIUM (MAKES 3L)**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seawater</td>
<td>2250 mL</td>
</tr>
<tr>
<td>Deionised H₂O</td>
<td>750 mL</td>
</tr>
<tr>
<td>Nitrate</td>
<td>3 mL</td>
</tr>
<tr>
<td>Vitamins</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>PII metals</td>
<td>7.5 mL</td>
</tr>
<tr>
<td>Soil Extract</td>
<td>7.5 mL</td>
</tr>
<tr>
<td>Phosphate*</td>
<td>1.5 mL</td>
</tr>
</tbody>
</table>

*Note: Phosphate to be added in Laminar Air Hood.*
Appendix III: PROTOCOL FOR THE EXTRACTION OF PHOTOSYNTHETIC PIGMENTS

This method uses 90% acetone as the extraction solvent because of its relative low toxicity and its efficiency for most types of algae.

Chlorophyll a is the principle pigment used in the estimation of phytoplankton biomass.

This method uses the fluorometric readings to evaluate the concentration of chlorophyll a.

Sample Collection

Enough water should be collected to concentrate phytoplankton on at least 3 filters (Whatman GF/F, Gelman A/E or equivalent) so that precision can be assessed. Filtration volume will depend on the particulate load of the water. 5L may be required in the open ocean, especially in the low productive areas like Perth Coastal Waters, whereas less than 1L can be sufficient for lakes or estuary waters. A sufficient volume has been filtered when a visible green or brown colour is visible on the filter. Record the volume. Filtering should be performed in subdued light as soon as possible after sampling due to the rapid degradation of chlorophyll a at increased temperatures and light intensities. Fold the filter in half with the particulate matter on the inside, fold it again and place into a labelled 8mL snap cap tube (or aluminium foil) and place in a dark cool place until they can be placed in a freezer or extracted straight away. Filters can be stored frozen for as long as 3 weeks without significant loss of chlorophyll a.

EXTRACTION BY GRINDING

Method

Take the samples from the freezer but keep them in a small esky on the bench, workspace lighting should be at a minimum. Remove filter from its tube with forceps and cut into small pieces into the grinding tube. Push the filter to the bottom of the tube and add 4mL of 90% acetone. Put the grinding tube into a beaker of crushed ice and grind the filter until it has been converted into a slurry. The ice should prevent overheating of the sample, which will cause degradation of the chlorophyll a. Pour the slurry into the 15mL glass centrifuge tube, use the 4mL pipette to rinse out the tube and the pestle and add the rinse to the centrifuge tube. Do this one more time ensuring all the slurry has been removed from both the tube and the pestle. Place the bung on the tube and shake vigorously, place into the freezer. Rinse grinding tube and pestle with acetone and wipe with a tissue before proceeding onto the next filter. Shake each tube before putting them into the freezer to steep. The samples should be allowed to steep for a minimum of 2 hours but not to exceed 24 hours. The tubes should be shaken at least once during this period. After steeping time, take samples from the freezer and shake again. Using a small (50mL) glass manifold filter the extraction through a GF/F and pour the filtrate into a clean 13mm glass vial. Samples should be allowed to come to ambient temperature before analysis. Ensure that for each run a blank sample is also analysed.

Sample Analysis

Ensure the instrument you are using has warmed up for the minimum time required. Place the tube into the calibrated fluorometer (the instrument will need to be recalibrated if there has been a
3°C temperature fluctuation since the last calibration). Once the reading has stabilized record as the Rb (before acid) result. Add 3 drops of 1N HCl to the tube, cap, shake and leave for 60 seconds. Place in fluorometer and record reading Ra (after acid). Calculate the chlorophyll a concentration from the following expression:

\[
\text{Chlorophyll a (mg/L)} = \frac{(r/r-1)(Rb-Ra)}{v/V}
\]

\(r\) = the before-to-after acidification ratio of a pure chlorophyll a solution.
\(Rb\) = fluorescence of a sample prior to acidification
\(Ra\) = fluorescence of a sample after acidification
\(v\) = volume (mL) of the extract
\(V\) = volume of the filtered sample
Appendix IV: CALIBRATION SCRIPT

%the purpose of this function is to calibrate an image, 
% based on the vertical data, y, and the unit of this data, unit.

function [Cal]=calibrationbridget(y,unit);

% define the length of vector containing data for calibration
Ly=length(y);

% define the z statistic to be used in determining confidence intervals
zed=1.96;

% for a number of different lengths, calculate the calibration:
for ii=1:2
    % define the number of units over which calibration will be calculated
    kk=floor(Ly/2)-2*ii;

    % find the difference between the pixels, divide by units
    eval(['diff',num2str(ii),'=(y(1:kk)-y(Ly-kk+1:Ly))/((Ly-kk)*unit);']);
end;

% calculate the overall mean confidence interval,
% in terms of pixel/mm
diff(1:length(diff1))=diff1;
diff(length(diff1)+1:length(diff1)+length(diff2))=diff2;

mdiff=mean(diff);
stdiff=std(diff);
Cal(1)=mdiff+zed*stdiff/sqrt(length(diff));
Cal(2)=mdiff-zed*stdiff/sqrt(length(diff));
Appendix V: WSSUM FUNCTION

% this function sums the contents of a number of matlab files
% in order for sinking rate, ws to be calculated.
% this file has been written for black and white images
%---------------------------------------------
% define parameters
%---------------------------------------------

% define the source of the images
file = 'E:\C2Ag22081_';  % Base file directory and name
% DEFINE HERE WHERE YOU WANT THE TRAIL FILE TO BE SAVED
% (the extension '_a.tif' will be added, where a is the trail number)
newfile='C:/matlabR12/Daniel/C117081trail1';

% define the total number of summed images, the number of final images
a=[1]';
% define the files summed in each images.
% if a=1, this is the file extensions you want summed in a single trail.
% if you want to sum a whole lot of trails at once, then define the
% extensions of the images you want in the first trail.
% all subsequent trails will use the same number of images, with the same offset from the first
% one.
b=[91:98]';
% number of files separation between images to be summed
sep=1;
% number of images offset from the start of the image collection
offset=0;
% define the minimum intensity for particle detection
cutoff=80;
% define the scaling factor for viewing data:
% this scales up the brightness of the particles relative to the b/g
scaler=60;

% initialisation image vectors:
%---------------------------------------------
filename=[file,'001.tif'];
I0=imread(filename,'tif');
temp=double(I0);
[h_pixels,w_pixels]=size(temp);

for ii=1:length(a)
    eval(['trail',num2str(a(ii)),']=zeros(h_pixels,w_pixels);']);
end;

%---------------------------------------------
% add images
%---------------------------------------------
%% Now loop through each image, adding them:
for ii=1:length(a)
    for jj=1:round((length(b)-offset)/sep)
        filename=[file,sprintf('%03d',((a(ii)-1)*length(b)*sep+offset+b(1)+(jj-1)*sep)),'.tif'];
        Im=imread(filename,'tif');
        %image(Im);
        temp=double(Im);
        %d=temp(upperLim:lowerLim,leftLim:rightLim);
        inc=or(temp>cutoff,0);
        eval(['trail',num2str(a(ii)),'=trail',num2str(a(ii)),'+inc;']);
        clear Im;
    end;
trailtif=uint8(trail1);
filenamenew=[newfile,'_',sprintf('%03d',a(ii)),'.tif'];
eval(['trailtif=trail',num2str(a(ii)),';']);
imwrite(trailtif,filenamenew,'tif');
end;

%------------------------------------------------
% display the image
%------------------------------------------------
for ii=1:length(a);
    figure;
    eval(['trail=or(trail',num2str(a(ii)),'>0,0);']);
    image(trail*scaler);
    title(['file=',file,' a=',num2str(a),' ,b=',num2str(b),' ,sep=',num2str(sep),'*dt s']);
    colormap(gray);
    clear trail;
end;

%------------------------------------------------
% save the summed image in a separate .tif file
Appendix VI: WSDATA FUNCTION

The purpose of this function is to read in pairs of data which define the edges of each particle. The vertical points are averaged to determine the mean particle positions, \( y \) which are then used to calculate the velocity, \( v \). The data is also evaluated in pairs to define the mean area, and upper and lower limits of area. This data is then recorded in file `filename`.

% clear unwanted variables
clear dm se base top v atemp dtemp;

% define parameter values
filename='C:/matlabR12/dataC2Ag2208_13';
zed=2.571;
mmpx=1/333.4;
mmpy=1/333.4;
dt=10;
Dt=dt*(length(y)/2-1);

% load data file
eval(['load ',filename,'.mat']);

% calculate velocity
base=0.5*(y(length(y)-1)+y(length(y)));
top=0.5*(y(1)+y(2));
delta=abs((base-top)*mmpy);
v=delta/Dt;
%% calculate area, equivalent diameter
%%
%% round the pixel locations, to use as indices in calculating area:
ry=round(y);
rx=round(x);

%% calculate the mean area, and confidence intervals for the area data
for ii=1:(length(ry)/2)
    atemp(ii)=sum(sum(trail(ry(2*ii-1):ry(2*ii),rx(2*ii-1):rx(2*ii))));
end;

%% convert area to mm^2, and find std error
a2temp=atemp*mmpx*mmpy;
se=std(a2temp)/sqrt(length(a2temp));
%% define the mean equivalent diameter squared
a2m(1,1)=mean(a2temp);
%% define upper and lower bounds on diameter
a2m(2,1)=a2m(1)-zed*se;
a2m(3,1)=a2m(1)+zed*se;

%% update data file
%%
%% update variables
a2(:,length(ws)+1)=a2m
ws(length(ws)+1)=v
counter(length(counter)+1)=length(y)/2
%% resave data file
eval(['save ',filename,'.mat a2 ws counter trail;']);