COMPARATIVE LABORATORY STUDY OF PHOTOACCLIMATION IN SELECTED DINOFLAGELLATE AND DIATOM SPECIES OF THE BENGUELA ECOSYSTEM

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DECLARATION

In this thesis, I report the results of original research carried out by myself between 2001 and 2005. All data collected during the experiments are my own. The ideas and conclusions presented are my own, but developed under the supervision and criticism of Dr. Ray Barlow (Marine and Coastal management), Dr Grant Pitcher (Marine and Coastal Management) and Prof. John Field (University of Cape Town).

Facilities of the Sea Point Aquarium of the Department of Environmental Affairs, South Africa, were used to grow the phytoplankton cultures up to the required cell densities. The dinoflagellate species were obtained from the Marine and Coastal Management culture collection held at the Sea Point Aquarium. The diatom species were collected from coastal waters at Saldanha Bay and were isolated by me. Samples were incubated in a light and temperature controlled environment, again using the Aquarium facilities. Incubations, using the photosynthetron, were carried out in the laboratory specifically dedicated for radioactive work. The radioactive samples were read using the scintillation counter in the PhysChem section of Marine and Coastal Management housed in the Foretrust Building, Cape Town. All culturing, sample preparations, experimentation, analyses for chlorophyll *a* readings, High Performance Liquid Chromatography, CHN, absorption and cell counts, were carried out by me. HPLC analyses were carried out by me under the critical eye of Dr. Ray Barlow.

Technical assistance was given by Desirée Calder with isolating diatom species and culturing, by Heather Sessions when using HPLC and CHN equipment. Assistance was given with the statistical analyses by Dawit Ghebrehiwet (University of Cape Town).

This work has not been submitted for a degree at this or any other university, and any assistance I received during the course of its completion is fully acknowledged.

Signature: _____ *Date:* _____

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The intuitive mind is a sacred gift and the rational mind is a faithful servant. We have created a society that honours the servant and has forgotten the gift.

Albert Einstein

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ABSTRACT

In their natural environment the diatoms (mixers) are exposed to fluctuations in incident irradiance due to vertical displacements in the water column induced by turbulence whereas the dinoflagellates (migrators and layer-formers) tend to control their vertical positions. In these two groups of phytoplankton physiological acclimation to these fluctuations results in the variation of a number of measurable photosynthetic parameters and variables which can be evaluated in controlled laboratory experiments. The processes can be expressed in time scales from seconds to hours e.g. carbon to chlorophyll ratio changes over several hours. Photoacclimative responses in five species of dinoflagellates (*Alexandrium catenella, Protoceratium reticulatum, Prorocentrum micans, Prorocentrum triestinum, Gymnodinium zeta*) and three diatom species (*Chaetoceros* sp., *C. capense, C.* cf. *pendulus*) were investigated with respect to parameters of *P versus E* curves (P^*_m, α* and E_k) and variability in chemical composition (C and N), photosynthetic capacity, pigment ratios, maximum quantum yield and chlorophyll to carbon ratio. These species were grown at irradiances of 33 (LL), 178 (ML) and 647 µmol quanta . m^{-2} . s^{-1} (HL) at a 12:12h day: night length at 17 ºC. The photosynthetic parameters, pigment concentration and Chl *a*-specific absorption were mostly affected by photoacclimational status. Species-specific differences were observed at the three different light levels in *P versus E* curves, pigment concentrations, absorption, carbon to chlorophyll *a* ratios and quantum yield. Photoprotective carotenoids (diadinoxanthin and diatoxanthin) were significantly different relative to Chl *a* in eight species of dinoflagellates and diatoms. Although the experimental data were variable, some trends emerged. Diatoms were more efficient in utilizing light at high intensities with P^*_m in *C. capense* peaking at 13.0 mg C . (mg Chl *a*)$^{-1}$. h^{-1} whilst small-celled dinoflagellates were most efficient in low light (LL). Changes in pigment concentrations under different light conditions were species-specific. Photosynthetic pigment (PSP) concentrations ranged up to 58% as observed in Chl *a* concentrations in the dinoflagellate cultures. Light absorption of diatoms and dinoflagellates was species-specific, increasing with higher light (HL) levels. Optimum absorption under high light was noted in *Chaetoceros capense* (mean 0.09 m^{-1} (mg Chl *a*)$^{-1}$). Maximum quantum yield increased during low light treatment in dinoflagellates, with a similar photoacclimation response in diatoms. The total cellular carbon content is greater in dinoflagellates than diatoms in laboratory cultures, the ratio of cellular carbon to chlorophyll *a* increases from LL to HL in both diatoms and dinoflagellates due to the decrease in Chl. Multivariate statistical analyses, using Multidimensional Scaling (MDS) and one-way Analysis of Similarity (ANOSIM) showed a significant difference ($p < 0.01$) between the two groups, diatoms and dinoflagellates, with respect to 10 physiological variables at three light levels.

CHAPTER 1. INTRODUCTION

Phytoplankton, the eukaryotic, single celled algae, include amongst other groups the diatoms (class Bacillariophyceae) and dinoflagellates (class Dinophyceae). They play a vital role in the ecology of the world oceans, being responsible for an estimated 45% of global productivity (Field *et al.* 1998). Phytoplankton organisms live in a constantly changing and turbulent system and are continually exposed to different light intensities within a short space of time (minutes to hours). To be able to survive and grow in an oceanic environment phytoplankton has developed an array of interrelated cellular mechanisms allowing them to optimise light harvesting and utilisation. These responses form the phenotypic process which are termed photoacclimation (Williams *et al.* 2003). Three factors have an important regulatory effect during photoacclimation i.e. light, temperature and nutrients. The regulatory effects include adjustment to optical properties involved in the "light reactions" of photosynthesis, the fine-tuning of RUBISCO levels (*1) to optimise electron flow in the "dark reaction" and providing optical and enzymatic safeguards against high-light damage. Their growth in the marine environment has been studied widely in an effort to describe and understand the dynamics of phytoplankton photoacclimation responses during photosynthesis which can be short-term (within minutes, e.g. xanthophyll cycle) and long-term (within hours, e.g. size and number of photosynthetic units). The photosynthesis *versus* irradiance response curve in which mass specific (chlorophyll *a*) photosynthetic rates are plotted *versus* light intensity is often used to characterise photoacclimation (Zonneveld 1997).

Changes in pigment ratios and cellular concentrations in response to different irradiances have been observed in several diatoms and dinoflagellates. In general all light harvesting pigments increase under low light and *vice versa* these include the carotenoids fucoxanthin of the diatoms and peridinin of dinoflagellates, in addition to all chlorophylls. Other carotenoids such as β-carotene follow the opposite course to irradiance in relation to photoacclimation since they function as photoprotective light filters (Wang *et al.* 2003, and others).

Typical photoacclimation responses are characteristic of different phytoplankton taxa. Also at inter-species level the responses are known to be very specific. To investigate the relationship between photoacclimation responses and cell volume, pigment complexes, absorption and carbon and nitrogen content during different light intensities in the two groups, uni-algal cultures of diatoms and dinoflagellates were used in laboratory experiments under controlled light, temperature and nutrient conditions.

*1) RUBISCO - Ribulose-1,5-bisphosphate carboxylase oxygenase : a plant protein which fixes carbon in photosynthetic organisms and accepts oxygen in place of carbon dioxide.

1.1 Photoacclimation

Periodically diatoms and dinoflagellates occupy an extreme environment within an upwelling system. For phytoplankton to survive within these turbulent marine regimes, different adaptive strategies for survival have been developed. Each species of phytoplankton has a different set of adaptive characteristics which defines its ecological niche. Cullen and MacIntyre (1998) point out that selective patterns of adaptation of phytoplankton species can be associated with depth regulation in different regimes of turbulence. Three general strategies, particularly in respect of depth regulation in the water column, can be classified *i.e.* mixing, migrating and layer-forming. In the two groups of phytoplankton, which are examined here in this thesis, the diatoms are considered to be "mixers" and the dinoflagellates are referred to as "migrators" and "layer-formers", a differentiation put forward by Cullen and MacIntyre (1998).

Strong upwelling is experienced in the Benguela Ecosystem. Upwelling, particularly in the southern part of the Benguela Current (Platt and Sathyendranath 1988) is very seasonal and is mostly experienced during certain wind conditions (particularly the south easterly winds) during the summer months (approximately October to March). During quiescent periods (very reduced to no turbulence) thermal stratification occurs when red tide blooms develop throughout the region.

Thermal stratification is reported to occur over the broader shelf area of the Benguela during quiescent periods and allow dinoflagellates and diatoms to adjust their vertical positions in the water column. In more stratified waters, during sampling along a 28 n mile transect off Lambert's Bay, pronounced diel vertical migration was observed between late afternoon and 2.00 hours with the motile dinoflagellates reaching a depth of 12 to 14 meters (Pitcher *et al.* 1998). It was noted that at 6 meter depth the velocity of surface southward and offshore flow can be up to 15 cm s^{-1}. Anderson and Sweeney (1977) have estimated the sinking speed of diatoms at 4 m d^{-1}. They also have the ability to reduce this to 0.4 m d^{-1} through buoyancy regulation.

Generally the mixers (diatoms) are associated with well mixed, high energy and nutrient-rich environments. Diatoms tend to have a high growth rate and cell sizes are usually large. In a strong turbulence the lack of mobility is not a disadvantage. Their physiological adaptive strategy, influenced by the nutrient concentrations in the water column, is to increase or decrease their sinking rates by changing their cell densities (buoyancy). Examples of morphological adaptations are setae and mucilage. This promotes aggregation and accelerates sinking rates (cell size) into depths from which the cells can be reintroduced into near surface layers by strong turbulent upwelling when nutrients have become more plentiful there.

Migrators and layer-formers (dinoflagellates) are generally associated with stratified, lower-energy, nutrient-poor environments. Motility may help to exploit the resources of different levels of the water column, both nutrient-depleted and well lit surface layers as well as deeper nutrient–rich layers at low

irradiance depths. This strategy is stated to result in the diel vertical migration of dinoflagellates (Watanaba *et al.* 1991, Pitcher *et al.* 1998) as well as cyclical and temporal vertical migration (Rivkin *et al.* 1984).

Some dinoflagellates, usually small-celled species, are layer-formers which migrate and aggregate in a restricted range where there is a maximum irradiance level such as at the surface, or they migrate to a nutrient replete zone at depth. An important adaptive strategy would be the efficient light utilisation for deep layer-formers where the irradiance is low and efficient nutrient utilization for surface layer-formers in nutrient depleted zones.

Quenching, associated with xanthophyll cycling, provides a partial protection from excessive irradiance (Olaizola *et al.* 1994). The physiological regulation of the xanthophyll pools is thought to be an adaptive feature of phytoplankton and reflects the different strategies of mixers, migrators and layer-formers (Demers *et al.* 1991, Ibelings *et al.* 1994).

Photophysiological responses of phytoplankton vary as a function of spectral scalar irradiance, temperature and nutrient status in the ocean. These variable environmental conditions have been found to alter the ratio of chlorophyll *a* (Chl *a*) to carbon (C) by a factor of 10 as in the diatom *Thalassiosira* sp. (Geider and Osborne 1987). To find convenient ecological parameters to define the growth and physiological responses of microalgae to environmental factors that would encompass all groups of microalgae is complex as a variety of adaptive mechanisms in response to irradiance in phytoplankton are taxon-specific as well as species-specific and operate over a range of time scales (Harris 1980).

Microalgae, as a group, show a high degree of phenotypic plasticity, which allows the cells to photoacclimate to changing irradiance-, chemical- and temperature conditions. MacIntyre and Cullen (2005) state that this reversible regulatory biochemical and/or physiological adaptation is genetically controlled, enabling the organisms to increase tolerance to external and internal changes. There cannot be a fixed parameterisation of cause and effect of the environmental influences on growth and survival of phytoplankton as each taxonomic group differs in structure and in photosynthetic components such as pigments, electron carriers and enzymes. Variability however in biochemical composition and physiological responses in phytoplankton is thought to be ordered. Raven and Kübler (2002) state that, as a result of the phytoplankton cells differing greatly in size and shape, the photosynthetic responses and pigment quota are to some extent scaled according to allometric laws. When the growth – size relationship of 5 classes of phytoplankton was investigated the results were very similar (Tang 1995). However the growth rates of dinoflagellates compared to diatoms of similar size show that dinoflagellates have a lower growth rate than other classes. This also reflects a lower photosynthetic capacity per unit biomass of dinoflagellates (Chan 1978, 1980). Tang (1995) calculated the allometric relationship of algal growth to be similar for different taxa, but also noted that dinoflagellates tend to have lower growth rates ($\mu = 3.45\ C^{-0.21}$) than other algae of similar size.

1.1.1 Photosynthesis

The physiological state of phytoplankton cells affects their photosynthetic activity and efficiency. Changes, for instance, in pigment concentrations such as chlorophyll *a* and carotenoids, in electron transfer chain components (Dubinsky *et al.* 1986) and in Calvin cycle enzymes (Kirk 1996) take place during acclimation to varying light intensities and during the different growth phases (e.g. active growth, senescence).

The process of photosynthesis *[1] involves the conversion of inorganic carbon (CO_2/HCO_3^-) from carbon dioxide into organic carbon in the form of glucose (CH_2O). Light, as the energy source, drives the enzymatic reactions and therefore affects the growth rate. The rate of this conversion (or carbon fixation), the *photosynthetic rate*, is expressed in terms of mass of carbon converted (fixed) per unit volume of water per unit time (mg C m^{-3} d^{-1}). The chlorophyll-specific photosynthetic rate for phytoplankton is mg C (mg Chl *a*)$^{-1}$ h^{-1}. The ecological term for the photosynthetic rate is the rate of primary production. Photosynthetic rates are related to irradiance in a non-linear fashion. To parameterise this relationship, photosynthesis *versus* irradiance data are required (Kirk 1996).

1.1.2 Photosynthesis versus irradiance curves

The photosynthesis *versus* irradiance curve (*P vs. E*) is used as a basis for predictive models of photosynthesis in phytoplankton. Two fundamental parameters are necessary to describe photosynthesis-irradiance relationships. They are the light-saturated rate of photosynthesis (P^*_m) usually normalised to chlorophyll *a* (as in this study) (also known as the 'assimilation number') and the initial slope of the light-limited portion of the photosynthesis *versus* irradiance curve (α*). Derived parameters that are needed are the light-saturation index E_k, which can be derived from the ratio P^*_m/ α* and β^* the photoinhibition parameter. The parameters describing the *P vs. E* curves contain information on the physiological state of the phytoplankton cells.

The shape and magnitude of the *P vs. E curve* therefore reflects the underlying biophysical, biochemical and metabolic processes that regulate photosynthesis (MacIntyre *et al.* 2002). Generally, variations in the parameters are the result of variations in the environmental conditions.

The *P vs E* response should ideally refer to instantaneous light and provide information on the photoacclimation state of the phytoplankton at the moment of sampling. Incubation time should therefore be as short as possible (JGOFS 2002).

The time chosen in determining *P vs E* relationships is therefore only 20 min so that no photoacclimation, or as little as possible, takes place, especially in the photoprotective apparatus of the cell. When ^{14}C is used to estimate photosynthetic carbon fixation and the duration of incubation is short, the newly incorporated

*[2]) Detailed descriptions of photosynthetic processes are found in Meyer *et al*. 1973; Salisbury and Ross 1985; Kirk 1996; Williams *et al*. 2003.

carbon is not respired or recycled in the cell. It can therefore be assumed that *P vs E* measurements yield results that are close to the gross carbon uptake rate and are consequently a measure of gross primary production (Williams 1993).

1.1.3 ^{14}C Techniques/methods

The rate of primary photosynthetic production is a critical parameter for the determination of energy and carbon flows through oceanic systems. The technique to measure total daily phytoplankton primary productivity was initially developed by Steemann Nielsen (1951, 1952). Further improvements of the ^{14}C-based measurements for phytoplankton production were advanced by Strickland (1965), Strickland and Parsons (1968) and Vollenweider (1971). Since 1972 numerous articles, including those by Peterson (1980) Carpenter and Lively (1980) and Fitzwater *et al*. (1982) contain recommendations and guidelines refining the method to obtain better quantitative measurements. In this context ICES (1981; 1993) has also published recommendations to refine the methodology. However, as recent as 1977, investigations to replace the radioactive isotope ^{14}C with a stable carbon isotope ^{13}C have been undertaken (Mateo *et al.* 2001) (Appendix 1).

The ^{14}C method uses the uptake of sodium ^{14}C- bicarbonate ($NaH^{14}CO_3$) to measure the incorporation of ^{14}C into particulate matter during incubation (10 min to 24 h duration) both in field and laboratory based experiments using an incubator such as a photosynthetron. The data obtained during short incubations, as described by Lewis and Smith (1983), are an approximation of the gross photosynthesis during the light period as no labelled carbon is lost by respiration (Williams 1993).

As the total inorganic carbon in the initial water sample is known, the uptake of carbon can be calculated from the equation:

$$carbon\ uptake = \frac{counts\ in\ particulate + dissolved\ organic\ form}{total\ counts}\ X\ available\ inorganic\ carbon\ X\ 1.05 \quad (1)$$

The isotope discrimination factor 1.05 corrects for the fact that $^{14}CO_2$ is "heavier" than $^{12}CO_2$ and is taken up more slowly than $^{12}CO_2$ (Evans *et al.* 1987). Steemann Nielsen and Kholy (1956) commented that the most serious shortcoming of this method is that no direct estimate of respiration is available from the normal incubation procedure.

1.2 Pigments

There are three chemically distinct types of photosynthetic pigments: chlorophylls, carotenoids and billiproteins. Chlorophylls and carotenoids are found in all photosynthetic plants, but billiproteins are only found in red and blue-green algae and cryptophytes. The major photosynthetic carotenoid, peridinin, is

characteristic of dinoflagellates while fucoxanthin is the key carotenoid in diatoms (Kirk 1996). Certain dinoflagellate species however, also contain fucoxanthin (Kirk 1996, Jeffrey *et al*. 1999).

The main role of Chl *a* in phytoplankton is to absorb light (photons or quanta) for photosynthesis. The accessory light harvesting pigments, such as Chl *b*, c_1, c_2, c_3, peridinin, fucoxanthin and various other photosynthetic carotenoids, have a significant function in extending the optical collection window at short and long wavelengths thus ensuring optimal absorption efficiency. Other carotenoids, such as diadinoxanthin, diatoxanthin and *β*-carotene, protect microalgal cells from the effects of high irradiances (Kirk 1996). The interconversion of the two xanthophylls diadinoxanthin (DD) and diatoxanthin (DT) during the xanthophyll cycle is an important strategy of photoacclimation for quenching photo-oxidative damage to phytoplankton cells (Fujiki *et al.* 2003). Reduction in the cellular content of accessory light harvesting pigments is generally observed in response to increasing irradiance. MacIntyre *et al.* (2002) found that Chl *b* and *c* decline by 80 – 90% and photosynthetic carotenoids and phycobillins by 50 – 90% over a similar range of increasing irradiances. These decreases possibly parallel the decrease in Chl *a* so that the ratio of Chl *a* : accessory pigments (AP) shows little variability with increased irradiance (Dubinsky *et al.* 1986, cited in MacIntyre *et al.* 2002). This is in contrast to observations made in the western English Channel (Aiken *et al.* 2004) in the Atlantic Ocean (Barlow *et al.* 2004) and in onshore (high Chl *a* corresponding to low AP) and offshore stations (low Chl *a* corresponding to high AP) in the Benguela Ecosystem which showed great variation in the ratios of Chl *a* to accessory pigments (AP). The decline in photosynthetic carotenoids and accessory pigments with higher irradiances is due to a decrease in the number of photosystem I (PSI) and photosystem II (PSII) reaction centres in both diatoms (Falkowski *et al.* 1985) and dinoflagellates (Falkowski *et al.* 1985; Prézelin and Sweeney 1978). In eukaryotic microalgae the cellular proportion of photoprotective xanthophylls increases at higher irradiances in diatom species such as *Thalassiosira weissflogii* (Fujiki and Taguchi 2001) and dinoflagellate species such as *Prorocentrum micans* (Dubinsky *et al*. 1986).

High performance liquid chromatography (HPLC), together with fluorometry and spectrophotometry, are often used for analysis of phytoplankton chlorophylls and other pigment suites (IOCCG report number 1, 1998; Stuart et al. 1998; Jeffrey *et al.* 1999). The HPLC technique is recommended by many researchers as it separates and accurately quantifies all major pigments and their degradation products such as chlorophyllide *a* (Bidigare *et al.* 1985; Trees *et al.* 1985; Jeffrey and Hallegraeff 1987; Jeffrey *et al.* 1999; Sigleo *et al.* 2000 and others). Besides Chl *a* concentrations, the technique provides information on a range of pigments which can be useful from a chemotaxonomic perspective in indicating phytoplankton community structure (Jeffrey *et al.* 1999). The HPLC technique is however not always a suitable method for Chl *a* analyses at sea and the standard fluorometric method, such as the Welschmeyer non-acidification technique (1994) is preferred as it was found to be quick and accurate (Trees *et al.* 2000).

It has been reported as far back as in the early 1960s that decomposition occurs in chlorophylls during extraction procedures. These degradation products are not necessary stable making chlorophyll

determination of water samples problematic. It is thought that chlorophyll *a* degradation takes place as a result of the methods used to extract pigments (Moreth and Yentsch 1970; Suzuki and Fujita 1987; Barrett and Jeffrey 1964, 1971; Jeffrey and Hallegraeff 1987; Jeffrey *et al.* 1999). Pigment extraction is done by soaking in acetone, methanol, or ethanol, and is aided by grinding, heating, or sonication. This causes breakage of the chloroplasts in the cells and these methods thus disorganise the thylakoid membranes inducing activation of the enzyme chlorophyllase (Moreth and Yentsch 1970) with varying results in test cultures. Generally in dinoflagellate species, for example *Prorocentrum minimum*, small amounts of decomposed components are found. In the diatom species, however, large amounts of chlorophyllide *a*, with insignificant amounts of pheophorbide *a*, are found in pigment extracts, for example *Skeletonema costatum*. Suzuki and Fujita (1986) reported that almost 65% of chlorophyllide *a* was found as a decomposition product in this species. Sigleo *et al.* (2000) reported chlorophyllide *a* levels up to 75% in oceanic waters towards the end of a diatom dominated bloom which in this case reflects that the cells have reached a mature or near senescent state. In many diatom cultures a high chlorophyllase activity has been found (Barrett and Jeffrey 1964, 1971), but not in dinoflagellates (Jeffrey and Hallegraeff 1987, Rodriguez *et al.* 2002). In a study of 10 classes of phytoplankton Jeffrey and Hallegraeff (1987) reported particularly high chlorophyllase activity in the diatom species of *Chaetoceros*, up to 100% of total Chl *a*. In contrast the dinoflagellate *Gymnodinium* sp. showed 0 – 2% chlorophyllide *a* of total Chl *a* formed and *Prorocentrum micans* showed 0% conversion.

The development of new techniques in HPLC analysis of phytoplankton have resulted in the discovery of a number of new pigments, but they have not been very effective in protecting the sample against chlorophyllase activity. Sigleo *et al.* (2000) however, reports that the effect of chlorophyllase activity may be minimised by reduced filtration time (less than 2 min) and by using 100% acetone for extractions.

1.3 Absorption

The absorption spectra of phytoplankton are useful for estimating the efficiency whereby a particular microalga can harvest light (photons or quanta) from the underwater radiation field. The Chl *a*-specific absorption coefficient at the wavelength of interest can be calculated since absorbance is proportional to the absorption coefficient (Kirk 1996). The large, flattened, membrane-bound sacs, the thylakoids, are the fundamental light absorbing organelles contained in the chloroplasts (Salisbury and Ross 1985). The absorbance spectra of these thylakoids are determined by the type and quantity of the chlorophyll/protein complexes present or attached to the membranes (Kirk 1996).

It has been reported that the pigment-specific absorption coefficient at any wavelength can differ considerably between species as a result of the size and shape of chloroplasts, cells or colonies, their pigment composition (Morel and Bricaud 1981; Osborne and Geider 1989; Kirk 1996; Raven and Kübler

2002) as well as the physiological state of the cells (Bricaud *et al.* 1995). These differences are due to the *package effect*, which is a consequence of the pigment molecules being contained in discrete packages within the chloroplasts, cells or colonies, instead of being uniformly distributed. The light harvesting capacity of the photosynthetic pigments is reduced when they are contained in packages, with a resulting reduction in cell growth/primary production (Kirk 1996). The most inefficient light collection takes place in large spherical colonies (58 µm diameter) whilst an increase in efficiency is achieved in long, cylindrical filaments. In picoplankton (<2 µm diameter) the package effect is of no consequence (Kirk 1996).

Variations in light harvesting efficiencies can also be brought about by self-shading in concentrated phytoplankton cultures and microalgal blooms, as light is rapidly attenuated due to the high number of individual cells present. On the other hand, in dense microalgal populations, the light scattering properties increase considerably, particularly in monospecific blooms of diatoms and coccolithophorids. This causes the backscattering of light to be more intense than that of algae enclosed in less refractile and thinner cell walls (Vaillancourt *et al.* 2004). All these factors have a significant effect on the microalgae's light harvesting efficiency for photosynthesis and therefore have an effect on cell growth (biomass) and primary production.

1.4 Quantum efficiency

Photosynthetic efficiency is generally quantified as the light-limited, maximum quantum yield in geophysical studies. The quantum yield of photosynthesis (ϕ) is an estimated measure of photosynthetic efficiency expressed in moles of CO_2 fixed per mole of photons absorbed. The maximum quantum yield (ϕ_m) is measured when photosynthesis is light-limited (Singsaas *et al.* 2001). Theoretically, ϕ_m is 0.125, meaning that 8 to 10 moles of photons are required to reduce 1 mole of CO_2 in the absence of photorespiration. Due to cyclic photophosphorylation, ϕ_m is usually closer to 0.112 in C_3 plants (Long *et al.* 1993). Values for ϕ_m of gross carbon uptake can typically be as low as 0.06-0.08 (Laws 1991; Sakshaug 1993). Karl *et al.* (2003) recorded ϕ_m values as low as 0.017 to 0.028 mol C mol quanta^{-1} during cruises conducted in winter, spring, summer and autumn. In natural communities ϕ_m can be <0.005 and highly variable (cited in JGOFS 1998). In other comparable studies, quantum yields were either found to be near the maximum theoretical value and invariable, or could be as low as 30% and varied among different species (Singsaas *et al.* 2001). Since there is a distinction between quantum yield calculated from O_2 evolution *versus* CO_2 assimilation (Singsaas 2001), it must be noted that the measurements of quantum yields in this study were calculated from CO_2 fixation and therefore are lower than ϕ_m values calculated from O_2 evolution.

1.5 Carbon : chlorophyll a ratio

The carbon : chlorophyll *a* ratio is one of the most widely used indices of the photoacclimated state of photosynthesis. The C : Chl *a* ratio is constantly changing as a result of fluctuations in environmental conditions with delayed responses at different time scales (Falkowski and Raven 1997; Geider *et al.* 1996). The ratio provides a link between phytoplankton growth rate and chlorophyll *a*-specific photosynthetic rates. Knowledge of the environmental factors that influence the parameters of the *P vs E* curve, α* and C : Chl *a* ratios of microalgae is particularly relevant to the modelling and mapping of phytoplankton production from ocean colour data (Platt and Sathyendranath 1988). As a result of photoacclimation, a decline in C : Chl *a* occurs in cells that maintain a constant P^*_m and α* (MacIntyre *et al.* 2002).

1.6 Production in the Benguela Current

Primary production is high in the southern Benguela Current as a consequence of upwelling in the region (Nelson and Hutchings 1983, Shannon 1985, Shannon and Nelson 1996). Satellite-based estimates of potential primary production in those areas with chlorophyll *a* greater than 1 mg m^{-3} showed the Benguela Current to be very productive ((370×10^6 tons C $year^{-1}$) closely followed by the Humboldt Current (200×10^6 tons C $year^{-1}$) (Carr 2002).

Several upwelling cells are formed as a result of wind/pressure fields. When these coincide with a topographical feature such as the shelf edge, convoluted upwelling filaments are formed. Upwelling events are very variable and seasonal (Nelson and Hutchings 1983; Hutchings 1992; Shannon and Nelson 1996). Maximum upwelling in the southern part of the Benguela Current ($33^0 – 34^0$ S) generally occurs in summer between the months October to March with surface temperatures of < 15^oC (Tilstone *et al.* 2002). Rates of production in the vicinity of the Cape Peninsula were estimated to be 2.2 g C m^{-2} d^{-1} in winter and 4.1 g C m^{-2} d^{-1} in summer (Brown 1984). Brown *et al.* (1991) calculated mean rates of 2.0 – 3.5 g C m^{-2} d^{-1} for the southern Benguela Current. Demarcq *et al*. (2008) reported midsummer production up to 5mg C m^{-2} d^{-1} with a yearly mean of > 1.4 mg C m^{-2} d^{-1} in the southern Benguela upwelling system. In the St Helena Bay region inshore production was estimated to be 4.0 - 5.6 g C m^{-2} d^{-1} within frontal blooms, but production in offshore waters beyond the upwelling front was lower, ranging from 2.4 - 4.0 g C m^{-2} d^{-1} (Mitchell-Innes *et al.*, 2000).

In terms of carbon phytoplankton populations are generally dominated by diatoms and dinoflagellates, although some studies have emphasized the importance of nanoflagellates (Mitchell-Innes and Winter 1987; Walker and Pitcher 1991; Pitcher *et al.* 1992). Diatoms (mixers) tend to dominate inshore in recently upwelled, nutrient-rich water, whereas dinoflagellates (migrators) and nanoflagellates are more important offshore on the seaward side of the fronts (Barlow *et al.* 2006, 2005, 2001).

Red tide blooms of toxic or non-toxic species of dinoflagellates generally occur during quiescent periods in aged, upwelled waters as the upwelling activity diminishes and thermal stratification increases (Pitcher *et al.* 1998). In the southern Benguela region, phytoplankton abundance is highly variable. Maximum concentrations occur in the vicinity of the greater St. Helena Bay region, 20 – 80 km offshore (Pitcher *et al.* 1998), although significant levels can extend to 100 km offshore following periods of active upwelling (Brown *et al.* 1991). Barlow (1982) reported phytoplankton biomass (chlorophyll *a*) in recently upwelled water, maturing upwelled water and aged water to be < 1, 1 – 20 and 5 – 30 mg m^{-3} respectively. Demarcq *et al.* (2003) estimated biomass concentrations of 0.1 mg m^{-3} to > 10 mg m^{-3} along the coastal shelf in oligotrophic waters from Cape Town to northern Namibia.

Dinoflagellates are able, to a certain extent, to regulate their position in the water column by using their flagella. This is an important feature that allows this phytoplankton group to have some control over its light environment and enables it to out-compete other species. Diatoms on the other hand, by regulating their cellular buoyancy, have very limited control over their position in the water column (Cullen and MacIntyre 1998) and tend to occur in recently upwelled or recently mixed water.

1.7 Objectives

In this study the object was to investigate the photoacclimation responses at three different light intensities of monocultures of selected dinoflagellate and diatom species which would be grown under controlled environmental conditions in a laboratory situation. Several species of dinoflagellates and diatoms in the southern Benguela region were isolated for this purpose. The experiments could be carried out at the Marine and Coastal Management laboratory in Sea Point. The laboratory was properly equipped and had been approved for radioactive isotope work by the Department of Health.

Measurements of the physiological acclimation of in situ phytoplankton, such as I had undertaken during ocean research cruises in the southern Benguela Current region, are usually difficult to interpret. This is due to the unknown history of the organisms before sampling and the algae may have been transported from a different environment as is often the case along the inshore regions of the Benguela ecosystem. In the laboratory, however, conditions can be controlled and the characteristics of algae as a function of environmental factors (irradiance, temperature and nutrients) can be investigated and parameterised. Growing microalgal cultures under laboratory conditions is, however, not entirely ideal. It has been reported, for instance, that measurements obtained from laboratory grown phytoplankton compared with those from field data may result in a two-fold overestimation of productivity (Kirk 1996). In view of these findings particular care has been taken to carry out experimentation as precisely and accurately as possible.

As mentioned above, the aim of the research reported in this thesis was to examine the photoacclimation strategies in selected marine diatoms and dinoflagellates that represented important components of the phytoplankton communities of the southern Benguela Ecosystem. Understanding the processes and

limitations of photoacclimation will aid in modern ventures such as commercial algal mass cultures and photo-bioreactors, as similar responses to changing light intensities take place (Dubinsky *et al.* 1995).

Measurements of the rate of photosynthesis, concentration of chlorophylls and carotenoids, particulate absorption and carbon content were undertaken in microalgal cultures fully *acclimated* [*3] to low, medium and high light intensities.

The following key questions were investigated using the methods and techniques discussed in Chapter 2.

1.7.1. Experiments - Photoacclimation

1) What are the changes in photosynthetic parameters (P^*_m, α^*, E_k,) in species of dinoflagellates and diatoms that are exposed to selected light intensities. Does cell size/volume influence these changes?

   Photosynthesis, in terms of carbon dioxide fixed at selected light intensities, can be measured. The changing photosynthetic parameters reflect the ability of the microalgal species to utilize light of a given intensity. The rate at which this photoacclimation takes place is, in turn, dependent on the light climate that the phytoplankton cells were exposed to during growth.

2) What are the changes in cellular pigment concentrations in species of dinoflagellates and diatoms that are exposed to selected light intensities?

   As the physiological state within the algal cells changes, the efficiency with which the photosynthetic apparatus can make use of the absorbed energy (quanta) to fix CO_2 depends on the concentration of the photosynthetic pigment Chl *a*, of additional accessory pigments Chl *b* and *c* and of various carotenoids. These extend the light-harvesting spectrum in phytoplankton.

3) Does the chlorophyll-*a* specific absorption coefficient in species change under varying irradiances?

   The light-harvesting efficiency, hence the absorption coefficient, of the photosynthetic pigments in microalgae is determined by the composition and concentration of the chlorophyll/carotenoid-protein attached to the thylakoid membranes. The total concentration of pigments and the relative amounts of the different pigments present can all be affected by light intensity. The specific absorption coefficient per unit pigment can vary markedly between and within different species at any wavelength. Chl *a*, including its allomers and epimers, represents the main light-harvesting pigment in photosynthetic microalgae.

*[3)] Acclimation: describes a change in the macromolucelar composition of an organism that occurs via synthesis breakdown of specific components, operating within limitations of the genetic make-up of the population. By way of contrast – adaptation describes an outcome of selection that involves changes in the genetic composition of a species or population (Williams *et al.* 2003).

1.7.2. Time series experiments - rate of photoacclimation

1) Does the C : Chl *a* ratio increase in dinoflagellate and diatom organisms when they are shifted from high to low irradiances and *vice versa* during short time spans *i.e*. 0.5 h.

   Under conditions of balanced growth, C : Chl *a* serves as a proxy for light absorption. Geider and MacIntyre (2003) showed that the ratio of carbon to chlorophyll *a* in natural phytoplankton populations constantly changes. For instance, the C : Chl *a* in the northern North Sea was reported to be at its lowest value of 20 : 1 in spring, decreasing to 100 : 1 in late summer reflecting the seasonality of the solar insolation. It is hypothesized that measurable changes in the ratio would take place over short time spans, *i.e*. 0.5 h.

2) What are the changes in C : Chl *a* ratio in selected species of dinoflagellates and diatoms when they are exposed to different irradiances during prolonged time spans?

   The ratio of cellular C to Chl *a* for a single species can vary by more than a factor of 10 in response to varying growth conditions such as irradiance, nutrient availability and temperature (Geider and Osborne 1987).

3) Is chlorophyll *a* to total pigment (Chl *a :* Tpig) fraction correlated with photosynthetic quantum efficiency (PQE) in phytoplankton species grown under varying light intensities during short time spans?

   Chl *a* is synthesised in preference to accessory pigments in response to limiting conditions, and the concentrations are maintained at a level needed for maximum growth rate. Chl *a*, therefore, is self regulating in response to changing environmental (light) conditions. As Chl *a* has a unique deep blue absorption spectrum (around 443 nm) and the accessory pigments absorb around 490 nm, the Chl *a* : Tpig ratio has a distinct optical signature. This is detectable in remotely sensed observations of ocean colour, useful for providing a bio-optical algorithm for photosynthetic quantum efficiency (Aiken *et al.* 2003).

   The dinoflagellates and diatoms occupy turbulent environments within marine upwelling systems. The objective of this thesis therefore is to examine the photoacclimation strategies of these two groups of microalgae under controlled laboratory conditions where the phytoplankton can be grown at stable temperatures (17.0 ^{o}C), in a nutrient replete medium and with cultures maintained in a state of exponential growth. The very variable responses of natural phytoplankton populations to changing environmental conditions, not only within taxa, but also between individual species, make it desirable to conduct laboratory experiments with single species. With a detailed understanding of how environmental light conditions influence microalgal growth for species in

culture, it may be possible to extrapolate to the different algal assemblages in the natural environment (Flynn and Fasham 2003). The information obtained from the experiments reported here will be useful in furthering the understanding of the photoacclimation mechanisms that occur when microalgae are exposed to varying irradiances and the effect they have on biochemical and physiological characteristics. Experiments with cultures, therefore, are central to our understanding of the physiological responses of microalgae to environmental variability such as those encountered in the Benguela ecosystem.

CHAPTER 2. MATERIALS AND METHODS

All laboratory techniques used in this project were based on published methods used in the study of phytoplankton photo-physiology. Uni-algal cultures were used to investigate the relationship between irradiance and photosynthesis (*P vs. E*) for eight algal species and was measured using the classical ^{14}C method initially put forward by Steemann-Nielsen (1951; 1952). Lewis and Smith (1983) recommended a small-volume, short-incubation time method for measuring this relationship using a photosynthetron. A more modern method is described by Evans *et al.* (1987) which considers refinements in methodology recommended by recent research. The relationship between photoacclimation responses and cell volumes, photosynthetic (PSP) and photoprotective (PPC) pigment concentrations and ratios, chlorophyll *a*-specific absorption coefficients (a*) and carbon and nitrogen contents were also investigated.

MacIntyre and Cullen (2005) give a detailed account of the techniques used for isolating single cells, maintenance of cultures, enumeration of cells and determination of acclimated growth. The HPLC method described by Barlow *et al.* (1997) was used for the analysis of phytoplankton pigments. Absorption measurements were conducted according to Tassan and Ferrari (1995) and Roesler (1998). Others are referenced below in the appropriate sections.

2.1 Cultures

Five dinoflagellate and three diatom species of phytoplankton isolated from the coastal oceanic environment (southern Benguela ecosystem) were used in the experiments. Five dinoflagellate species were obtained from the Marine and Coastal Management culture collection, which were isolated and maintained by Desirée Calder. For the three diatom species, single cells of the individual species were isolated by me from seawater samples taken in August 2001 from Saldanha Bay on the west coast of South Africa. An inverted light microscope with a stage designed for a multiwell plate was used. Target cells were isolated with a Pasteur pipette which was drawn out at the tip. For the isolation method, a flexible latex tube was attached to a mouthpiece at one end and the micropipette at the other. An enriched natural seawater medium (see "2.1.1 Growth medium") was used for culturing the isolated cells. Cultures were incubated in a growth chamber equipped with cool-white fluorescent light, set at a temperature of $17^{o}C$. The cultures were maintained at 30-60 µmol quanta m^{-2} s^{-1} on a light-dark cycle of 12:12 hours.

The dinoflagellate species previously isolated from the west coast of South Africa were:

> *Alexandrium catenella*, a thecate species known to produce paralytic shellfish poisoning (PSP) toxins (Pitcher 1998), (Hamasaki *et al.* 2001), Cape Town Culture Collection 05, Yzerfontein 2000;

Protoceratium reticulatum ((Claparède and Lachmann) Bütschli, 1885. p. 1007, pl. 52), thecate. Cape Town Culture Collection 01, Lamberts Bay 2000;

Prorocentrum micans (Ehrenberg, 1833. p.307), thecate. Cape Town Culture Collection 03, Lamberts Bay 2000;

Prorocentrum triestinum (Schiller), thecate. Cape Town Culture Collection 08, Lamberts Bay 2000;

Gymnodinium zeta, athecate (or 'naked"). Cape Town Culture Collection 09, Lamberts Bay 2000.

Diatom species isolated were:

Chaetoceros cf. *pendulus*, Cape Town Culture Collection 029, Saldanha Bay 2001;

Chaetoceros capense, Cape Town Culture Collection 030, Saldanha Bay 2001;

Chaetoceros sp., Cape Town Culture Collection 031, Saldanha Bay 2001.

The most numerous diatom species observed in samples of the southern Benguela Ecosystem were of the genus *Chaetoceros* Ehrenberg (Author's unpublished data). Samples were collected over a period of 12 months during June 2000/July 2001 (Appendix 2.1). The genus *Chaetoceros* is considered to be the most variable genus of truly planktonic diatoms, containing over 160 known species (Boden 1989).

The linear measurements of the dinoflagellate and diatom species, obtained microscopically during cell counts, were used to estimate the cell volume of the different species. Cell volumes were calculated using the following formulae as routinely used in the laboratories of Marine and Coastal Management, Sea Point, Cape Town:

		Volume	Length
Alexandrium catenella:	$V = \pi . D^2 . L / 6$	(238 μm^3)	(18-19 µm)
Protoceratium reticulatum	$V = \pi . D^3 / 6$	(358 μm^3)	(30-50 µm)
Prorocentrum micans	$V = \pi . D^2 . L / 6$	(740 μm^3)	(30-48 µm)
Prorocentrum triestinum	$V = 2 (\pi . D^2 . L) / 12$	(69 μm^3)	(7-20 µm)
Gymnodinium zeta	$V = 2 (\pi . D^2 . L) / 12$	(28 μm^3)	(6-20 µm)
Chaetoceros cf. *pendulus*	$V = \pi . D^2 . L / 4$	(253 μm^3)	(24-48 µm)
Chaetoceros capense	$V = \pi r^2 * L$	(166 μm^3)	(18-37 µm)
Chaetoceros sp.	$V = \pi r^2 * L$	(513 μm^3)	(24-53 µm)

Where πr^2 = area

L = length

D = diameter

2.1.1 Growth medium

Most species in marine culture collections are maintained in natural seawater enriched with major inorganic nutrients, trace metals and vitamins. Although natural seawater provides a good base for broad spectrum media, variations in its quality throughout the year need to be monitored and corrected to keep certain delicate species in a healthy state when the water inhibits growth (Harrison et al. 1980). Jeffrey and LeRoi (1997) state however that natural seawater is the preferred base for micro algal culture media, because artificial seawater contains large quantities of salts that can add excessive quantities of unknown and unwanted contaminants to the final media. Natural seawater, properly filtered and purified has always given excellent results. It should be obtained offshore to minimize the concentration of metal and organic pollutants and fine sediment, as inshore water can be highly variable in salinity and in organic and inorganic loads. This variability can produce very variable algal growth rates (Jeffrey and LeRoi 1997). These could be minimised, but not entirely eliminated, by charcoal treatment (Harrison et al. 1980). The Marine and Coastal Management Research laboratory in Sea Point, Cape Town, collects seawater from approximately 1 km distance off-shore south west of Cape Town at approximately 10m depth. The seawater is purified by filtering it sequentially through three filters. The purified seawater is stored at 18^{o}C in the dark. The temperature is set by the Sea Point Aquarium, Marine and Coastal Management.

To ensure the growth medium was sterile the natural seawater was filtered twice, using 0.22 µm filters, to obtain a particle-free medium, after which it was autoclaved in 1 L polycarbonate bottles for 30 min at 120^{o}C. Keller (K) medium was added after the seawater had cooled.

Table 2.1: Keller *et al*. (1987) Marine Enrichment Basal Salt mixture used for microalgal culturing, tabulated in sequence for ease of mixing.

Major nutrients and trace metals	**mg L^{-1}**
Ammonium Chloride	2.6750
Cobalt Sulfate Hydrate	0.0141
Cupric Sulfate Pentahydrate	0.0025
Disodium EDNA Dihydrate	37.220
Ferric-Sodium EDTA	4.2950
Glycerophosphate Disodium Hydrate	3.0610

Manganese Chloride Tetrahydrate	0.1800
Sodium Molybdate Dihydrate	0.0060
Sodium Nitrate	75.000
Sodium Selenite	0.0017
Trizma Base	121.10
Zinc Sulfate Heptahydrate	0.0220
Vitamins solution	0.5
Component of Keller medium for diatom culturing	
Sodium Metasilicate (vacuum-dried)	6.5890
Primary stock solutions:	
Biotin	5 mg in 50 mL distilled water
B_{12}	10 mL in 100 mL distilled water
Vitamin working solution:	
Thiamin HCl – B1	0.2 mL in 100 mL distilled water
To this solution was added:	
Biotin (primary stock)	1 mL
B_{12} (primary stock)	1 mL

The medium used here was prepared according to Keller *et al*. (1987) using Marine Enrichment Basal Salt mixture (K) without silicate (Sigma Product No. K1630) for culturing dinoflagellates. For culturing diatoms, 1 mL of sodium metasilicate solution per litre of natural seawater was added to the Keller medium.

The components of the medium are shown in Table 2.1 above. Thiamine, biotin and vitamin B_{12} were added to all culture media as in a procedure described by Jeffrey and LeRoi (1997).

2.1.2 Culture maintenance

The monocultures were maintained using the batch culturing method as described by Guillard (1975). This approach requires the transfer of small amounts of exponentially growing cells to a new flask of enriched medium as a means of keeping the cells in exponential growth and nutrient replete. This was done at regular intervals of 2 – 3 weeks or more frequently, depending on the requirements of the faster growing species of phytoplankton. Although this method requires many new culture bottles, it does ensure that the medium remains fresh, as well as providing the cells with a continuous supply of nutrients and preventing nutrient limitation and nutrient stress. The cultures were grown in 250 mL culture bottles in a temperature controlled room (17 ^{o}C) on a 12:12h light-dark cycle and were shaken daily. At no time was there any

sign of diatoms or dinoflagellates sticking to the sides of the flasks as the cultures were transferred to a new flask at regular intervals.

Depending on the culture bottle's position on the shelf, the irradiance (cool-white fluorescent lights measured inside the flask with a QSL-100 4π quantum scalar irradiance sensor, Biospherical Instruments, San Diego, CA, USA) ranged between 40-80 µmol quanta m^{-2} s^{-1}.

2.2 Photoacclimation experiments

The monocultures of the diatoms and dinoflagellates were incubated for 4 days in temperature controlled waterbaths (17 ^{o}C) under light intensities of 647, 178 and 33 µmol quanta m^{-2} s^{-1}. Light was provided by three overhead growth lamps enclosed in highbay aluminium reflectors (Unit model HB50 with Osram 400W MHE lamp). The lamps were shaded with neutral stainless steel mesh screens of different mesh densities to produce low, medium and high light intensities. The light intensities simulated approximately 32% (high light), 9% (medium light) and 1.6% (low light) of surface irradiance at midday (2000 µmol quanta m^{-2} s^{-1}). Before acclimation in the waterbaths under the three different growth conditions, the culture samples were diluted, thus maintaining the cultures in exponential growth. The temperature in the waterbaths was maintained at 17 ^{o}C (± 0.1 ^{o}C) by circulating water at a high rate through a thermostatically controlled cooling system.

2.2.1 Sampling

After the cultures were acclimated for 4 days at a designated light level, subsamples were withdrawn for incubation in the photosynthetron. Every experimental session was started at midmorning (9.30 am local time) to keep diel photic cycle variations to a minimum. Additional subsamples were used for cell counts, phytoplankton absorption, Chl *a* measurements, pigment concentration, and establishing carbon and nitrogen content. All subsamples were kept refrigerated and processed in dim light.

2.3 Photosynthesis *versus* Irradiance Experiments

2.3.1 Photosynthetron

The design of the photosynthetron used for the incubations was adapted from the model described by Lewis and Smith (1983). The apparatus consisted of two separate compartments to allow for duplicate measurements. They were side by side, with a built-in cooling system for independent temperature control (Figure 2.1). The cooling system provided a constant flow of water through the incubation chambers, keeping the temperature at 17 ^{o}C (±0.1 ^{o}C). Each compartment consisted of an upper aluminium block provided with water passages. The block contained 30 tubes with an inner diameter equivalent to the outer diameter of the 20 mL scintillation vials used to hold the sample material. Cooling water was directed through the ported aluminium block and a plate glass compartment consisting of two sheets of plate glass,

each 0.5 cm thick. This glass compartment attenuated most of the infrared radiation. The light source (General Electric 500W tungsten halogen lamp) was placed below the glass, illuminating the bottom of the vials from underneath. The spectral signature of the tungsten-halogen lamp used in the incubator is illustrated in Figure 2.2. The scintillation vials were exposed to light ranging from 0 to 1200 µmol quanta m^{-2} s^{-1} by randomly placing various grades of neutral density filters at the bottom of the aluminium tubes. Irradiance measurements were made with a QSL-100 4π quantum scalar irradiance sensor (Biospherical Instruments, San Diego, CA, USA) placed inside a scintillation vial with 1 mL filtered seawater.

2.3.2 Photosynthetron incubation

The ^{14}C technique for marine waters is outlined by Evans *et al.* (1987) and Strickland and Parsons (1968). The method differs little from Steemann-Nielsen's (1951, 1952) original procedure except that liquid scintillation counting has replaced Geiger-Müller counting. The purpose of this method is to estimate the uptake of dissolved inorganic carbon (DIC) from the water by phytoplankton as it photosynthesises in the light. The carbon taken up either remains in the plankton as particulate organic carbon (POC) or is excreted into the water as dissolved organic carbon (DOC), a fraction of which may be hydrolised and converted to $^{14}CO_2$. This fraction may escape from the solution and is not counted. DOC was not measured during these experiments. The ^{14}C method measures the loss of dissolved inorganic carbon (DIC) by acidifying the whole seawater sample and adding scintillation cocktail directly to it. This technique does not discriminate between dissolved and particulate ^{14}C and therefore measures the sum of particulate organic carbon (POC) synthesis and any DIC loss (Carpenter and Lively 1980; Lewis and Smith 1983).

(a)

(b)

Figure 2.1: (a) Photosynthetron with dual control providing for two simultaneous incubations at different individual temperatures. The instrument is easily transportable and can be safely used at sea. (b) View of the open compartment on the left showing 30 tubes in a water-cooled ported aluminium block illuminated from below. (The photosynthetron was manufactured by Scientific Manufacturers CC Cape Town).

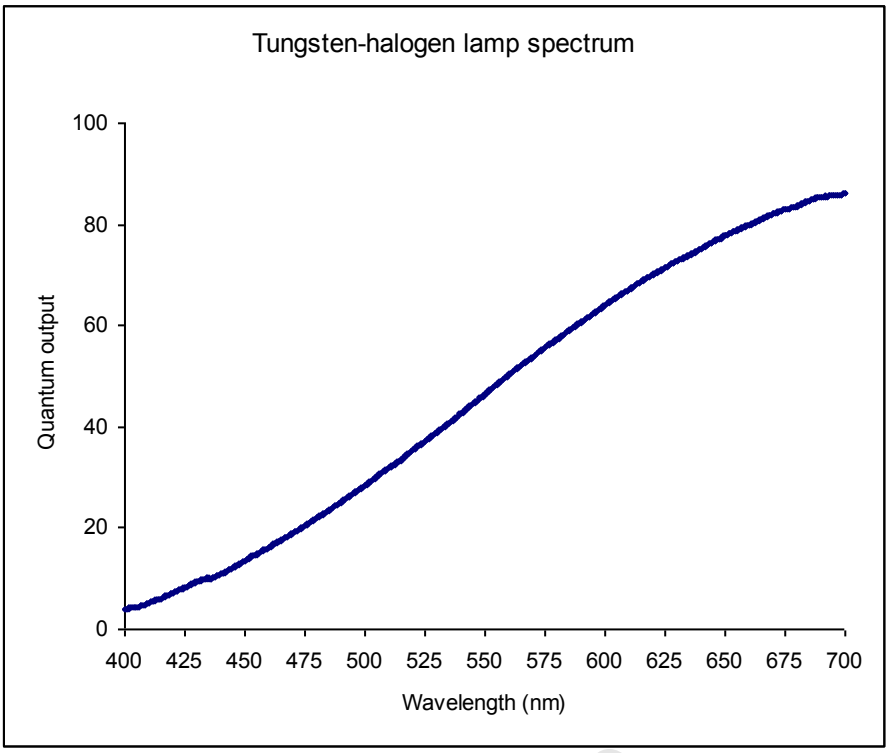

Figure 2.2: Spectral signature of the tungsten-halogen lamp measured and used as a light source in the photosynthetron.

2.3.3 Technical problems

To ensure that reliable data were collected during the experiments, the following precautions were taken.

1) Doty and Oguri (1959) recommended that to prevent a decrease in the uptake of $NaH^{14}CO_3$, care had to be taken to prevent contact of water samples with toxic materials. Hence sterile polypropylene culture bottles and hard glass scintillation vials were used in the experiments.

2) To prevent samples from becoming photo-inhibited, exposure to strong light prior to the experiments was avoided.

3) The solution used to dilute the concentrated stock solution of $NaH^{14}CO_3$ was buffered at basic pH (9.5) so that most of the ^{14}C present in the carrier HCO^-_3 would not lose counts on exposure to air.

4) MilliQ water was used in making up the stock solutions to prevent, as far as possible, other dissolved substances influencing the results. To prevent silica leaching from glass containers into the stock solution, the stock solutions were stored in Polypropylene Eppendorff tubes and kept frozen until use.

5) After 10 mL scintillation fluid was added and before scintillation counting, the subsamples were kept in the dark for 12 h to prevent undue excitation by reducing chemoluminescence (Hawkins and Steiner 1994).

6) The total activity of ^{14}C in the inoculated culture sample is needed to calculate the fraction of the ^{14}C taken up. To prevent any loss of ^{14}C, a small volume of the whole culture sample was counted by liquid scintillation with the added CO_2-binding base, ethanolamine.

7) A zero-time blank sample was counted to assure that contaminants did not bias the production estimates.

8) Each sample was counted with a known efficiency, external standard quench correction.

2.3.4 Experimental procedure

Fifty mL of algal culture was placed in a beaker and $NaH^{14}CO_3$ added with stirring. Total activity was determined using triplicate aliquots (0.1 mL) mixed with 10 mL scintillation fluor plus 0.01 mL 6N NaOH and 0.1 mL ethanolamine. A small volume (1 mL enriched with 37000 Bq mL^{-1} $NaH^{14}CO_3$) **(*1)** of acclimated phytoplankton culture was dispensed by repetitive pipetting into 30 glass scintillation vials that were placed in the photosynthetron and illuminated from below for 20 min. At the end of the incubation, the samples were acidified with 0.5 mL 6N HCl, shaken for 1 h to purge inorganic carbon, after which the solution was neutralized with 0.5 mL of 6N NaOH. The scintillation fluor (Packard Optima Gold, XR) was added directly to the vials. The five zero-time controls were treated identically except that they were acidified immediately at the beginning of the incubation. (See Appendix 2.2 for detailed procedure).

After the samples were neutralized and the fluor added, the vials were stored in the dark for 12 h before counting took place. The ^{14}C disintegration per minute (DPM) was measured on a Beckman LS 1800 liquid scintillation counter using the external standard and the channel ratio methods to correct for quenching.

The data was fitted by a least squares non-linear parameter estimation routine using the statistical package Statistica 6 (1984) to the following photosynthesis-irradiance model of Platt *et al.* 1980(a):

$$P^B(E) = P^B m\,[1 - \exp(-\alpha^B E / P^B m)] \qquad (2.1)$$

where P is the instantaneous rate of primary production per unit volume of water, B is the photosynthetically active biomass, E the irradiance, parameter α^B is the light-limited slope of the curve as E

*[1] 1microcurie (1 µCi) = 3.7 x 10^{-4} disintegrations per second = 37000 Becquerel (Bq) (A Dictionary of Chemistry, Oxford University Press).

tends to zero and $P^B m$ is the observed maximum photosynthetic rate normalised to chlorophyll *a (*mg C mg Chl *a* h^{-1}) if there is no inhibition (Lewis and Smith 1983). The goodness of fit for all curves had values of R^2 in excess of 0.97. All experiments were replicated in triplicate where possible.

2.4 Quantum yield

The *P vs E* relationship was used to calculate the quantum yield, ϕ, which represents the efficiency with which the absorbed light is converted to carbon. The parameter α^* is defined in terms of ambient (irradiance) light. ϕ_m is defined in terms of absorbed light. Since the *P vs E* curve was expressed in terms of photosynthetically active radiation (PAR), the maximum quantum yield, $\phi_{m,}$ should be equivalent to the quantum yield in the region of the initial slope, α, normalised to chlorophyll *a* (JGOFS 1998). The equation used was:

$$\phi_m = \frac{\alpha^{Chl}}{A^{Chl}} \qquad (2.2)$$

where:

α^{Chl} = initial, light limited rate of photosynthesis in the *P vs E* curve;

A^{Chl} = integrated, Chl *a*-specific absorption coefficient over PAR;

(400 -700 nm).

The units used for ϕ_m were CO_2 fixed per mol quanta absorbed photons.

2.5 Cell counts

The Utermöhl method was used for counting dinoflagellate and diatom cells (Utermöhl 1958). The samples were fixed with 2% formalin and counted within two days of preservation. Before drawing the samples, the main volume of culture was gently mixed to give a homogeneous suspension of the single celled species of dinoflagellates, of diatoms and of chain-forming diatoms, giving a random distribution in the counting chamber. A 10 mL sample, measured volumetrically, was settled into a 2 mL volume circular counting chamber. Before counting the contents of the chamber, the distribution of the settled organisms was examined to make sure that the distribution was even. In the case of chain-forming diatoms and clumped dinoflagellates, it was sometimes necessary to dilute the sample with filtered seawater (dilution factor is final volume/initial volume). To estimate the number of cells mL^{-1}, the microscope stage was slowly moved from left to right over a measured distance and the cells counted. At the beginning of each new sweep, the slide was very slightly turned on the microscope stage covering randomly the circular area of the chamber. Depending on the total count obtained, five to seven sweeps of equal length were made for each sample. All samples were counted using an IM35 Zeiss Inverted Microscope. The concentration of the cells in the culture was calculated as:

cell concentration (cells L^{-1}) = slide area (mm^2)/area counted (mm^2) x 1000/volume settled (mL); (2.3)

area counted (mm^2) = graduation width x number of sweeps x length of sweep (mm); (2.4)

slide area = 490.87 mm^2.

2.6 High performance liquid chromatography

High performance liquid chromatography (HPLC) made it possible to separate and quantify simultaneously the complex range of chlorophylls and carotenoids and their degradation products. It provides a powerful tool for studying the changes in phytoplankton pigment pools. Numerous HPLC techniques have been published to date, each with their own advantages and limitations. The reverse phase C-8 HPLC method used here, as described by Barlow *et al.* (1997) and Barlow and Sessions (2000) with suitable sample collections and extraction, yielded adequate detection of pigments present in low concentrations.

During each incubation experiment, 10 mL aliquots of suspended algal cells were taken for pigment analysis. These were filtered through 25 mm Whatman GF/F filters under low vacuum (200 mmHg). The filter was carefully dried between absorbent paper towels, folded, placed in a cryovial, immediately frozen and stored in liquid nitrogen until analysis.

The HPLC system consisted of a Varian ProStar tertiary pump, a Thermo Separation Products (TSP) AS3000 autosampler, a TSP UV6000 diode array detector and computer with ChromQuest software.

For extraction, the frozen filter containing the algal sample was placed in a graduated centrifuge tube with 2 to 4 mL acetone-carotenal solvent and ultra-sonicated for 30 s with a VirTis VirSonic 60 sonicator equipped with a 2 mm diameter probe operated at about 50 W. After the sample was clarified by centrifugation in a refrigerated centrifuge, an aliquot of about 1 mL was carefully removed with a pipette, pipetted into a dark glass autosampler vial and loaded into the autosampler cooling tray. The autosampler incorporated a column compartment containing a 3 µm reversed-phase C_8 monomeric column (Hypersil MOS2) and the autoinjector. Both were heated to 25°C. Prior to injection, the autosampler was programmed to vortex mix 300 µL of extract with 300 µL of 1M ammonium acetate buffer. 100 µL of the extract-buffer was injected onto the chromatography column.

The individual pigments were separated at a flow rate of 1 mL min^{-1} over 30 min, using binary solvents in a linear step gradient. Before each subsequent injection, the column was reconditioned to its original condition over an additional 7 min.

Solvent A consisted of 70 : 30 (v:v) methanol : 1M ammonium acetate and Solvent B was 100% methanol. The gradient used is shown in the table below.

Step	Time min.	Solvent A [%]	Solvent B [%]
Start	0	75	25
2	1	50	50
3	20	30	70
4	25	0	100
End	32	0	100

An internal standard (*trans-β*-apo-8´-carotenal) was used to correct for losses in sample handling to improve estimations of pigment concentration.

Pigments were detected by absorbance at 440 nm and any chlorophyll transformation products were simultaneously monitored at 665 nm. Spectral data were collected on every sample between 400 – 700 nm. Peak areas were initially and automatically integrated using instrument default conditions. Every chromatogram was then reintegrated by manually moving peak markers and baselines to optimise the integrations.

The primary pigment standards, chlorophyll *a* and carotenal, were purchased from Sigma-Aldrich Limited. Other pigments were obtained from the DHI Institute for Water and Environment, Denmark.

2.7 Phytoplankton absorption

Absorption was measured with a UV – 2501 PC spectrophotometer using an 8 mm integrating sphere attachment (ISR - 2200). The concentration of phytoplankton samples in suspension was not sufficient for measurements in cuvettes. Depending on the particle loading, 5 – 10 mL aliquots, obtained from the stock culture used for the *P vs E* experiments, were concentrated onto 25 mm Whatman GF/F glass fibre filters under less than 12 mm mercury pressure. The filters were placed in covered Micropore petri dishes, immediately frozen and stored in liquid nitrogen until further analysis.

Six blank filter pads, for reference and baseline correction, were prepared by filtering through a volume of twice filtered sea water, equivalent to the volume filtered for the phytoplankton samples. A new set of blanks were prepared for each set of samples.

The filter pad absorption was recorded between 350 and 750 nm, initially with the concentrated phytoplankton material on the pad and secondly after the filter pads were bleached with sodium hyper chloride (NaClO) to remove the phytoplankton pigments (Tassan and Ferrari 1995).

To obtain an accurate absorption coefficient, a correction factor accounting for path-length amplification due to scattering by the glass fibres, was used following the method of Roesler (1998), with a null absorption point set to 750 nm.

The equations used were as follows:

particulate absorption coeff. =

$$\frac{(a_p(\lambda) - a_p(750\text{ nm}) - \text{bleached pad})}{(2 \times \text{volume filtered (L)}) / (\text{filter area (m}^2) \times 2.303)} \quad (2.5)$$

minus

detrital absorption coeff. =

$$\frac{(a_d(\lambda) - a_d(750\text{ nm}) - \text{bleached blank})}{(2 \times \text{volume filtered (L)}) / (\text{filter area (m}^2) \times 2.303)} \quad (2.6)$$

where

$a_p(\lambda)$ = particulate absorption reading;

$a_d(\lambda)$ = residual absorption of depigmented filter;

2 = path-length amplification factor for filter pads (Whatman GF/F glass fibre filter); (Roesler 1998);

2.303 = coefficient representing the transformation from $\log_{10}$ to $\log_e$.

2.8 Determination of particulate carbon and nitrogen

Samples were analysed on a LECO CHNS analyser 932 using the method of Ehrhardt and Koeve (1999) to determine total carbon and nitrogen in particles in the size range of approximately 0.45 and 300 μm.

Since carbon and nitrogen were to be measured, filters had to be made of inorganic material. Glass fibre filters are, therefore, commonly used to collect particles for total carbon determinations and analyses. The Whatman GF/F filters with a nominal pore size of 0.7 μm are recommended because of their better sampling efficiency (UNESCO 1994, Karl *et al.* 1991). Before use, the filters (diameter 2.5 cm) were precombusted for 24 h at 450 ^{o}C. Known sample volumes were filtered through the clean fibre glass filters under slight vacuum (0.0027 MPa) to prevent cell rupture and were immediately frozen in liquid nitrogen. Prior to analysis the filtered samples were freeze-dried for 24 h. Great care was taken to use clean sampling equipment only and all filters were handled with clean tweezers.

Small discs were cut out of the fibreglass filters with a cork borer, encapsulated in tin boats, pelletalized and finally analysed on the LECO CHNS 932 analyser with high purity Acetanilide as a standard. To prevent extraneous contributions to the analytical signal, pre-combusted, unused filters were analysed as blanks and subtracted. The procedural blanks were kept below 5 μg of carbon and 0.5 μg of nitrogen.

2.9 Chlorophyll *a* measurements

2.9.1 Fluorometry

The unique photosynthetic pigment chlorophyll *a* (Chl *a*), present in all photosynthesising organisms, has been used as a simple biomass indicator of marine phytoplankton since the early 1950's. Chl *a* is usually measured using a fluorometer which provides a simple, cost effective and sensitive measurements of extracted Chl *a*. A new fluorometric method has been advanced by Welschmeyer (1994) whereby the sample does not need to be acidified. This technique is free from errors associated with the conventional acidification technique used to assay Chl *a*. The method does not discriminate chlorophyllide *a* from Chl *a* because the two pigments have identical spectral properties (Lorenzen and Newton Downs 1986). All measurements of chlorophyll *a* in this study were made using the fluorometric methodology as described below.

Aliquots of the Chl *a* samples were collected in triplicate, filtered immediately on Whatman GF/F filters and extracted overnight in cold 90% acetone. Samples were analysed on a Turner Designs 10 – AU Fluorometer equipped with a highly selective lamp and optical filter combination to optimise the Chl-*a* measurement according to Welschmeyer (1994). The fluorometer was calibrated with Chl *a* standard purchased from Sigma-Aldrech.

2.9.2 High performance liquid chromatography

Pigment analysis by high performance liquid chromatography (HPLC) was used to provide accurate information on all chlorophylls, accessory pigments, degradation products and carotenoid concentrations since a complete separation can be achieved with the HPLC technique (Barlow *et al.*1997; Jeffrey *et al.*1997).

2.10 Statistical analysis

Statistical analyses were performed on the experimental data using a two-way fixed effect ANOVA (Zar 1999) using the following tests where appropriate:

1) Test of normality assumption (identifies violations of assumptions that threaten the validity of results) (Zar 1999).

2) Test for homogeneity of variances (Levene's test) tests that the variances in the different groups are equal (homogeneous), Bartlett's *Chi*-square test (with appropriate degrees of freedom and *p*-levels) also tests the homogeneity of variances assumption in the univariate cases) (Zar 1999).

3) Test of sum of squares (SS) Whole Model vs. SS Residual (tests the relationship between the separate slopes and analysis of covariance models) (Zar 1999).

4) Tukey HSD test (*Post hoc* test (or multiple comparison test) tests the significance of range and can be used to determine the significant differences between group means in an analysis of variance setting. Hence it assesses the probability under the null hypothesis (Zar 1999).

5) Kruskal-Wallis test, a non-parametric One-way Analysis of Variance (ANOVA) test was used when the assumption of the ANOVA was not met (Zar 1999).

6) Least squares non-linear estimation routine (STATSOFT 2004 – Statistica 7).

7) Ordination by non-metric multidimensional scaling (MDS) using Euclidean distance (Clarke and Warwick 2006).

8) One-way Analysis of Similarities (ANOSIM) (Clarke and Warwick 2006), a non-parametric multivariate analogue to ANOVA.

9) One-way Analysis of Variance (ANOVA) were conducted with R statistical analytic environment (R Development core team, 2009) in Chapter 5. The lattice figures in chapter 5 were produced using the R package "lattice" (Sarkar, 2009).

10) All the multivariate analysis in chapter 6 were conducted using PRIMER (Clarke and Warwick, 2001; Clarke and Gorley, 2006).

CHAPTER 3. PHOTOPHYSIOLOGICAL RESPONSES OF SELECTED DINOFLAGELLATES

Monocultures of five different dinoflagellate species isolated from the southern Benguela Current along the west coast of South Africa were grown in a temperature controlled laboratory where they were exposed to low (33 µmol quanta m^{-2} s^{-1}), medium (178 µmol quanta m^{-2} s^{-1}) and high light (647 µmol quanta m^{-2} s^{-1}) conditions. The species used were *Alexandrium catenella, Protoceratium reticulatum, Gymnodinium zeta, Prorocentrum micans* and *Prorocentrum triestinum.* The photoacclimation responses of the individual species recorded at different light levels in terms of changes in chlorophyll *a* concentration, photosynthetic rates, pigment composition and absorption coefficients were compared. All the experiments were carried out following the design described in detail in Chapter 2 and the cultures were low-light acclimated (40 µmol quanta m^{-2} s^{-1}) prior to experimentation for four days.

3.1 Chlorophyll *a* measurements

Two methods were used to measure the Chl *a* concentration of the five dinoflagellate species, fluorometric analysis and high performance liquid chromatography (HPLC). As recommended, the HPLC analysis was carried out on the same subsamples of the stock culture as those which were used for the fluorometric method (Bidigare 2005; Bidigare *et al.* 2005, 2003). Chl *a* values, measured fluorometrically, were used for all standardisation in the following chapters. Pigment analyses were done using the HPLC method.

In all the experiments carried out during this study with the species *Prorocentrum micans, Protoceratium reticulatum, Alexandrium catenella, Prorocentrum triestinum* and *Gymnydinium* cf. *zeta* the total chlorophyll *a* (Chl *a*) concentrations per unit volume (L), measured by fluorometry (in triplicate), showed a steady increase in the low light treatment and a decrease at high irradiances which is in agreement with other published findings. There were two exceptions which occurred in the experiment with the two larger-celled species *i.e. Prorocentrum micans* which showed an increase of Chl *a* concentration from LL to HL. (Appendix 3.2) and *Protoceratium reticulatum* where the Chl *a* concentration decreased slightly at ML, increasing again at high light (Expt. 11). Figure 3.0 (a) shows the Chl *a* : cell volume relationship. It illustrates that the Chl *a* density is significantly less in large-celled compared to small-celled species of dinoflagellates. These results correspond to similar findings in literature (Menden-Deuer and Lessard 2000). In calculating the Chl *a* concentration per cell a constant increase was noted in all five dinoflagellate cultures. Paralleled to the increases in Chl *a* concentrations were cell numbers which showed similar increases from LL to HL (Appendix 3.3).

The total chlorophyll *a* concentrations per cell for all the species used corresponded systematically with the individual cell volumes (Figure 3.0 (b)). *Protoceratium reticulatum* (±360 μm^{-3}) which is approximately half the size of *Prorocentrum micans* (±740 μm^{-3}), was the only exception as the Chl *a* concentration here was greater by approximately one third compared to *Prorocentrum micans* (range from LL to HL was 21 to 32 pg $cell^{-1}$ and 18 to 25 pg $cell^{-1}$ respectively) (Figure 3.0 (b)). The lowest Chl *a* content was recorded at HL in one of the experiments with the smallest species, *Gymnodinium zeta*, (22.0 pg $cell^{-1}$ and the highest concentration was noted in the larger celled *Protoceratium reticulatum* at LL (49.9 pg $cell^{-1}$) although in terms of cellular content this species generally followed the same pattern as the other dinoflagellate species with decreasing Chl *a* concentrations at high irradiances.

Differences in Chl *a* readings measured fluorometrically were noted compared to those determined using HPLC analysis (Appendix 3.1). Possibly the differences may be due to the extraction method used in the two methods (sonication *versus* soaking) since pigment recovery from soaking alone is often low and variable. In his review Jeffrey *et al* (1997) mentions the possibility of interference by accessory pigments.

Traces of chlorophyllide *a* were found in the dinoflagellate species ranging from 0.11 to 1.98% which were insignificant. Similarly very low percentages or absence of chlorophyllide *a* have been reported in literature for dinoflagellate species (Lorenzen and Jeffrey 1980; Jeffrey and Hallegraeff 1987).

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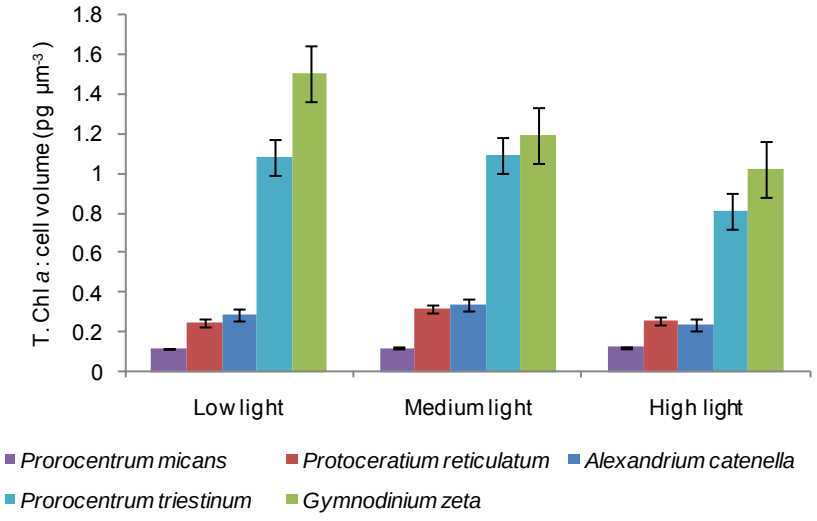


Figure 3.0 (a): Comparison of total Chl *a* : cell volume, measured fluorometrically, of the five dinoflagellate species acclimated to low, medium and high irradiances. Bars represent mean values. The species shown in the graph are arranged according to cell size.
(1) *Prorocentrum micans* (~740 μm^3), (2) *Protoceratium reticulatum* (~360 μm^3), (3) Alexandrium *catenella* (~240 μm^3), (4) *Prorocentrum tirestinum* (~70 μm^3) and (5) *Gymnodinium zeta* (~40 μm^3).
Error bars indicate ± one standard error.

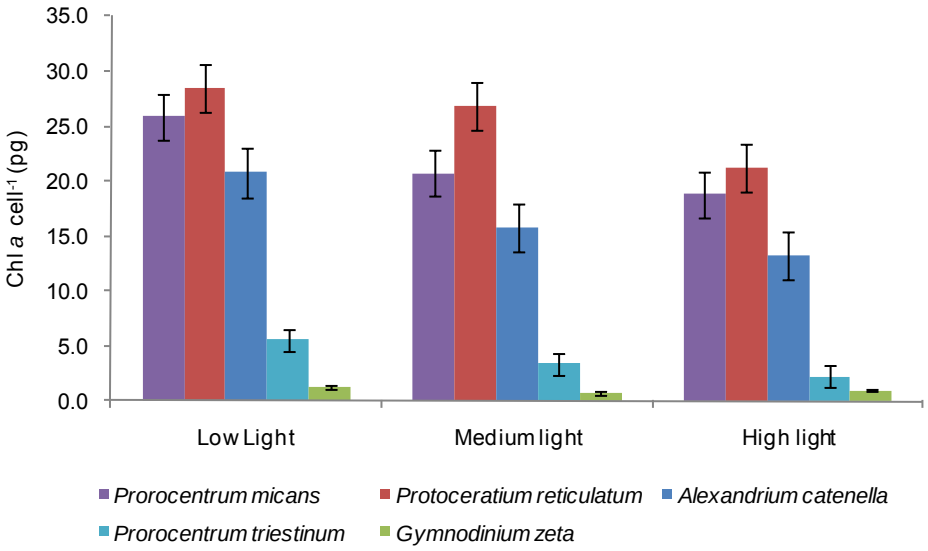


Figure 3.0 (b): Comparison of total fluorometric Chl *a* concentrations per cell, measured fluorometrically, of the five dinoflagellate species. The species shown in the graph are arranged according to cell size. (1) *Prorocentrum micans* (~740 µm^3), (2) *Protoceratium reticulatum* (~360 µm^3), (3) *Alexandrium catenella* (~240 µm^3), (4) *Prorocentrum tirestinum* (~70 µm^3) and (5) *Gymnodinium zeta* (~30 µm^3). *Error bars indicate ± one standard error.*

3.2 Cell counts

The concentrations of the cells (cells L^{-1}) in the samples, which were used in the experiments are tabulated in Appendix 3.3. Generally the cell density ranged between 1.1 x 10^6 to 10.0 x 10^6 cells L^{-1} for the larger species. In the samples of the smaller celled species, *Prorocentrum triestinum* and *Gymnodinium zeta* densities ranged from 20 x 10^6 to 99 x 10^6 cells $^{-1}$. The samples used for the experiments were examined using a microscope and selected for their cell density and viability (Appendix 3.3). The distribution of cell size/volume nor the changes in the cell volume in the cultures grown under LL, ML and HL during a 4 d period was not investigated.

3.3 Photosynthesis *versus* irradiance (*P vs E*) curves and photosynthetic parameters

The *P vs E* response curve, describing the rate of photosynthesis as a function of irradiance (*E*), is based on radio-labelled carbon assimilation. *P* represents the carbon fixation rate expressed in mg C m^{-3} h^{-1} and *E* the incident irradiance in terms of photosynthetic available radiation (PAR) expressed as µmol quanta m^{-2} s^{-1}. The shape and magnitude of the *P vs E* curve reflects the processes (biophysical, biochemical and metabolic) that regulate photosynthesis. The rates of gross photosynthesis, as a function of irradiance, were measured in a photosynthetron using the ^{14}C technique. The data were fitted to the model of Platt *et al.*

(1980), with the fit being forced through zero, using a non-linear estimation routine (STATSOFT 2004, Statistica 7).

Examples of the *P vs E* curves of the five species of dinoflagellates, *Alexandrium catenella* (a–c), *Prorocentrum micans* (d-f), *Protoceratium reticulatum* (g-i), *Gymnodinium* cf. *zeta* (j-l) and *Prorocentrum triestinum* (m-o) are given in Figure 3.1. The coefficient of determination (R^2) of the samples ranged between 0.96-0.99, showing a good fit to the *P*/*E* model. The *P vs E* curve obtained from *in vitro* incubations of dinoflagellates in the photosynthetron showed a wide variation at the different light levels in the different experiments using the same species and between species. Subsequent incubations were carried out with the same species only when sufficient culture material became available again. This time lapse of up to several months could have been one of the factors causing variability as certain species such as *Alexandrium catenella* were noted to have a definite seasonal growth-rate pattern. Methodological differences could not have been responsible for the observed variability. Differences were noted in the light-limited and light-saturated portions of the curve (Figure 3.1). Scatter of the data points along the curve were essentially small and curve fitting was very good for the larger-celled species *Prorocentrum micans*, *Protoceratium reticulatum* and *Alexandrium catenella* (approximate cell volumes for these species were: ~740 μm^3, ~ 360 μm^3 and ~240 μm^3 respectively) as well as for the smaller-celled species. The scatter of data points with large-celled species using small incubation vessels, such as mentioned by Tilzer *et al.* (1993) and Lewis *et al.* (1988), was not experienced during these small volume, short-incubation time experiments.

The initial slope of the light curve of chlorophyll-specific photosynthesis, α* (alpha, the light utilization coefficient), varied for each species particularly at low light levels (Table 3.1). The values of α* ranged from 0.01 to 0.06 mg C m^{-3} h^{-1} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$. Five examples of the *P vs E* curves of the incubation experiments are shown in Figure 3.1. In the microalgae species *Alexandrium catenella* and *Protoceratium reticulatum* α* ranged from 0.04 to 0.05 ±SE 0.002 and 0.03 to 0.06 ±SE 0.02 mg C m^{-3} h^{-1} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$ respectively. The initial slope in these two species was greater than that of the other low light acclimated species used *i.e. Prorocentrum micans, Prorocentrum triestinum* and *Gymnodinium* cf. *zeta*. The initial slope of medium light acclimated microalgae, *Alexandrium catenella, Protoceratium reticulatum* and *Prorocentrum micans* were very similar (α* ranged from 0.02 ±SE 0.01 to 0.04 ±SE 0.02 mg C m^{-3} h^{-1} (mg Chl *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$, but were generally greater than those of the smaller species. At high light levels the initial slope of the larger-celled species, *i.e. Alexandrium catenella, Protoceratium reticulatum* and *Prorocentrum micans* was greater by 0.01 mg C m^{-3} h^{-1} (mg Chl *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$ compared to the smaller-celled species (*Prorocentrum triestinum* and *Gymnodinium* cf. *zeta*) (Figure 3.1).

As the initial slope of the light curve of Chl *a*-specific photosynthesis, α* (alpha), represents the rate of photosynthesis per unit biomass per unit of incident irradiance (Kirk 1996) and thus is a measure of the efficiency with which the organism utilises light at low irradiances to fix CO_2, it demonstrates that

Alexandrium catenella and *Protoceratium reticulatum* were the most efficient followed by *Prorocentrum micans, Prorocentrum. triestinum* and *Gymnodinium zeta* in low light conditions. The least efficient in high light were the smallest species *Prorocentrum triestinum* and *Gymnodinium zeta* generally showing a difference of < 0.01 mg C m^{-3} h^{-1} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$ between the light conditions the species were exposed to.

Statistically, in two-way ANOVA, species responses to different light levels were significant (F =7.4, df = 4, pr = 0.0003433) as well as light level (F = 9.8, df = 2, p = 0.000619). *Gymnodinium zeta* had the smallest α* value of all species as shown in the Tukey HSD *Post hoc* pair-wise comparison (Appendix 3.4 (a-1)).

Findings reported by Conrad (1992, cited in ICES 1993) show that the initial slope of the light curve of chlorophyll *a*-specific photosynthesis of deep-living phytoplankton is greater than in algae from near surface water. The results in this study may reflect that the dinoflagellate species used in my experiments have a preferential depth in their natural habitat and are more adapted to a low light environment. The ability to function efficiently at lower light levels is a characteristic which presumably may help to reduce resource-based, inter-specific competition (Heath *et al.*1988).

Table 3.1: Comparison of the photosynthetic parameters, normalised to fluorometrically obtained Chl *a*, of different species of dinoflagellates (*Alexandrium catenella*, *Protoceratium reticulatum*, *Gymnodinium zeta*, *Prorocentrum triestinum* and *Prorocentrum micans*). Sample statistics are mean ± standard error of three different experiments.

		Low light (33 µmol quanta m^{-2} s^{-1})			**Medium light** (178 µmol quanta $m^{-2}s^{-1}$)			**High light** (647µmol quanta m^{-2} s^{-1})		
	Experiment number	**Maximum photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1}) P^*_m	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1} (mg Chl *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$ α*	**Light saturation Parameter** (µmol quanta m^{-2} s^{-1}) E_k	**Maximum photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1}) P^*_m	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1} (mg Ch *a*) (µmol quanta m^{-2} s^{-1})$^{-1}$ A*	**Light Saturation Parameter** (µmol quanta m^{-2} s^{-1}) E_k	**Maximum photosynthetic rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1}) P^*_m	**Maximum light utilization coefficient** (mg C m^{-3} h^{-1} (mg Ch *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1}) [1] α*	**Light Saturation Parameter** (µmol quanta m^{-2} s^{-1}) E_k
Alexandrium catenella	5	8.5	0.04	191	4.6	0.03	151	4.7	0.02	253
Alexandrium catenella	8	10.0	0.05	216	6.5	0.03	234	6.8	0.02	376
Mean		**9.3**	**0.05**	**204**	**5.5**	**0.03**	**193**	**5.8**	**0.02**	**314**
± SE		1.1	0.002	17	1.3	0.002	59	1.5	0.0003	87
Protoceratium reticulatum	6	4.5	0.03	173	2.7	0.02	154	2.8	0.02	153
Protoceratium reticulatum	7	11.4	0.06	195	8.7	0.04	194	8.8	0.03	284
Protoceratium reticulatum	11	6.2	0.03	219	5.3	0.03	207	5.2	0.02	323
Mean		**7.4**	**0.04**	**195**	**3.6**	**0.03**	**185**	**5.6**	**0.02**	**253**
± SE		3.6	0.02	23	3.0	0.01	27	3.0	0.01	89
Gymnodinium zeta	3	0.7	0.01	79	2.1	0.02	133	1.2	0.01	154
Gymnodinium zeta	9	3.0	0.02	153	5.0	0.02	268	2.4	0.01	481
Gymnodinium zeta	12	2.4	0.02	139	3.1	0.01	242	1.3	0.01	340
Mean		**2.0**	**0.02**	**124**	**3.4**	**0.02**	**214**	**1.7**	**0.01**	**325**
± SE		1.2	0.002	39	1.5	0.002	72	0.7	0.002	164
Prorocentrum triestinum	4	3.7	0.02	187	3.3	0.02	165	4.2	0.01	292

		Low light (33 µmol quanta m^{-2} s^{-1})			**Medium light** (178 µmol quanta $m^{-2}s^{-1}$)			**High light** (647µmol quanta m^{-2} s^{-1})		
	Experiment number	**Maximum photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1} (mg Chl *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light saturation Parameter** (µmol quanta m^{-2} s^{-1})	**Maximum photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1} (mg Ch *a*) (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light Saturation Parameter** (µmol quanta m^{-2} s^{-1})	**Maximum photosynthetic rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization coefficient** (mg C m^{-3} h^{-1} (mg Ch *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1})^{1}	**Light Saturation Parameter** (µmol quanta m^{-2} s^{-1})
		P^*_m	α*	E_k	P^*_m	A*	E_k	P^*_m	α*	E_k
Prorocentrum triestinum	9	5.4	0.03	202	4.6	0.02	296	5.4	0.01	435
Prorocentrum triestinum	13	6.2	0.03	209	6.8	0.02	282	6.0	0.01	439
Mean		**5.1**	**0.03**	**199**	**4.9**	**0.02**	**248**	**5.2**	**0.01**	**388**
± SE		1.3	0.01	11	1.8	0.001	72	0.9	0.001	84
Prorocentrum micans	3	3.2	0.03	107	3.2	0.03	110	2.8	0.02	147
Prorocentrum micans	8	1.1	0.01	7.6	1.7	0.02	83	2.4	0.01	206
Prorocentrum micans	11	9.9	0.03	306	7.3	0.04	178	5.7	0.03	186
Mean		**4.7**	**0.03**	**163**	**4.0**	**0.03**	**124**	**3.6**	**0.02**	**180**
± SE			0.01	125	2.9	0.01	49	1.8	0.01	30

Low light (33 µmol quanta $m^{-2} s^{-1}$) Medium light (178 µmol quanta $m^{-2} s^{-1}$) High light (647 µmol quanta $m^{-2} s^{-1}$)

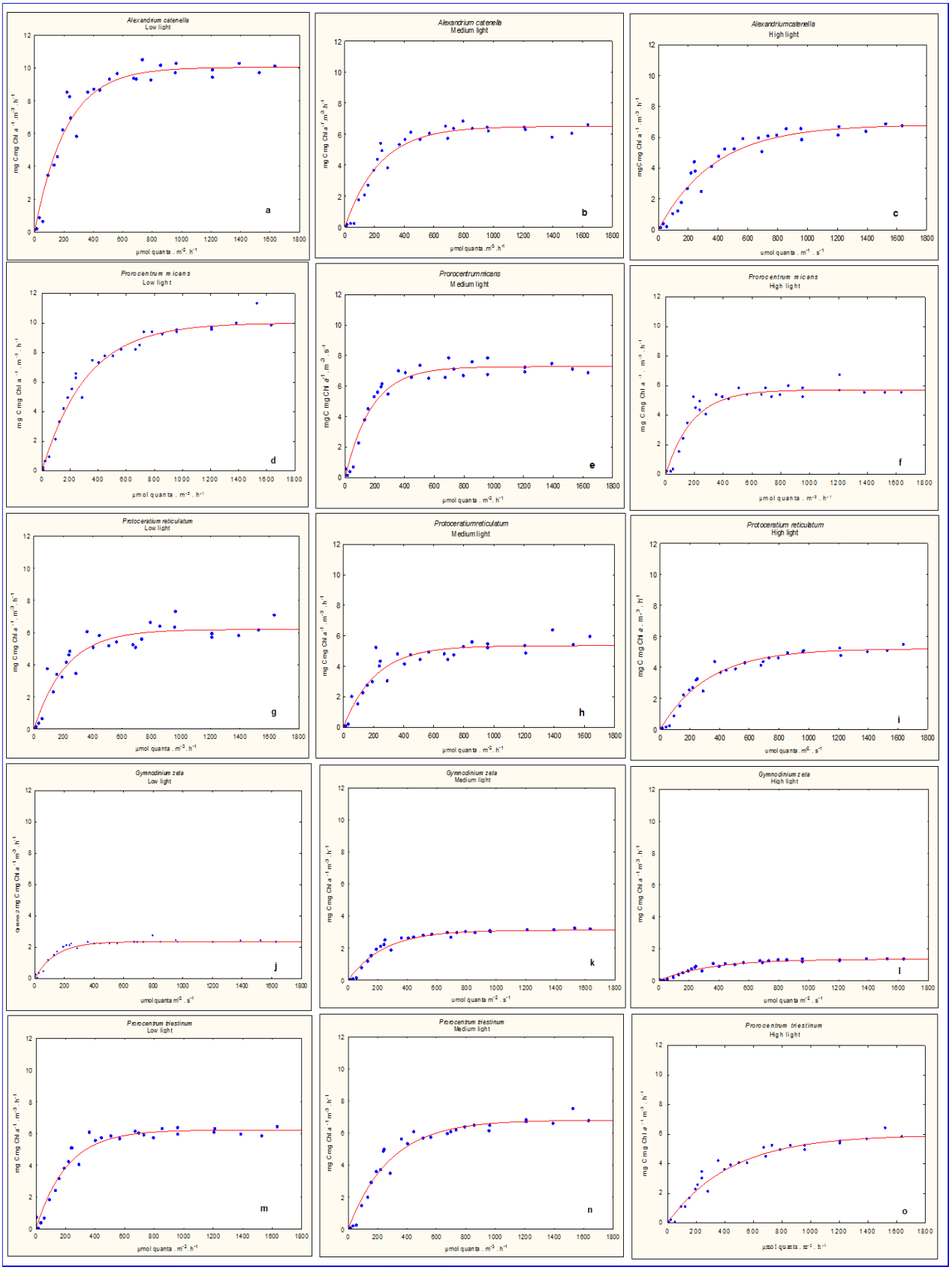


Figure 3.1: Examples of *P versus E* curve experiments for dinoflagellate species *Alexandrium catenella* (Expt. 8 (a-c)), *Prorocentrum micans* (Expt. 11(d-f)), *Protoceratium reticulatum* (Expt. 11(g-i)), *Gymnodinium* cf. *zeta* (Expt. 12(j-l)) and *Prorocentrum triestinum* (Expt. 13 (m-o)). The coefficient of determination, R^2, in all cases was > 0.95 and < 0.99.

The light saturated photosynthesis parameters, P^*_m (maximum photosynthetic rate), normalised to chlorophyll *a*, varied between 0.7 to 11.4 mg C . (mg Chl *a*) $^{-1}$ h^{-1}) in the experiments during this study. Variances were observed between the different species as well as between subsequent experiments carried out at different times during the year using the same species. The lowest values of P^*_m were measured at high light levels, decreasing at medium light levels with a slight increase at low light levels. The exception was *Gymnodinium* cf. *zeta* which showed an overall increase of 1.4 (± SE 1.5) mg C m^{-3} (mg Chl *a*) $^{-1}$ h^{-1} at medium light levels ((Table 3.1), decreasing again to 1.7 ((± SE 0.7) mg C m^{-3} (mg Chl *a*) $^{-1}$ h^{-1}.

Lewis *et al*. (1985) pointed out that productivity is greater during light-limited photosynthesis compared with light-saturated photosynthesis. Under light-limited conditions, the maximum photosynthetic rate (P^*_m) is determined by the light absorption properties of the pigment complexes of the algae. As P^*_m is related to the number of photosynthetic units (*n*), that is the complexes of reaction centres and antennal pigments (JGOFS 1998), damage to the photosynthetic units may lower *n* and correspondingly P^*_m (Maxwell *et al.* 1994, Olaizola *et al*. 1994 and Vassiliev *et al.* 1994). Although the photosynthetic parameter values, P^*_m for the five dinoflagellates used in the experiments decreased from low light to high light (see Figure 3.2 (a)) in agreement with other research such as undertaken by Tilzer (1993) and others, no photoinhibition was apparent which seems to indicate that the exposure time to high light was too short to cause damage to the light harvesting pigment complexes. Therefore, other processes associated with e.g. the Calvin cycle in light acclimated cells, which are not immediately apparent, must be activated during exposure to different light intensities.

The maximum photosynthetic rate (P_m*) for the large-celled species, *Prorocentrium reticulatum* and *Prorocentrum micans* was generally reached at a light intensity between 275 to 380 µmol quanta m^{-2} s^{-1} for the LL, ML and HL acclimated cultures. In the smallest species, *Prorocentrum triestinum* and *Gymnodinium zeta*, the maximum rate was reached at a lower light intensity between 200 to 300 in LL, between 300 to 400 in ML and 475 to 550 µmol quanta m^{-2} s^{-1} in HL, which seems to indicate that larger species require a greater light intensity to reach the optimum P^*_m value.

Using the mean photosynthetic data, of the cultures, P^*_m did not vary systematically with cell size (Figure 3.2 (a)). The variability observed could be related to both packaging and composition of accessory pigments. Cell diameter correlated negatively with the concentration of cellular pigments, which is thought to be a strategy to minimise self-shading (Agusti 1991). The mean photosynthetic parameter data of α* (Figure 3.2 (b)) showed a good relationship with cell size with the larger celled species showing a 1.5 to 2.5-fold decrease from low light to high light. The smaller celled species decreased by >2.0-fold.

Statistical analysis of the log transformed data, using a two-way fixed effect ANOVA, showed that light level did not have an effect on the maximum photosynthetic rate only the species responses were significant ($F = 0.28$, $df = 2$, $p = 0.7521$) with *Prorocentrum triestinum* having a significantly higher

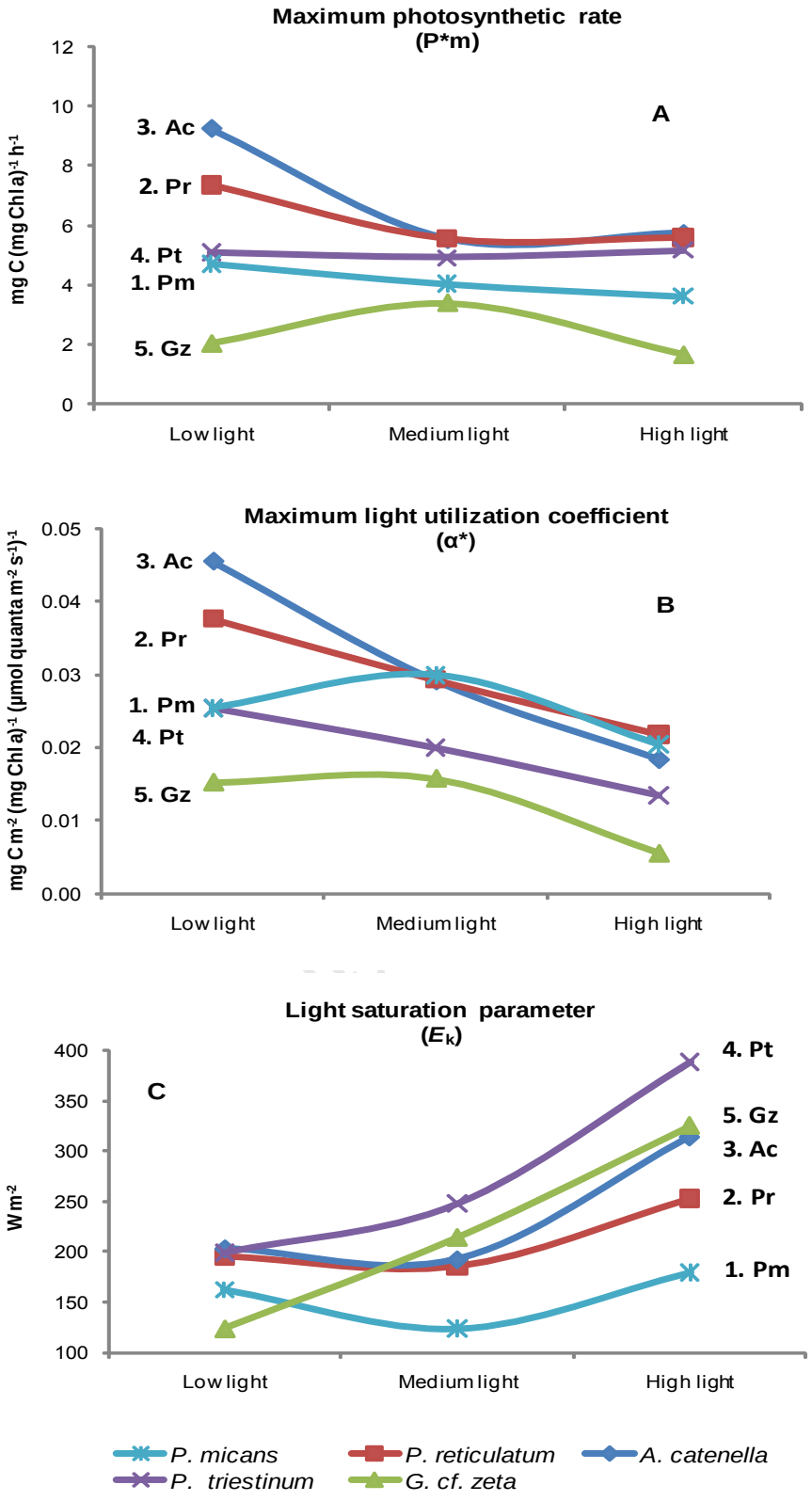


Figure 3.2: Comparison of the mean photosynthetic parameters, P^*_m (A), α* (B) and E_k (C) of the different dinoflagellate species (The approximate cell volumes are: *Prorocentrum micans* 740 µm^{-3}, *Protoceratium reticulatum* 360 µm^{-3}, *Alexandrium catenella* 260 µm^{-3}, *Prorocentrum triestinum* 70 µm^{-3} and *Gymnodinium zeta* 40 µm^{-3}).
JGOFS (1998) recommended units have been used.

P^*_m rate. The different species responded to changing light levels which was significant (p = 0.0014), (Appendix 3.4(a-2)).

The irradiance value, E_k (P^*_m / α^*), represents the parameter corresponding to the onset of light saturation of photosynthesis. Microalgae endeavour to maintain an optimum balance between light and dark reactions of photosynthesis, *i.e.* a balance between the rate of photon absorption by PSII and the rate of electron transport from water to CO_2. E_k therefore indicates the irradiance at which control of photosynthesis passes from light absorption and photochemical energy conversion to reductant utilisation. In phytoplankton, E_k is adjusted upwards with increased irradiance and *vice versa*, although the photosynthetic rate (P^*_m) is lower. At higher irradiances the photosynthetic rate does not correspondingly decrease (JGOFS 1998). A comparable trend was demonstrated in the results of my experiments. The highest values of E_k were observed for high light levels being > 300 µmol quanta m^{-2} s^{-1}. The light acclimated species *Prorocentrum triestinum* and *Gymnodinium zeta* had the highest light saturation rates at high irradiances. The mean was 388 ±SE 84 and 325 ±SE 164 µmol quanta m^{-2} s^{-1} respectively (Table 3.1). This was followed by *Alexandrium catenella* (mean 314 ±SE 87 µmol quanta m^{-2} s^{-1}). The mean values of E_k for all five species of dinoflagellates gradually increased when exposed to low, medium and high irradiances which was significant (F = 9.19, df = 2, p = 0.0009). No interaction was apparent between species and light level *i.e.* all species responded in similar fashion to changing light levels (Appendix 3.4 (a-3)).

The intrinsic variations in P^*_m and α* (Figure 3.2 (a & b)), and therefore the value of E_k, in this data set, seem to reflect the differences in cell volume of the various species used in my experiments. This assumption is well supported at medium and high light with respect to cell size (Figure 3.2 (c)) and demonstrates that small-celled species have a physiological advantage over large-celled species resulting from the combined effects of a group of variables. For instance, with an increased area to volume ratio, a small cell has an increased light absorption efficiency and reduced intracellular shading from a lower pigment concentration. A small cell also has a thinner diffusion boundary layer which exists close to the cell wall which aids the fluxes of solutes between cell surface and cell contents. In turbulent conditions the thickness of the boundary layer is reduced and the resistance to diffusion is decreased effectively aiding in the diffusional transport across and chemical reactions within a boundary layer. Cell size also affects sinking speed and the energy cost of flagellar motility (Raven and Kübler 2002). These attributes aid dinoflagellates to photosynthesise efficiently at lower light levels in the water column. The effect of cell size on E_k is also mentioned by Tilzer (1989), as well as the changes in chlorophyll *a* concentrations, due to photoacclimation, as discussed by Tilzer *et al.* (1993), Platt *et al.* (1992), Prézelin *et al.* (1991) and others. The photosynthetic parameters indicated that generally the dinoflagellates photosynthesise most efficiently in a low light environment, whilst diatoms (Chapter 4) are most efficient at high irradiances. A decrease from P^*_m at high light, *i.e.* photoinhibition, which usually occurs after light saturation of photosynthesis, was not observed under high irradiance conditions in any of the five dinoflagellate species. This may be due to the relatively low light levels available in the photosynthetron (< 1200 µmol quanta m^{-3} h^{-1}) as compared to the natural environment at latitude 34° S.

Comparing the photosynthetic parameters of dinoflagellate and diatom (see Chapter 4) species with natural populations in the southern part of the Benguela Ecosystem similar values for P_m*, α* and E_k were recorded for inshore stations 1 and 2 and offshore stations 7 and 8 (Balarin 2000, unpublished data of author). The *P vs E* relationships were investigated by means of the classic ^{14}C technique as described in Chapter 2 using the photosynthetron (Figure 2.1). Surface samples were taken along an offshore transect consisting of 12 to 15 stations covering a maximum distance of 221 km (depth approximately 200m) from the coast (Appendix 3.4 (b). On board incubations were terminated after 20 min as was done with the laboratory culture samples.

The P_m* values recorded for cruises Algoa 79 and 83 were very variable although they showed a definitive trend which could be correlated with the distance offshore where the samples were taken. Low values were recorded close to shore, increasing midway over the shelf and decreasing rapidly in the open ocean beyond the shelf edge (200m isobar). For Cruise 79 the values for inshore, midway and open ocean were 5.05 , 18.56 and 4.49 mg C (mg Chl *a*)$^{-1}$ h^{-1}) respectively and for Cruise 83 the values were 3.06 , 20.32 and 0.55 mg C (mg Chl *a*)$^{-1}$ h^{-1}) (Appendix 3.4 (b)). The high values at the midway stations (approximately 100 km offshore) corresponded with high T Chl : T pig readings. In this region the ratio of T Chl to T pig ranged between 0.6 to 0.8. The mean values for P_m* for both cruises were virtually identical (8.88 and 8.91 mg C (mg Chl *a*)$^{-1}$ h^{-1}).

The α* parameters of the natural phytoplankton populations (range 0.007 to 0.115, mean 0.032 to 0.041 mg C (mg Chl *a*)$^{-1}$ h^{-1}) corresponded well with the values observed in the laboratory cultures of the dinoflagellates and diatoms species (range 0.006 to 0.103, mean 0.005 to 0.048 mg C (mg Chl *a*)$^{-1}$ h^{-1}) particularly in the inshore and far offshore stations.

The values of the parameter E_k were in a similar range as recorded for the cultured species and followed a similar trend as mentioned above with high values for the mid stations 4 and 5 during August 2000.

An investigation conducted during February/March 1996 at the Lambert's Bay monitoring stations at the west coast of South Africa produced a similar range of photosynthetic parameters from *P vs E* studies for surface samples as mentioned in my study above (Mitchell-Innes *et al.* (2000)). Interestingly the highest values for P_m* were recorded for samples collected at approximately midday between 12.30 and 14.30 hours (range 1.86 to 5.69 mg C Chl^{-1} h^{-1}) which demonstrates the cyclic and temporal nature of photosynthesis. The α* values ranged from 0.14 to 0.25 mg C Chl^{-1} h^{-1} (µmol m^{-2} s^{-1})$^{-1}$. Calculated values of E_k in this study ranged from 102 to 285 µmol quanta m^{-2} s^{-1}.

A comparison of the E_k data reported by other researchers for different coastal oceanic environments showed ranges between 66 to 286 µmol quanta m^{-2} s^{-1} for the months of January and February in the Gulf of Coquimbo, Chile (Montecino *et al.* 1996). Mitchell-Innes *et al.* (2000) recorded a range of 155 to 227 µmol quanta m^{-2} s^{-1} in Lamberts' Bay, South Africa, during the same months. The data collected during the months of August and September during my investigation in St Helena Bay, west coast of South Africa,

were slightly higher (range 217 – 277 μmol quanta m^{-2} s^{-1}) than those reported for similar months by Montecino *et al.*(1996) (range 103 – 156 μmol quanta m^{-2} s^{-1}) (Appendix 3.4 (c)). It is difficult to compare data as the way experiments are conducted and results are calculated by other researchers varies in each case.

3.4 Pigment composition

Photo-pigment indices were quantified to assess the changing contributions of chlorophylls and carotenoids to the total pigment pool. The dinoflagellates used in the experiments contained the light harvesting pigments (PSP) chlorophylls *a*, c_1 and c_2, and the photosynthetic carotenoid peridinin. Chlorophyll *a* (Chl *a*), which constituted most of the chlorophyll present, changed with variations in the relative concentrations of accessory pigments, generally increasing with decreasing light intensities. Examples of the accessory photosynthetic pigment ratios per total pigment are shown in Figure 3.3(a) for the five dinoflagellate species. It indicates that the Chl *a* proportion of total pigments constitutes the highest concentration with very slight variations at low, medium and high light treatments. Similarly, peridinin remains fairly constant at all light levels as does Chl c_1 and c_2. *Protoceratium reticulatum* shows a slight departure from this pattern as the Chl *a* increases at medium light with a slight increase in peridinin but a corresponding decrease in Chl c_1 and c_2.

All photoprotective pigments show more definitive increases at higher light levels ranging from 12 to 60% with *Protoceratium reticulatum* showing the highest increase. This increase was generally coupled with a decline in photosynthetic pigments (PSP) (range 11 to 48%) at increasing light levels. The exception was *Prorocentrum micans* where a decrease is shown in the photoprotective pigments (PPC) concentration from medium light to high light levels, which is contrary to expectations.

At low light elevated proportions of PSPs was observed by Lutz *et al.* (2003) with an increase in PPCs at high light, which is generally in agreement with this study. The variation in accessory pigments provides an insight into photoacclimation strategies to changing light conditions.

The mechanism of photoacclimation appears to vary depending on the phytoplankton species. The pigment pool does not change appreciably but the individual intracellular pigments do, either increasing or decreasing depending on the light environment (Figure 3.3(b)). The Chl *a* concentration per cell in the species *Prorocentrum micans, Gymnodinium zeta and Protoceratium reticulatum* declined slightly from low light to high light by approximately 30% (range 28 to 34%). These results are consistent with models that describe photoacclimation of cell pigment content in terms of a dynamic energy balance (Kana *et al.* 1997). *Alexandrium catenella* and *Prorocentrum triestinum* also showed a similar decline differing however from this pattern by a far greater decrease up to 60% in the cellular Chl *a* content. The peridinin content per cell declined by approximately 10% less compared to Chl *a* in the three species noted above. The genus *Prorocentrum* however, seems to demonstrate a definite attribute with the greatest decline in the photosynthetic pigment peridinin at high light (58 to 73%) coupled with a substantial decline in the

photoprotective pigment diadinoxanthin from low light to high light which was not paralleled in the other three species studied where the opposite was observed.

The concentration of PPCs generally showed an increase at higher irradiances in *Alexandrium catenella*, *Protoceratium reticulatum*, *Gymnodinium* cf. *zeta* and *Prorocentrum triestinum* (Figure 3.3) species. The exception was *Prorocentrum micans* where the highest concentration of PPC was noted under medium light conditions (See also 3.4.1 on xanthophyll cycling). The highest total contents of the PPC pigments were observed under high light conditions in *Protoceratium reticulatum* (range 16.9 – 41.9 µg L^{-1}) (Appendix 3.6). Dinoxanthin and *β*-carotene ratios were variable depending on the different dinoflagellate species (Figure 3.3).

Chl *a* derivatives, the epimers and allomers, as well as chlorophyllide *a* were summed together to calculate total chlorophyll *a* (TChl *a*) concentrations. Chlorophyllide *a* was found in very low concentrations in the dinoflagellate species (0.03 - 3.93%), 4% being the exception, which is in agreement with other researchers reported values for dinoflagellates (Jeffrey and Hallegraeff 1987). The calculated ratios did not vary significantly among the species nor between the different light levels (1-way ANOVA, $p > 0.05$).

Statistically the ratios of the photoprotective carotenoids (PPC), diadinoxanthin, diatoxanthin, dinoxanthin and *β*-carotene were found to differ between the phytoplankton species. Using a 2-way fixed effects ANOVA (Zar 1999) showed that there was a significant difference for both diadinoxanthin ($F = 2.306$, $df = 4,30$, $p = 0.026$) and diatoxanthin ($F = 4.806$, $df = 4,30$, $p = 0.00001$) among all 5 species at three light levels.

A comparison of pigment concentrations per cell in the different species illustrates more clearly that the Chl *a* content is higher at low light treatment decreasing at high light, with peridinin following a similar trend. *Protoceratium reticulatum* has a high content of chlorophyll c at low light compared to the other species. Diadinoxanthin shows a significant increase in cellular concentration at high irradiance levels in *A. catenella*, *P. reticulatum* and *G. zeta*. The opposite was indicated in the *Prorocentrum* species (*P. triestinum* and *P. micans* with diadinoxanthin decreasing from low light to high light by 56 and 49% respectively. As was evident from Figure 3.3 (a) the photosynthetic pigment ratios to total pigments remained fairly constant at the different irradiance levels

Statistically it was shown that there was a significant difference in the ratio of photosynthetic pigments (PSC) to total pigments between the dinoflagellate species' in their individual responses to different light intensities using a two-way ANOVA.(Species, $F = 10.1$, $df = 4$, $p = 2.59E-05$ and Light , $F = 22.8$, $df = 2$, $p = 9.52E-07$) Appendix 3.7 (a-1). The effects of species and light on the ratios between photosynthetic pigments (PPC) to total pigments and PSCs to PPCs were similarly significant (Appendix 3.7 (a-2 and a-3).

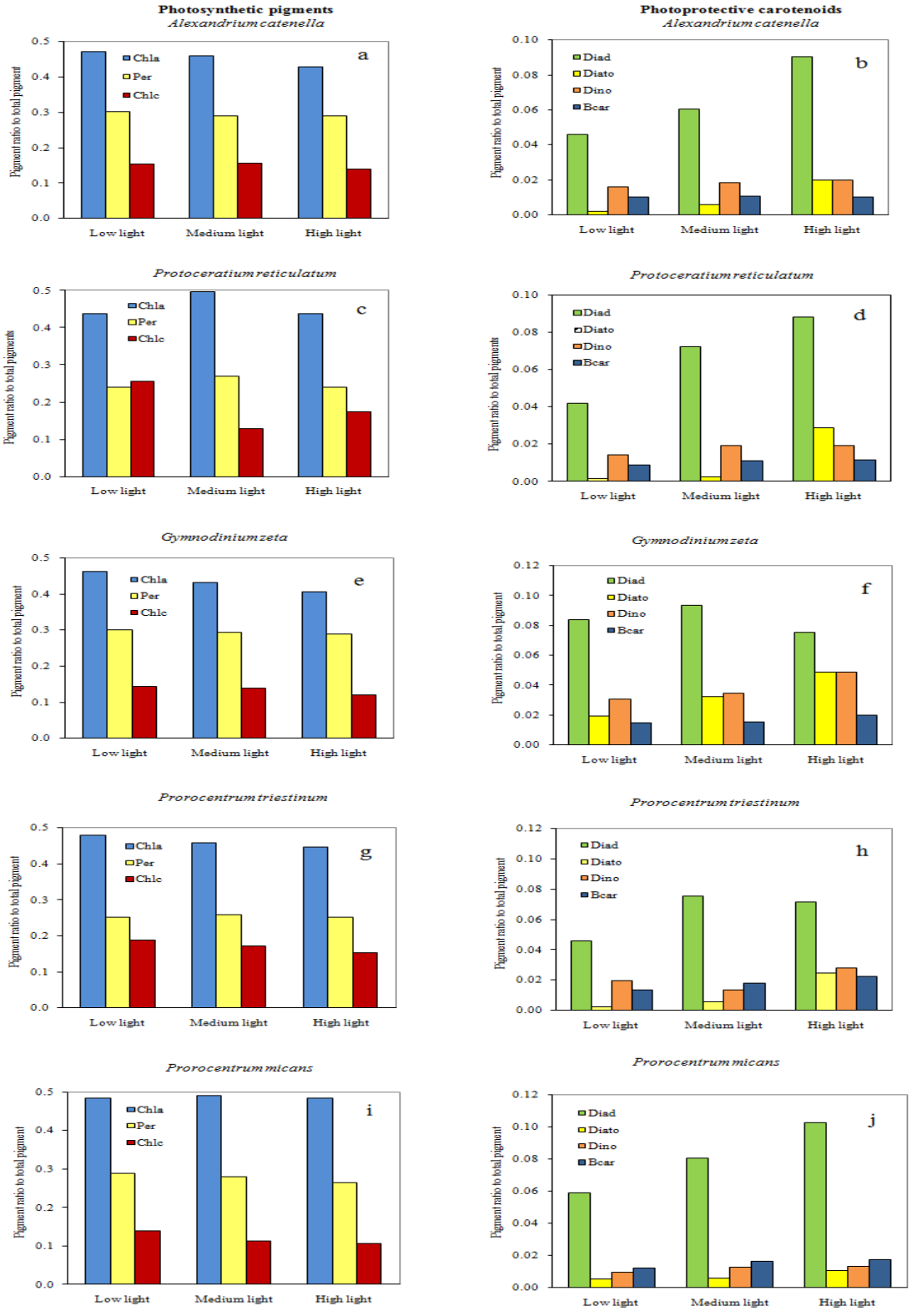


Figure 3.3 (a): Pigment ratios to total pigment (µg:µg) for the five dinoflagellate species incubated under three irradiances as indicated. *Alexandrium catenella* (a and b), *Protoceratium reticulatum* (c and d), *Gymnodinium* cf. *zeta* (e and f), *Prorocentrum triestinum* (g and h), *Prorocentrum micans* (i and j). Chl *a* = chlorophyll *a*, Per = peridinin, chl c = chlorophylls $c_{1,2}$, Diad = diadinoxanthin, Diato = diatoxanthin, Dino = dinoxanthin and βcar = β-carotene.

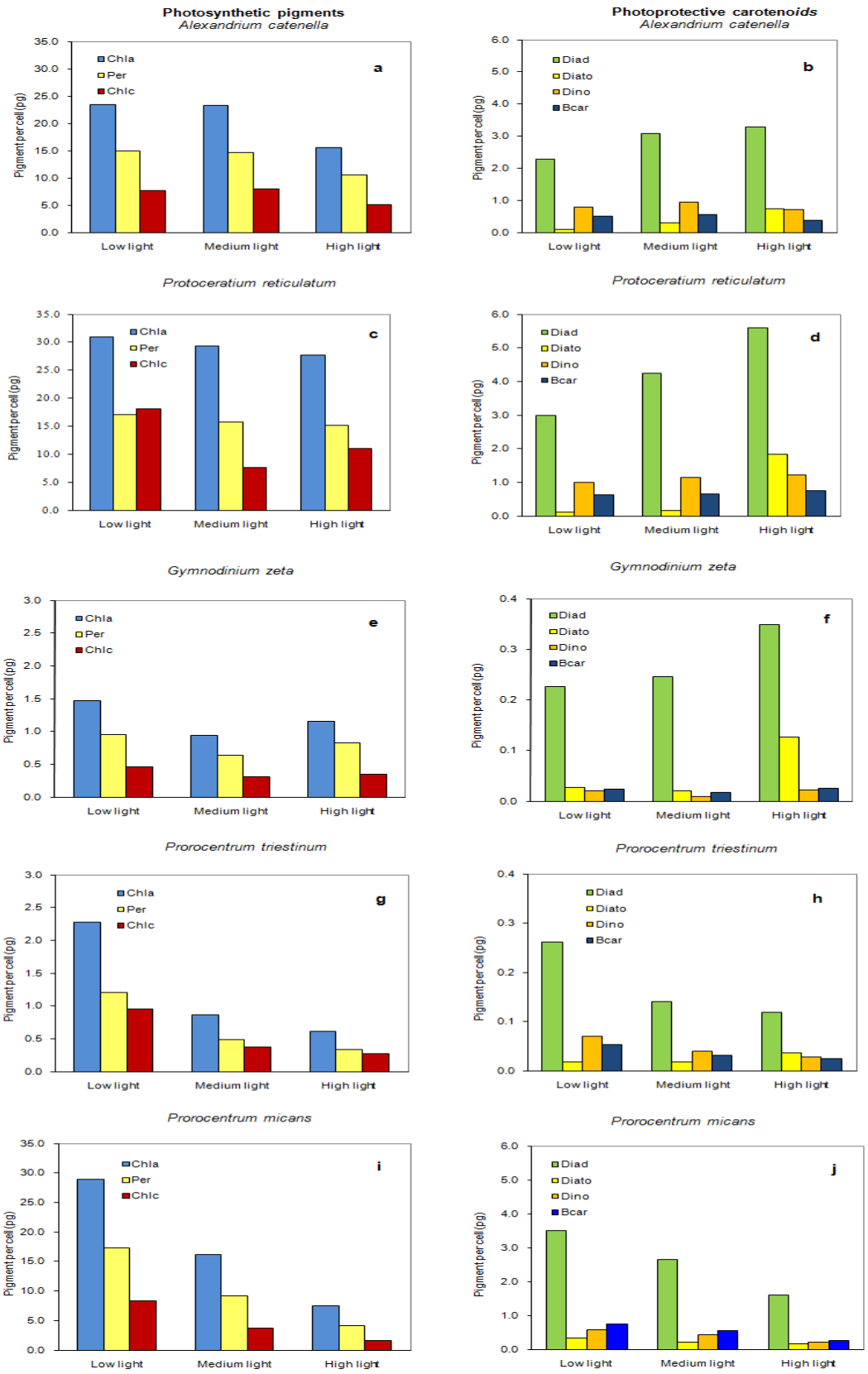


Figure 3.3 (b): Pigment contents per cell (pg) for the five dinoflagellate species grown under varying irradiances as indicated. Note different scales for the smaller celled species (e,f,g,h). (*Alexandrium catenella* (a and b), *Protoceratium reticulatum* (c and d), *Gymnodinium* cf. *zeta* (e and f), *Prorocentrum triestinum* (g and h), *Prorocentrum micans* (i and j). Chl *a* = chlorophyll *a*, Per = peridinin, Chl c = chlorophylls c $_{1,2}$, Diad = diadinoxanthin, Diato = diadinoxanthin, Dino = dinoxanthin, Bcar = β-carotene.

3.4.1 Xanthophyll cycling

The physiological regulation of the xanthophyll pool is considered to be a characteristic feature of photoacclimation and is an important strategy used by migrators and layer-formers (Cullen and MacIntyre 1998). Xanthophyll cycling has been shown to be an effective quenching mechanism (Moisan *et al.* 1998; Lohr and Wilhelm 1999; Lavaud *et al.* 2004). It does not affect the light harvesting efficiency of microalgae and reduces the cost of synthesising other light harvesting pigments.. The rates of interconversion between the xanthophylls diadinoxanthin (DD) and diatoxanthin (DT) and the ratio of DD/DT can be used as an indirect means of measuring the abilities and rates of phytoplankton photoacclimation and photoprotection (Demers *et al.* 1991). Only diatoms, dinoflagellates, prymnesiophytes and some chrysophytes have the ability to convert DD to DT during the transition from low light to high light via a de-epoxidation reaction (Olaizola *et al.* 1992; 1994). This is a reversible reaction. It involves the dissipation of excess energy by non-photochemical fluorescence quenching via pathways that lead to non-destructive thermal de-excitation of pigments (Fujiki 2003). Different species of microalgae exhibit differential rates of xanthophyll cycling (Moisan *et al.* 1998). Meyer *et al.* (2000) reported that changes in xanthophyll cycling occurred within the first five minutes of the initial shift to high light conditions in semi-continuous cultures of *Phaeocystis globosa* and *Thalassiosira* sp., generally resulting in a decrease in the DD pool and an increase in the DT pool. The sum of the DD + DT pool however stayed constant, which indicated that no *de novo* synthesis of the two xanthophylls occurred during light shifts.

Comparing the experiments discussed in this thesis it was found that the xanthophyll pool expressed as chlorophyll *a*-specific ((DD + DT)/Chl *a*) was higher in monocultures of *Alexandrium catenella* species grown under high light irradiances than in those grown under low light over a period of 6 days (Figure 3.4 (a)). Under HL irradiance *A. catenella* adapted rapidly for the first two days. Under low light conditions the xanthoplyll pool remained fairly constant. These results are in accordance with other studies conducted with monocultures of phytoplankton species such as *Chaetoceros gracilis* and *Thalassiosira weissflogii* (Goericke and Welschmeyer 1992; Fujiki and Taguchi 2001). It has been suggested by Moline (1998) and Sigleo *et al.* (2000) that the xanthophyll pool can be used as an index to assess phytoplankton light histories and the degree of water column stability.

During a period of high irradiances Fujiki *et al.* (2003) recorded (DD + DT) /Chl *a* ratios of 0.0053 – 0.18 in natural phytoplankton assemblages. Bricaud *et al.* (2004) recorded ratios of PPC/ TClh *a* corresponding to eutrophic waters (Morocco and Benguela upwelling areas) mostly below 0.2. Compared with figures quoted in references my data showed mean ratios for the dinoflagellate species grown under different light conditions ranging from 0.098 – 0.185 (low light), 0.156 – 0.274 (medium light) and 0.219 – 0.362 (high light) (Appendix 3.7). Low values in natural assemblages could be the result of phytoplankton species which are without DD and DT (Fujiki *et al.* 2003).

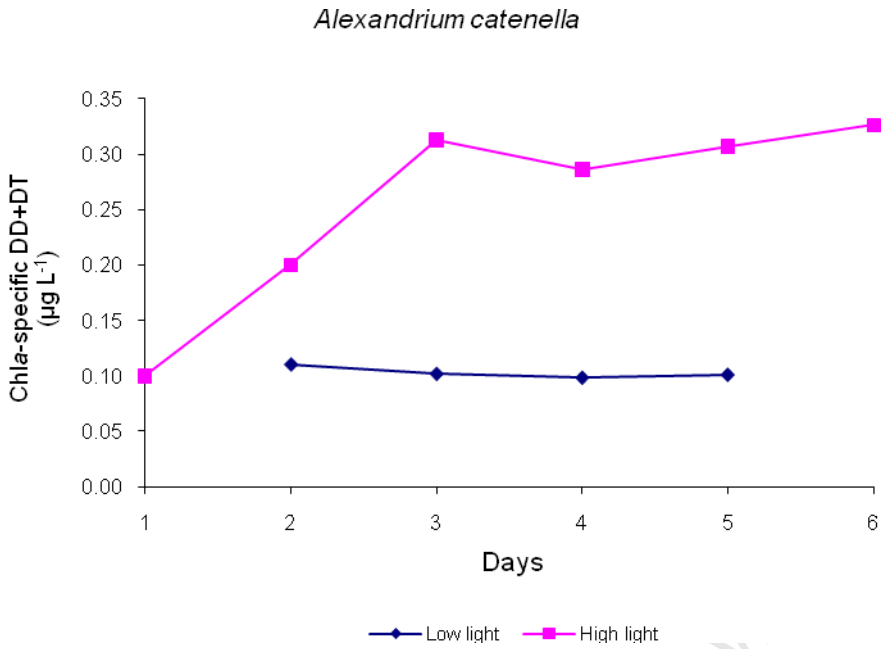


Figure 3.4 (a): Changes in chlorophyll *a*-specific xanthophyll pool (DD+DT/Chl *a*) values, measured by HPLC, in the dinoflagellate *Alexandrium catenella* over a period of 6 days. The xanthophyll pool is higher in the cells grown under high light (647 µmol quanta m^{-2} s^{-1}) than under low light (33 µmol quanta m^{-2} s^{-1}) conditions indicating an important strategy for quenching photo-oxidative damage.

The sum of the DD+DT pool generally showed an increase in all species when cultures were exposed to higher light levels (Figure 3.4 (b), Appendix 3.7). This agreed with the rapid increase within hours in both DD and DT observed in the diatom *Skeletonema costatum* (Anning *et al.* 2000) during high light conditions. It does not agree with other research findings *i.e.* Meyer *et al.* (2000) and Fujiki *et al.* (2003). *De novo* photoprotective pigments are therefore formed in the dinoflagellate species used here for the experiments. The percentage of diadinoxanthin to total carotenoids ranged between 4.1 - 18.1%, 10.0 - 26.2% and 7.5 - 25.8% in the five phytoplankton species grown at low, medium and high light respectively. A similar range was reported by Jeffrey *et al.* (1975) (Appendix 3.8).

Statistical analysis indicated a significant difference in the xanthophyll pools among the 5 species, also across the light levels (Species F = 19,2, df = 4,30, p = 0.0000; Light level F = 97.7, df = 2,30, p = 0.0000). There was some interaction effect (F = 0.956, df = 8,30, p = 0.48) indicating that light levels affect xanthophylls pools differently in different species. The data were square root transformed to meet the assumptions (Appendix 3.7 (a)). The diadinoxanthin percentage was significantly different among the 5 dinoflagellates affected by light level and species, but in this case there was no interaction effect (Species F = 4.7, df = 4,30, p = 0.0045, Light F = 9.6, df = 2,30, p = 0.0005) (Appendix 3.8 (a)).

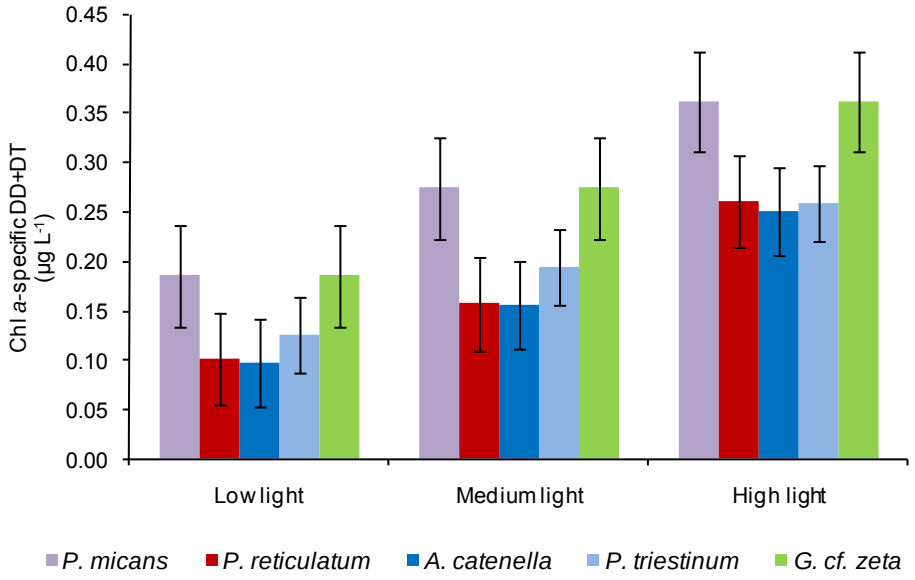


Figure 3.4 (b): Chlorophyll *a*-specific xanthophyll pool (DD+DT/Chl *a*) values of the five dinoflagellate species acclimated to three different irradiance levels as indicated for four days. The same trend is recorded for the five species as was noted for *A. catenella* during the time series shown in Figure 3.4 (a). The mean xanthophyll pool values (n=3) increases at each irradiance level reaching the highest concentration at high light. *Error bars indicate ± one standard error.*

Generally it was found that the higher the light intensity, the higher were the concentrations of light-protective xanthophylls (Figure 3.5). This is probably related to an evolutionary adaptation to the substantial environmental fluctuations dinoflagellates are subjected to in their natural environment (Appendix 3.8).

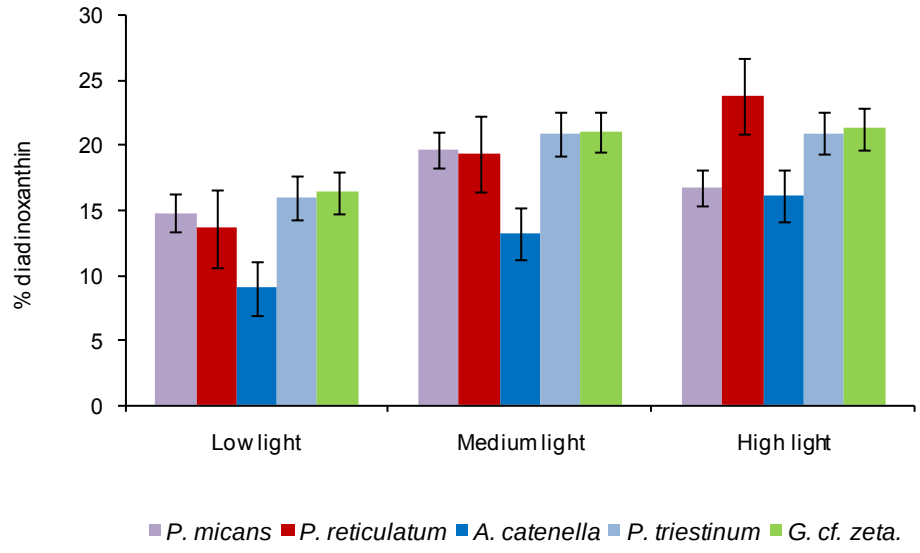


Figure 3.5: Diadinoxanthin percentage of total carotenoids in the five dinoflagellate species grown at three different levels of irradiance (low light (LL), medium light (ML) and high light (HL). *Error bars indicate ± one standard error*.

3.5 Absorption

Subsamples for absorption measurements were taken from the same photoacclimated culture sample on the day the *P vs E* experiments were carried out. The samples were filtered and frozen immediately (See Chapter 2 for details). The absorption measurements were normalized to Chl *a* concentrations, to give the chlorophyll-specific absorption coefficient a^* (m^{-2} (mg Chl *a*)$^{-1}$ after subtracting optical density at 750 nm to correct for residual scattering. Examples of absorption spectra are shown in Figure 3.6. The spectra indicate absorption at phytoplankton's major absorption peaks *i.e*. in the red region (wavelengths 671 nm), due to chlorophyll *a* and in the blue region (wavelengths 436 and 457 nm) with the expected two distinct peaks. Chlorophyll *a*, c_1 and c_2 and the carotenoids exhibit high absorption in this blue region. Absorption is minimal in the green region of the spectrum (571 – 644 nm) (Kirk 1996).

Generally absorption increased with increased irradiance in the dinoflagellate species between the wavelengths 436 – 457 nm and at 671 nm. *Protoceratium reticulatum* (Expt. 11) was an exception with highest absorption values in medium light (Figure 3.6.5). This corresponded to the presence of a high Chl *a* concentration (mean 113.0 µg L^{-1}). This species also had a high Chl *a* content per cell (2.5 to 2.9 pg cell $^{-1}$). Cleveland and Perry (1994) state that Chl *a* normalised spectra emphasise shape rather than magnitude with variability in spectral shape being the highest at pigment absorption peaks due to the ratios of accessory pigments to Chl *a* which varies with light levels and species (Figure 3.6). A pair-wise comparison among the species showed that there was no significant effect due to species ($F = 1.7$, df. = 4, $p = 0.17$) nor light. There was no interaction effect between species and light level. (Appendix 3.9 (a-1)).

The variations in the chlorophyll *a*-specific absorption coefficients for the five species grown under the different experimental light conditions are shown in Figure 3.7. Chl *a*-specific mean spectral absorption coefficients are tabulated in Appendix 3.8. The range of values is mainly the result of the optical properties of the individual cells in the sample. Comparing the results of the five dinoflagellate species indicate that at 440 nm wavelengths the value of the mean spectral absorption coefficient of *Gymnodinium zeta* is the highest, particularly when grown under medium and high irradiances (the values were 0.106 and 0.083 m^2 (mg Chl *a*)$^{-1}$ respectively)(Figure 3.7 (c)). The values at wavelength 675 nm however remained fairly constant for all light levels. *Protoceratium reticulatum* followed a similar pattern with a mean spectral absorption value at 440 nm of 0.080 m^2 (mg Chl *a*)$^{-1}$ at medium irradiance and 0.047 m^2 (mg Chl *a*)$^{-1}$ at 675 nm under high light (Figures 3.7 (b). The value of the mean spectral chlorophyll *a*-specific absorption coefficient at wavebands 440 nm and 675 nm of *Prorocentrum micans* (Figure 3.7e) remained fairly constant at all light levels.

The mean spectral absorption value at 440 nm normalised to Chl *a* at low, medium and high light ranged from 0.017 to 0.078, 0.019 to 0.080 and 0.024 to 0.093 m^2 (mg Chl *a*)$^{-1}$ respectively (Figure 3.8)), but

although the dinoflagellate species showed this wide variation in absorption the resulting data of this study corresponds with a similar variation observed for *a** of phytoplankton at 440 nm (Hoepffner and Sathyendranath 1992) and at 440 nm and 675 nm (Maske and Haardt 1987).

The absorption coefficient did not vary systematically with cell size as was noted in the *P vs E* results. This may reflect that a medium light level is optimum for these phytoplankton species and that other factors besides cell volume affect the absorption capacity of the species used in the experiments. This has not been measured in this study.

In literature the theoretical maximum values of *a** (675 nm) for non-packaged cells is 0.023 m^2 mg^{-1}. In comparison some of the *a** (675 nm) values recorded in the experiments are rather higher than the theoretical maximum except *Alexandrium catenella*. Here the range, with 0.017 at low light and 0.024 m^2 mg^{-1} at high light (Figure 3.8), fell close to the theoretical maximum (Kirk 1996). Nelson *et al.* (1993) made the observation that the higher values of *a** may be due to 'missing pigments' (carotenoids or phycobiliproteins) not measured by the standard fluorometric method or other light absorbing compounds (cytochromes, flavins or quinones) extracted by methanol and thus included in the $a^*(\lambda)$ spectrum. The cultures used in these experiments were in a state of exponential growth and were healthy with adequate algal material on the filters used for absorption measurement, the increased values in Chl *a*-specific absorption may be due to unidentified light-absorbing compounds not measured by fluorometry nor by HPLC methodology, as hypothesised by Bricaud *et al.* (2004). In this study 100% acetone with carotenal as internal standard (following the Marine and Coastal Management method (Barlow *et al.* 2000)) was used which may give different results in the five phytoplankton species.

Generally, the value of the mean spectral chlorophyll *a*-specific absorption of the five dinoflagellate species is greater with increased irradiances, with the highest absorption taking place in the wavebands 440 nm (blue region) and 675 nm (red region). The increase in Chl *a*-specific absorption in *Prorocentrum triestinum* relative to the similar sized *Gymnodinium zeta* may be due to its higher quota of photosynthetic carotenoids (Figure 3.7 and Table 3.2).

Table 3.2: Pigment data for the phytoplankton species shown in Figures 3.6 and 3.7. Size is approximate length (µm).

Pigment : Chl *a* ratios in µg L^{-1}. Total chlorophyll *a* per cell in pg. (Chl *a* measurements were made fluorometrically).

		Low light					**Medium light**					**High light**				
Species	**Size µm**	**T.Chl *a* Cell^{-1}**	**Chlc: Chl*a***	**[a]PSC: Chl *a***	**[b]PPC: Chl *a***	**[c]Xanth: Chl *a***	**T.Chl *a* Cell^{-1}**	**Chlc: Chl *a***	**[a]PSC: Chl *a***	**[b]PPC: Chl *a***	**[c]Xanth: Chl *a***	**T.Chl *a* Cell^{-1}**	**Chlc: Chl *a***	**[a]PSC: Chl *a***	**[b]PPC: Chl *a***	**[c]Xanth: Chl *a***
Alexandrium catenella	18 to 90	23.1	0.327	0.642	0.102	0.022	12.4	0.341	0.629	0.145	0.023	11.4	0.326	0.678	0.258	0.024
Protoceratium reticulatum	30 to 50	49.3	0.363	0.541	0.102	0.022	41.1	0.385	0.543	0.171	0.025	37.67	0.351	0.554	0.275	0.029
Gymnodinium zeta	6 to 20	1.4	0.309	0.650	0.171	0.017	0.8	0.322	0.676	0.282	0.018	1.2	0.295	0.712	0.410	0.021
Prorocentrum triestinum	7 to 20	3.0	0.420	0.531	0.123	0.023	1.6	0.425	0.566	0.183	0.037	1.1	0.451	0.553	0.251	0.041
Prorocentrum micans	30 to 48	31.4	0.290	0.597	0.133	0.025	23.5	0.374	0.563	0.178	0.039	22.0	0.220	0.548	0.233	0.036

Chlc, the sum of all c chlorophylls

PSC, photosynthetic carotenoids

PPC, photoprotective carotenoids

Xanth, xanthophylls

[a] peridinin

[b] diodinoxanthin and diatoxanthin

[c] β-carotene

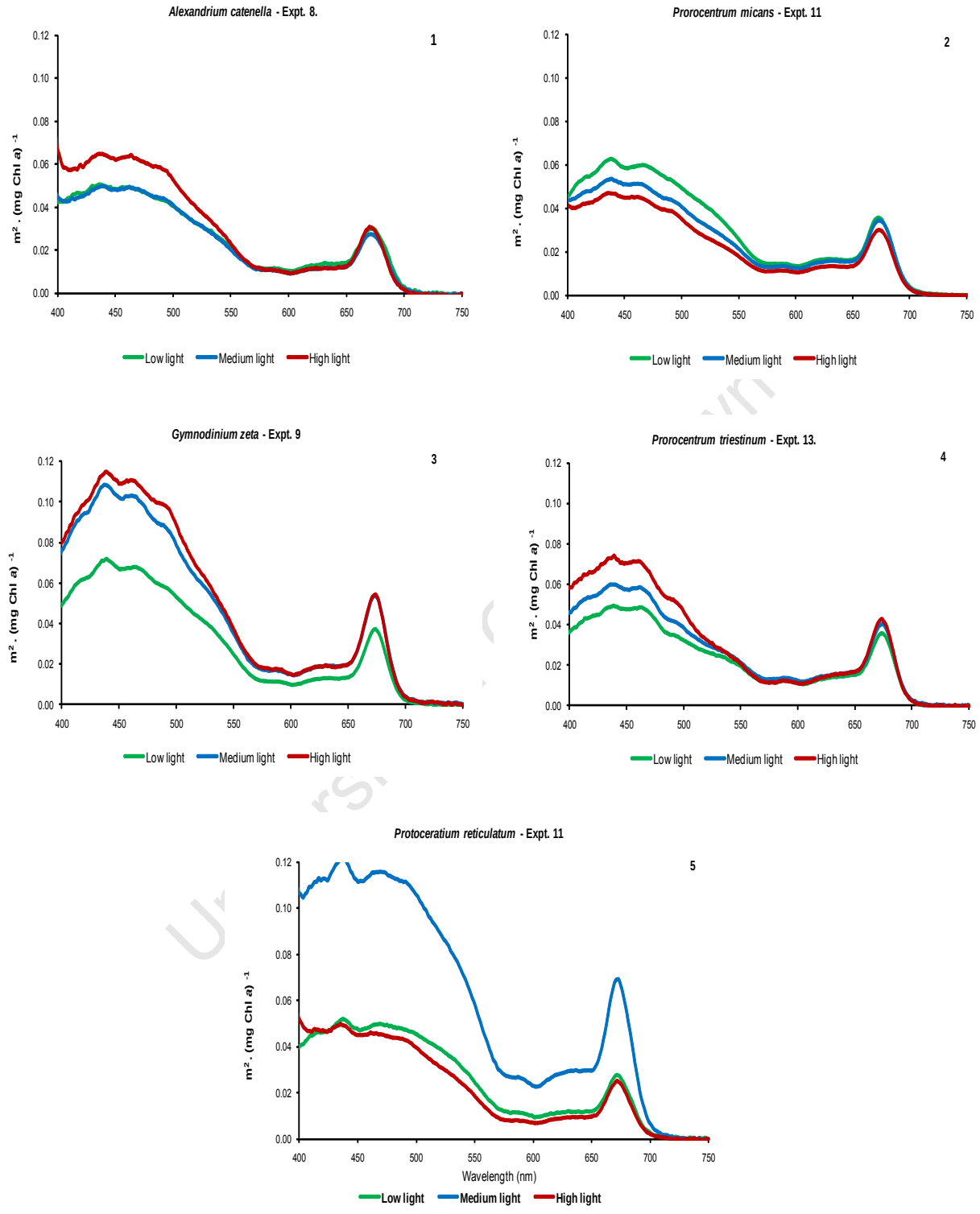


Figure 3.6: Examples of Chl *a*-specific absorption spectra for five dinoflagellate species grown under different experimental irradiances. Note shape of Chl *a* normalised spectra. (*Alexandrium catenella* (1), *Prorocentrum micans* (2), *Gymnodinium zeta* (3), *Prorocentrum triestinum* (4) and *Protoceratium reticulatum* (5). (Absorption was normalised to fluorometrically measured Chl *a*).

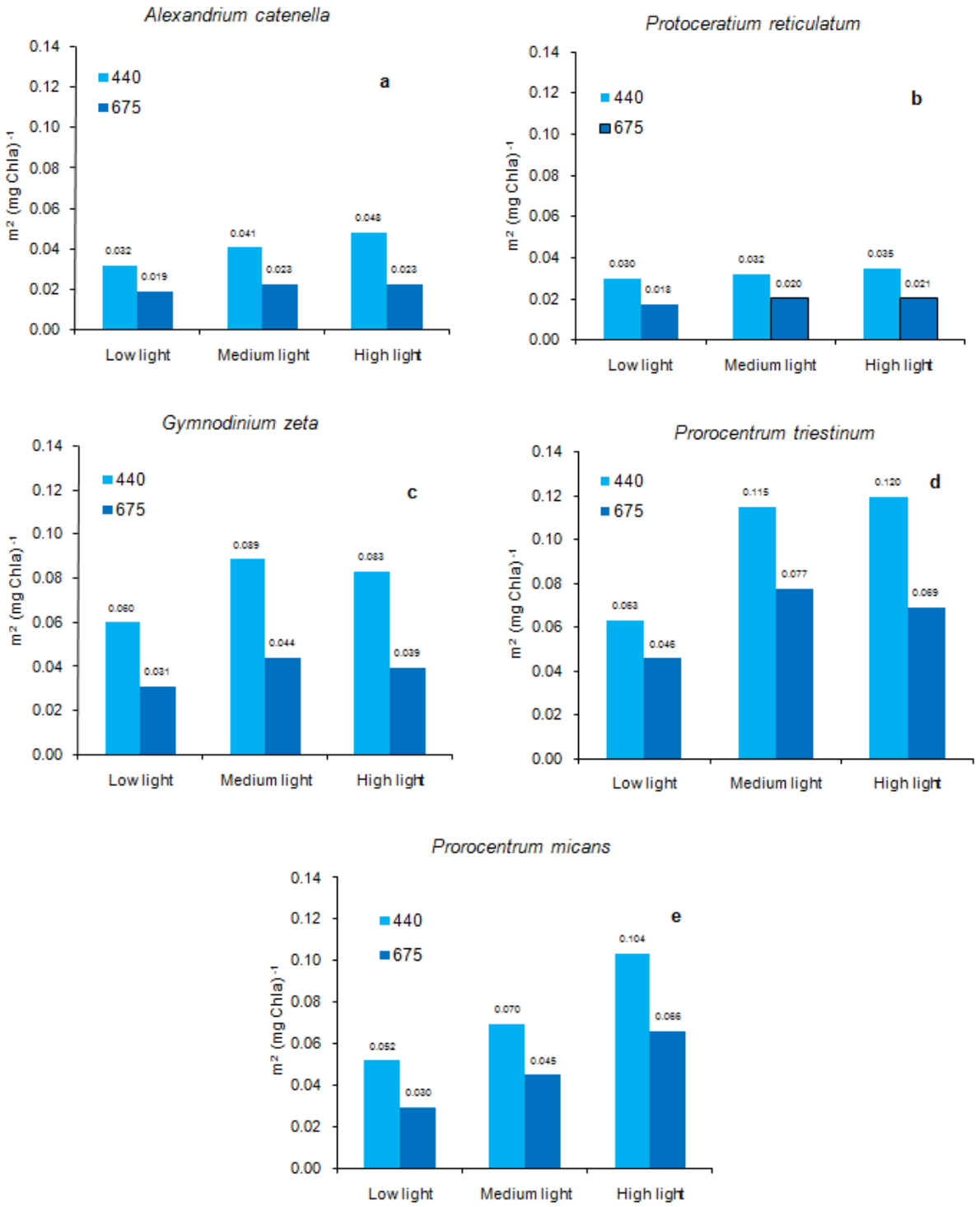


Figure 3.7: Examples of Chl *a*-specific absorption coefficient values of five dinoflagellate species (*Alexandrium catenella* (a), *Protoceratium reticulatum* (b), *Gymnodinium zeta* (c), *Prorocentrum triestinum* (d) and *Prorocentrum micans* (e)) at wavelengths 440 nm and 675 nm. (Absorption was normalised to fluorometrically measured Chl *a*).

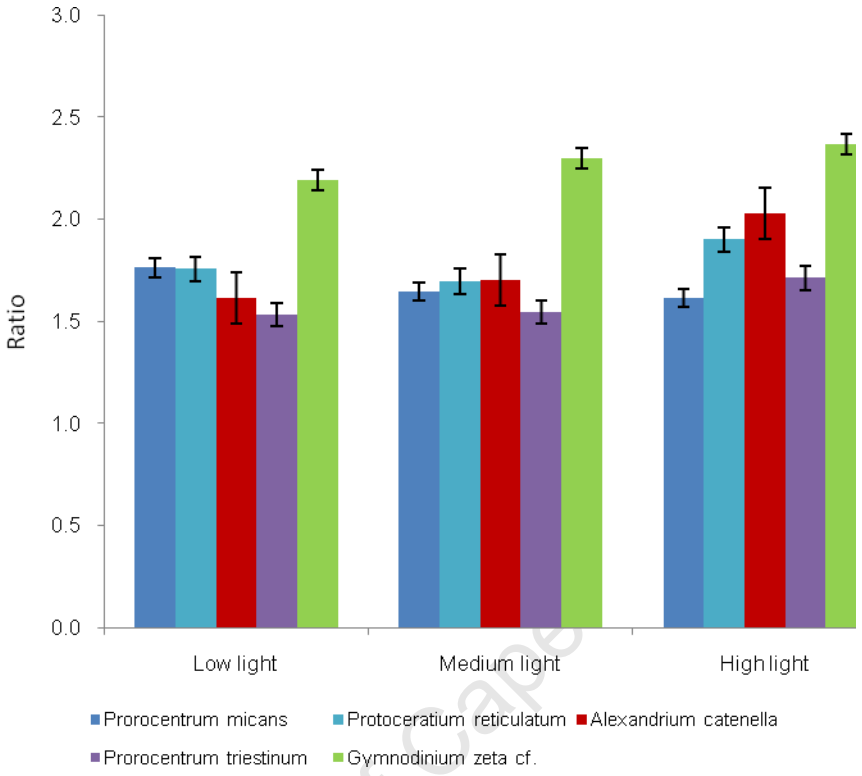


Figure 3.8 (a) : Ratio of Chl a-specific absorption coefficient at 440 nm to 675 nm of the five dinoflagellate species measured at low, medium and high light conditions. (Absorption was normalised to fluorometrically measured Chl *a*). *Error bars indicate ± one standard error.*

The variation in the absorption spectra can be attributed to the different light harvesting complexes (LHCs) of the five different species of phytoplankton used in these experiments. The LHCs are responsible for most of the absorption of light in the cell. The molecular structure of the pigments, present as chromoproteins in the cell, which make up the LHCs, determines the absorption spectra of each species of phytoplankton. Interestingly, studies in respect of theoretical control mechanisms of optimising pigment concentrations in microalgae under conditions of different light intensities and other environmental factors, the *dynamic balance theory*, suggest that the redox state of electron transport pathways between PS II and PS I act as a *light sensor* which sets the pigment level in the cell (Kana *et al.* 1997). This regulation of pigment synthesis controls the level of photoacclimation (Escoubas *et al.* 1995). Cellular pigment concentrations therefore constitute a balance between the rates of PS II loss and gain which in turn balances the light energy input with biochemical energy use (Kana *et al.*1997; Anning *et al.* 2000).

Different species of phytoplankton have different LHCs containing different pigments therefore the absorption spectrum of each species will be the result of the sum of the spectra of its LHCs. During

photoacclimation the same species changes the number of photosynthetic units (PSUs) and/or the proportion of different parts of the PSU in response to changing light fields which is a cause of variability. Another source of variability in the absorption characteristic of phytoplankton species is the packaging effect. Absorption is lowered when the chromoproteins (forming the different components of the photosynthetic units, reaction centres, subantennae and LHCs) are contained in discrete parcels compared to the same material held in solution. This packaging effect is also related to cell size, cell shape and the intracellular concentration of pigments (Sathyendranath *et al*. 1987, Kirk 1994, Raven and Kübler 2002, Bricaud *et al.* 2004). An increase in a cell's radius lowers the mean value of the average Chl *a*-specific absorption coefficient and affects the thickness of the diffusion boundary layer (Raven 1986, 1999; Fogg 1991). This could explain the reason that the smallest species *Gymnodinium zeta* (Figure 3.7) showed greater absorption values at the indicated wavebands, although that does not seem to be relevant in two of the larger celled species *Prorocentrum micans* and *Protoceratium reticulatum*. It merely underlines the very variable characteristics of the individual phytoplankton species under investigation. It is suggested by Hoepffner and Sathyendranath (1993) that knowledge of the spectral characteristics of phytoplankton absorption coefficients can be used to reconstruct the *in vivo* absorption spectrum of a mixed algal community. Inversely, it can also be used to evaluate the concentration of pigments in the sample.

As the absorption data illustrated, the ratio of absorption of the samples of dinoflagellate cultures at 440 nm and 675 nm was generally between 1.00 and 2.00 indicating that the cultures were in a healthy state (Kiefer *et al.* 1979) (Figure 3.8). No significant increase in absorption at 440 nm relative to 675 nm indicative of an ageing culture was observed (Kiefer *et al.* 1979; Yentsch and Phinney 1982). Three species showed peak-height ratios > 2.00 *i.e. Gymnodinium zeta* (Experiment 3, 9), *Alexandrium catenella* (Experiment 8 in HL) and *Protoceratium reticulatum* (Experiment 11 in HL) (Appendix 3.10). Analysis of the data using a two-way fixed effect ANOVA revealed that only the effect of light level was significant, not species ($F = 17.6$, $df = 2$, $p = 1.65E\text{-}05$). There was no interaction effect between species and light (Appendix 3.10 (a)).

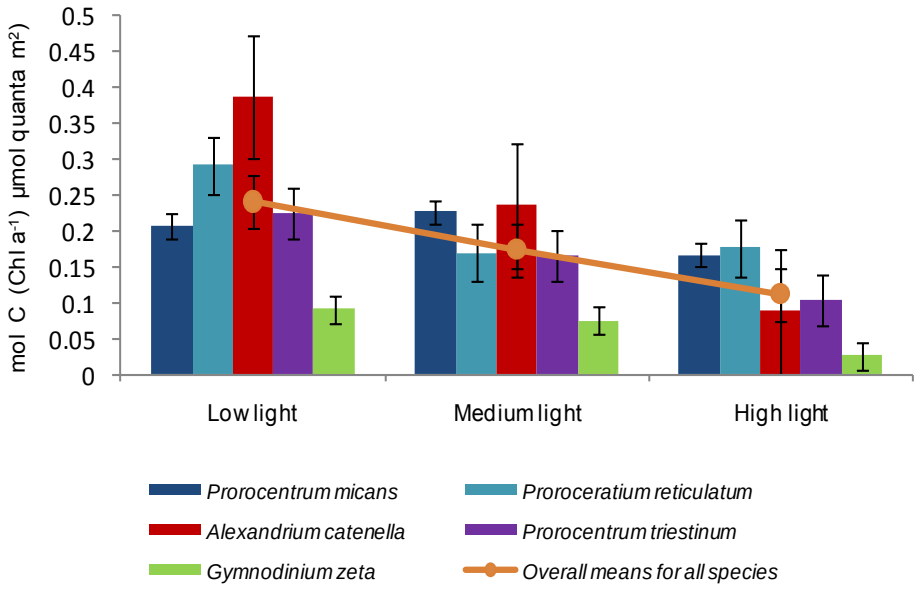


Figure 3.9 (a): Comparison of the estimated maximum quantum yield for the five dinoflagellate species acclimated to different irradiances as shown. Overall mean (n= 5) for the dinoflagellates used in the experiments is also indicated.
Error bars indicate ± one standard error.

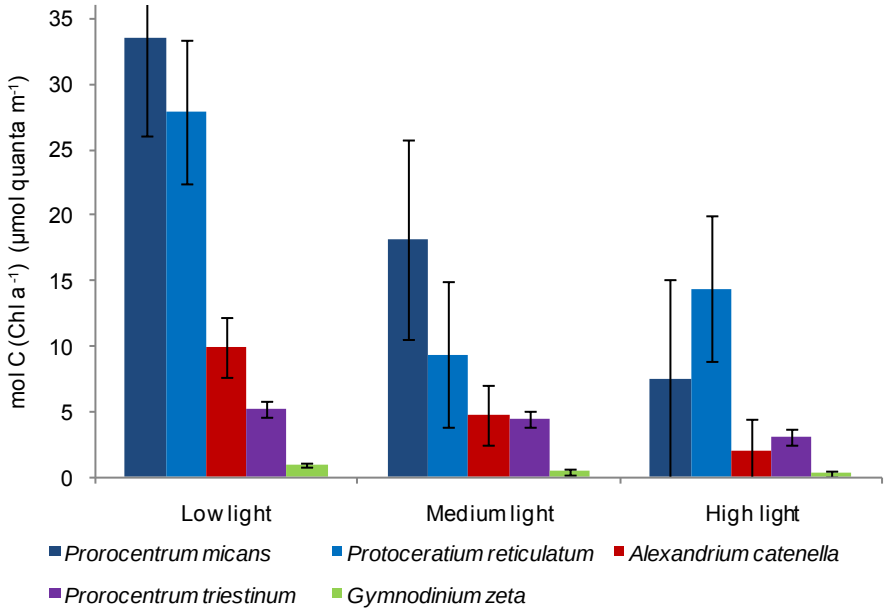


Figure 3.9 (b): Comparison of the estimated maximum quantum yield for the five dinoflagellate species acclimated to the different irradiances calculated per cell.
Error bars indicate ± one standard error

3.6 Maximum quantum yield

The quantum yield of carbon fixation is an index of the efficiency with which the energy is transferred from the pigment to the electron transport carriers in the phytoplankton cell and an indicator of the photochemical mechanism (Bannister and Weidemann 1983; Kirk 1996 and others). A comparison of the estimated maximum quantum yields (ϕ_m) of the five different dinoflagellates, derived from the initial slope of α of the *P versus E* relationship and the absorption cross section *a** relative to chlorophyll *a*, in m^2 (mg Chl *a*)$^{-1}$, is given in Figure 3.9 (a) and Appendix 3.10.

The maximum quantum yield (ϕ_m) for the three experiments using the same dinoflagellate species generally showed a higher maximum quantum (θ_m) yield value for the lower irradiances (range 0.29 – 0.11 mol C (Chl *a*)$^{-1}$ µmol quanta m^{-2} s^{-1}). This is in agreement with other published results (JGOFS Report 1993; Kirk 1996). Generally the maximum value of ϕ_m occurs at diminishing irradiances and decreases with increasing light (Figure 3.9 (a)). A few exceptions occurred in the larger celled species *i.e. Prorocentrum micans* showed an increase at medium light (range (LL) 0.21, (ML) 0.23, (HL) 0.17 mol C (Chl *a*)$^{-1}$ µmol quanta m^{-2} s^{-1})). This increase was not apparent when the data were analysed per cell (Figure 3.9 (b). *Protoceratium reticulatum* showed a decrease from low light to medium light ranging from 0.29 (LL) to 0.17 (ML) and 0.18 (HL) mol C (Chl *a*)$^{-}$1 µmol quanta m^{-2} s^{-1}. Comparing the θ_m of the five dinoflagellate species per cell illustrated a definite correlation with cell volume as the species were systematically arranged according to cell size with the largest species (*Prorocentrum micans*) achieving the highest quantum yields and the smallest (*Gymnodinium zeta*) the lowest. Mean maximum quantum yield per cell for these species taken over all light levels was 0.19 and 0.6 mol C (Chl *a*)$^{-}$1 µmol quanta m^{-2} s^{-1} respectively. Statistical analysis using a two-way fixed effect ANOVA indicated that there was a significant difference between species responses to changing light conditions, also light level was significant (Species, F = 9.7, df = 4, p = 7.89E-05; Light, F = 9.5, df = 2, p = 0.0008) (Appendix 3.11 (a-1)). In the analysis for the maximum quantum yield per cell the slight difference between species was significant (p = 0.06) (Appendix 3.11 (a-2)).

The maximum quantum yield data showed great variation for the different experiments with each species which was supported by the variation in the data for the ratios of total peridinin to total pigment. The theoretical maximum value of ϕ_m which plant species in principle might achieve is ~ 0.125 mol C fixed mol^{-1} photons absorbed (8 moles of photons are required to reduce 1 mole of CO_2 in the absence of photorespiration (Kirk 1996). ϕ_m may be closer to 0.112 because of photophosphorylation (Long *et al*. 1993). The values of the maximum quantum yield in my data set are comparatively high. This could possibly be attributed to the species sensitivity to light quality as maximum quantum yield was found to be wavelength dependent in certain dinoflagellate species (Schofield *et al.* 1996; MacIntyre and Cullen 2005). The incubation light source used was a tungsten-halogen lamp which produced a fairly flat spectrum, especially in the blue-green region (Kyewalyanga 1997) where phytoplankton have a major absorption peak. Another

factor could be the differences in the species' light harvesting complexes (LHC) *e.g*. when peridinin is present in greater concentrations as was the case in *Gymnodinium zeta* and *Alexandrium catenella* grown under a low and high irradiances. The total peridinin to total pigment (μg L^{-1}) ratio recorded here was 0.3 compared with the other species which fell in the range 0.25 to 0.3. Researchers report that the presence of spectrally distinct peridinin/Chl *a*-protein (PCP) or Chl *a*/Chl c_1/peridinin-protein (ACP) complexes can also constitute an important factor (Jovine *et al.* 1993). Many causes for the variation in maximum quantum yield are discussed in literature (Cleveland *et al.*1989; Sosik and Mitchell 1991), but due to the complexity of the different processes involved, it makes interpretation of these data difficult.

3.7 Carbon to chlorophyll *a* ratio

The total carbon (TC) concentrations for the five dinoflagellates grown under low, medium and high irradiances were very variable (Appendix 3.12). Generally the concentrations increased at higher irradiances (Figure 3.10 (a)). The highest values were found in the culture of the large celled species *Protoceratium reticulatum* (mean 16.09 (LL), 18.24 (ML) and 21.80 mg L^{-1}) increasing by 26%. A similar increase was noted in the small-celled species *Prorocentrum triestinum* (mean 15.66 mg L^{-1}) grown in medium light increasing by 11%. *A. catenella* showed little change at the three light levels with a slight decrease of carbon content at high light (range 13.67–12.35 mg C L^{-1}). *Prorocentrum triestinum, Gymnodinium zeta* and *Prorocentrum micans* all showed a greater increase at medium light. All species responded to changing light levels in a different fashion ($F = 2.8$, $df = 4$, $p = 0.04$) although light level itself was not significant ($p > 0.05$), (Appendix 3.12 (a-1)).

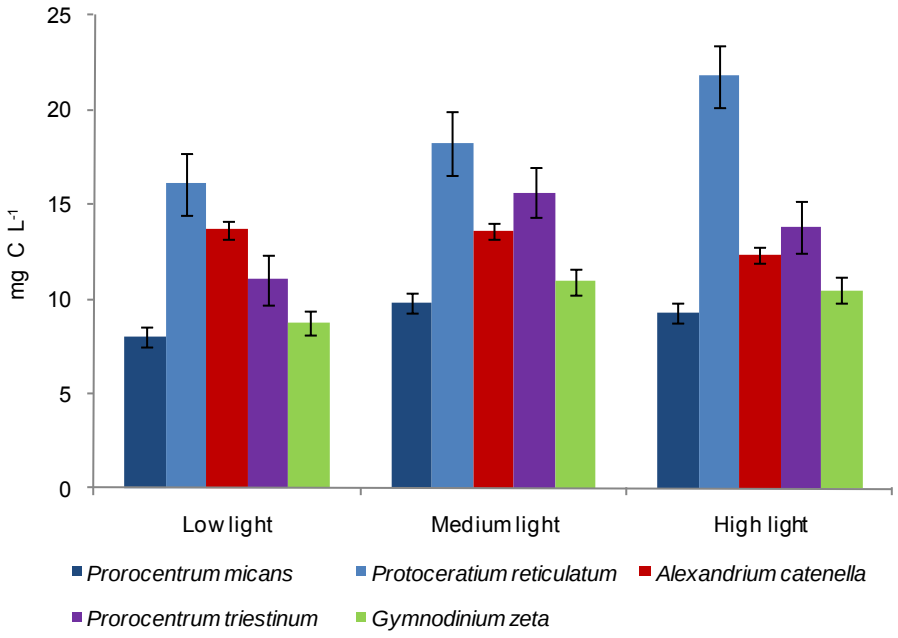
The total carbon to chlorophyll *a* ratios generally increased from low light to high light levels. In some cases the ratio doubled as observed in the small celled *Gymnodinium zeta* (Figure 3.10 (b). Increases in the species *Protoceratium reticulatum* and *Alexandrium catenella* ranged from 25 to 38%. The high ratios correlated with the low values of chlorophyll *a.* Statistical analysis indicated that again only species had a significant effect whereas light level and the interaction effects were not significant ($F = 2.99$, $df = 4$, $p = 0.04$, Appendix 3.12 (a-2)).

The total carbon to volume relationship for the phototrophic dinoflagellate species at low, medium and high light levels is shown in Figure 3.10 (c). The comparison shows that the C density is not constant but decreases significantly with increasing cell volume. This variance is highly species-specific. In this study the dinoflagellates were found to be denser than diatoms (See Chapter 4). Similar results have been reported in literature (Menden-Deuer and Lessard 2000).

In these analyses mentioned above it was apparent that cell volume is an important factor in relation to the cells' carbon content since the species were arranged systematically according to cell size (Figure 3.10 (c). This was also definitely demonstrated in the maximum quantum yield values. All species responses to changing light levels were significant ($F = 34.3$, $df = 4$, $p = 8.08E\text{-}10$) although light level itself was not significant ($p > 0.05$), (Appendix 3.12 (a-3)).

Changes in pigment composition and intracellular concentration do not co-vary with cell size (Richardson *et al.* (1996) so other factors such as physiological responses of the unicellular algae to irradiance probably come into play. The responses fall into two categories *i.e.* those that improve the cell's light energy harvesting/utilization abilities and those that reduce energy required for growth. In the first instant an increase or decrease in pigment content takes place including some adjustment to the photosynthetic apparatus. In the second category phytoplankton response to increasing or decreasing irradiance fields causes changes in the energy-saving mechanisms of the cell. Thompson *et al.* (1991) documented that changes in pigment concentrations, and hence carbon content, causes changes in cell volume in phytoplankton species grown under different light intensities.

The ratios of total carbon to chlorophyll *a* were highest at high irradiances in the cultures of the larger celled species *Alexandrium catenella* and *Protoceratium reticulatum* ranging up to 346.5 (Figure 3.10 (b)). Those cultures which had a high total carbon to chlorophyll *a* ratio had low Chl *a* L^{-1} content. This influences the ratio calculations and resulted in an unusually high ratio particularly in the case of *Protoceratium reticulatum* (Expt. 7) (Appendix 3.12). In literature the particulate carbon to chlorophyll ratios reported for natural populations, ranging from 27 – 67 (Riemann *et al.* 1989), are much lower than those observed in this study although these ratios in this study are in agreement with other documented variations in carbon to chlorophyll *a* ranging from 40 to > 200 (Wang *et al.* 2009) depending on the available light intensity. Species-specific increases and declines were noted for the C : Chl *a* ratios at the different irradiance levels which corresponded to similar fluctuations in P^*_m and α* values.



3.10 (a): Total carbon concentrations of the five dinoflagellate species grown under low (~33 µmol quanta m^{-2} h^{-1}) Figure, medium (~ 178 µmol quanta m^{-2} h^{-1}) and high light conditions (~ 647.0 µmol quanta m^{-2} h^{-1}). *Error bars indicate ± one standard error.*

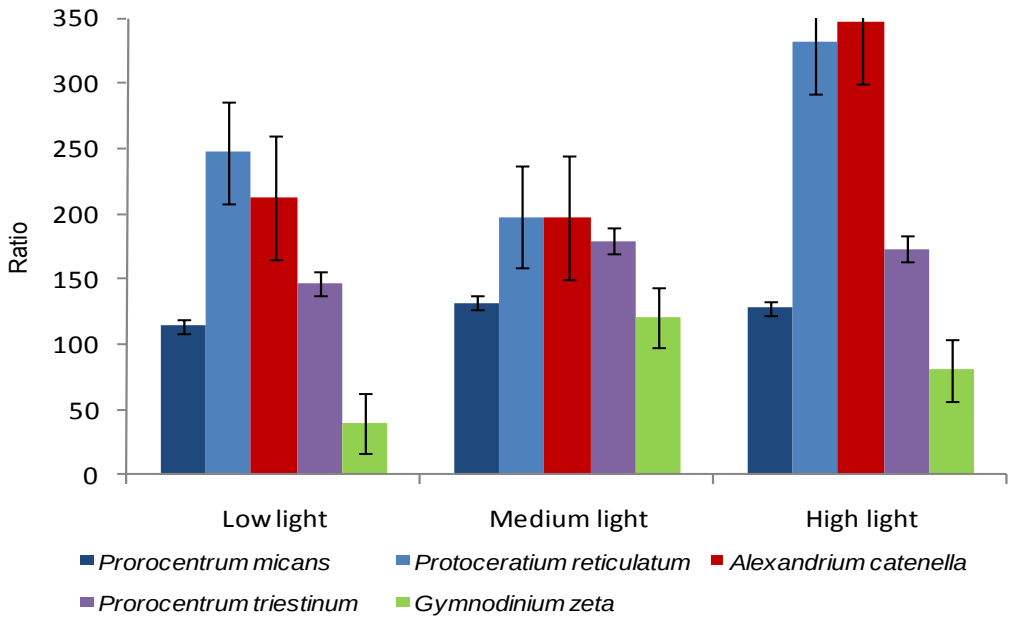


Figure 3.10 (b): Comparison of total carbon to total chlorophyll *a* ratios of the five dinoflagellate cultures acclimated to three different irradiance levels as illustrated. (Chl *a* measurements were made fluorometrically). *Error bars indicate ± one standard error.*

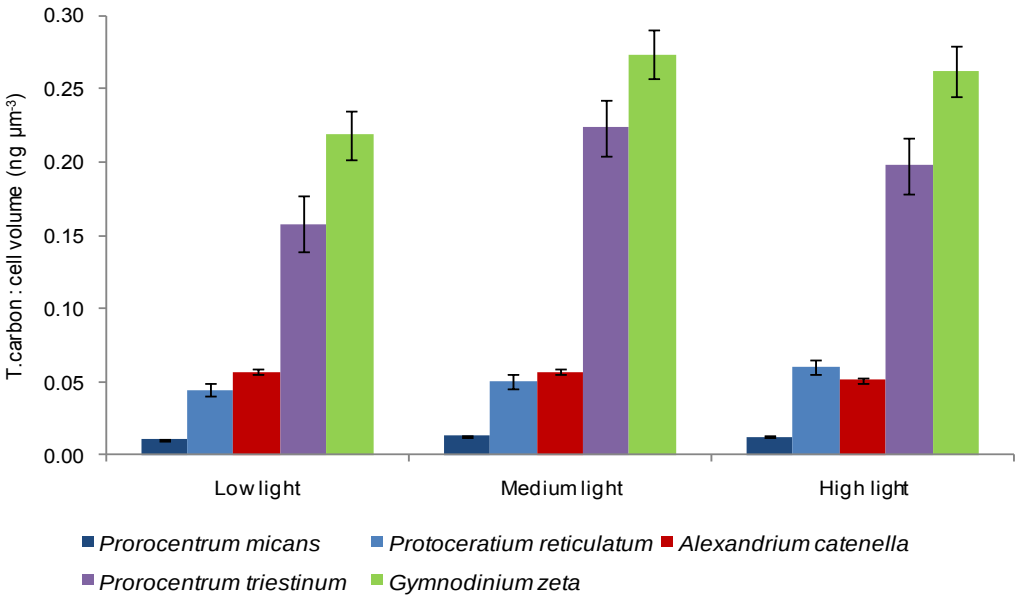


Figure 3.10 (c): Comparison of total carbon : cell volume relationshipfor the five dinoflagellate species grown under low, medium and high light conditions (~33 µmol quanta m^{-2} s^{-1}) , (~178 µmol quanta m^{-2} s^{-1}) (~647 µmol quanta m^{-2} s^{-1}). *Error bars indicate ± one standard error.*

3.8 Summary

The dinoflagellates (migrators and layer-formers) examined in this thesis exhibited definite trends characteristic of phytoplankton existing in a lower light environment in the water column. The *P versus E* light saturated rate normalised to Chl *a* (P^*_m) ranged from 3.0 to 14 mg C (mg Chl *a*)$^{-1}$ h^{-1}). Comparing the *P versus E* data of cultures of the different species it was noted that photophysiologically and photosynthetically *Alexandrium catenella,* a toxic, bloom forming species, was able to optimise both high irradiance environments (mean photosynthetic rate was 6.8 mg C (mg Chl *a*)$^{-1}$ h^{-1}) as well as a low irradiance level best, followed by *Protoceratium reticulatum* and *Prorocentrum triestinum* with a mean photosynthetic rate of 6.2 and 5.0 mg C (mg Chl *a*)$^{-1}$ h^{-1}). By extension, efficient vertical depth regulation is important for these species to exploit available light at surface as well as at depth in a turbulent oceanic environment. The data of *Alexandrium catenella* and *Protoceratium reticulatum* cultures indicate that these two species are the most adapted to deeper depths in the water column compared with the other species.

Interestingly, analysis of the parameters E_k, P^*_m and α* shows that cell size has a definitive effect since the species from medium light to high light varied systematically with cell volume. This trend was also mentioned by Tilzer *et al.* (1989). In the two smallest dinoflagellate species, E_k increased from 248 μmol quanta m^{-2} s^{-1} at medium light to 346 μmol quanta m^{-2} s^{-1} at high light in *Prorocentrum triestinum* and from 214 (ML) to 385 μmol quanta m^{-2} s^{-1} (HL) in *Gymnodinium zeta*. In the largest species, *Prorocentrum micans*, E_k was lowest (123 in ML) increasing to 180 μmol quanta m^{-2} s^{-1} in high light. The effect of cell size on P^*_m showed a similar trend but not as distinct. The α* values were very variable and not correlated with cell size.

In the photosynthetic pigment analysis of the five dinoflagellate species it was demonstrated that the pigment pool did not change appreciably but the individual pigments within the cell either increased or decreased depending on the light intensity the cultures were exposed to. Chl *a* showed the highest concentration of the photosynthetic pigments remaining fairly constant at all light levels with a slight decrease at high light levels with peridinin following a similar pattern (Figure 3.3 (a)). However, pigment concentrations per cell as reflected in Figure 3.3 (b), gave a clearer indication of the pigment concentration changes in the individual cells. At high light levels there is a definite decrease of Chl *a* particularly in *P. micans* and *P. triestinum* (up to 50%) coupled with a similar decline in peridinin and chlorophyll c_1 and c_2. Both these species show a decline in the concentration of photoprotective pigment diadinoxanthin from LL to HL which is not the case in the other species. This is in contrast to expectation. In this study there was clear evidence that the genus *Prorocentrum,* represented by the two species *Prorocentrum micans* and *Prorocentrum triestinum,* possessed a definitive attribute which was noted in all the replicate experiments with these two species. The decline in the photosynthetic pigment peridinin at high light was coupled with a substantial decline in the photoprotective pigment diadinoxanthin from low light to high light which was not paralleled in the other

three species used in this study as the opposite took place, *i.e.* diadinoxanthin usually increased at higher light levels.

The analysis of the absorption data illustrated species-specific photoacclimation trends. Although the spectral absorption values remained fairly constant at all light levels there was always a slight increase from low light to high light with the main increase taking place under medium light conditions. Analysing the Chl *a* normalised absorption values (a^* (λ)) at wavelengths 440 nm and 675 nm compared with non normalised values (m^{-1}) reflected the same trend i.e. that the medium irradiance used under the laboratory conditions in continuous cultures was generally the optimum level for the dinoflagellate species used.

The maximum quantum yields (denoted by θ_m) that were obtained illustrated clearly that cell volume is an important factor in respect of yields as the data systematically correlated to cell size. This trend was also particularly evident in the $E_{k.}$ values.

Species-specific declines and increases were noted in the total carbon content and the carbon to Chl *a* ratios at the different light levels. Generally increases were noted at high light with ratios in some cases doubling as observed in the small-celled *Gymnodinium zeta*). Generally increases noted ranged from 25 to 38% as in Alexandrium *catenella* and *Protoceratium reticulatum*. The high ratios usually correlated to low Chl *a* L^{-1} concentrations. Variation in the C to Chl *a* ratio have been reported in literature ranging from 40 to >200 and were not constant as usually is presumed (Wang *et al.* 2009).

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CHAPTER 4. PHOTOPHYSIOLOGICAL RESPONSES OF SELECTED DIATOMS

The response to different irradiance levels, photoacclimation, in the phytoplankton group diatoms was investigated using three diatom species isolated from the southern Benguela Current along the west coast of South Africa. Photoacclimation typically manifests itself as a graded reduction in photosynthetic pigments in response to increased light levels. The object of this investigation was to measure the species-specific variations due to light limiting and light saturating photosynthetic rates in photoacclimated monocultures of the three diatom species, *Chaetoceros* sp., *Chaetoceros capense* and *Chaetoceros pendulus*, using the classic ^{14}C method (Strickland 1965 and others). The laboratory conditions under which the photosynthetic responses were determined were kept constant throughout all the experiments so that the observed parameters (P^*_m, α^* and E_k) could be meaningfully compared. It is well known that the irradiance required to saturate photosynthesis is, to a great extent, dependent on the concentration of CO_2 in the culture's nutrient medium and temperature, so short incubation times lasting 20 min were used for the *P versus E* curves experiments. The data thus obtained would reflect gross photosynthesis (Williams 1993). The unit of mass, Chl *a*, to which the photosynthesis data was normalised in this study, was fluorometrically obtained and compared with HPLC analyses conducted on subsamples of the same cultures used for each experiment. Light absorption and carbon assimilation with a view to be able to assess and compare the species-specific photoacclimation responses were also measured. The experimental design followed is described in detail in Chapter 2.

4.1 Chlorophyll *a* measurements

In the experiments with the diatom species *Chaetoceros* sp., *Chaetoceros capense* and *Chaetoceros* cf. *pendulus*, the total chlorophyll *a* concentrations measured by fluorometry (in triplicate) generally showed higher values at low light intensities decreasing in high light treatment (Figure 4.0 (a)). The concentration of Chl *a* in the three diatom species decreased from low light to high light by approximately 35% (range from largest cell to smallest was 26 to 35%). The decrease in the largest-celled *Chaetoceros* sp. from LL to HL was the lowest (26%). A similar photoacclimation response was noted in the dinoflagellate species.

A comparison of the Chl *a* : cell volume relationship for the three species of diatoms is given in Figure 4.0 (b). It shows that the total Chl *a* concentration for each species corresponded well to cell size as they varied systematically with cell volumes. *Chaetoceros* sp. had the least Chl *a* : cell volume quota decreasing significantly with increasing cell volume. The difference in the Chl *a* density is not only reflected in the calculated cell volumes which were approximately: *Chaetoceros* sp. - 520 μm^3, *Chaetoceros pendulus* - 260 μm^3 and *Chaetoceros* cf. *pendulus* - 170 μm^3, but also depends on the species' shape, packaging effect, and other morphological differences which need to be investigated further. Statistically light level was

shown to be significant in respect of the Chl *a* cell quota of the different diatom species (F = 4.7, df = 2, p = 0.02). (Appendix 4.1 (a-2)).

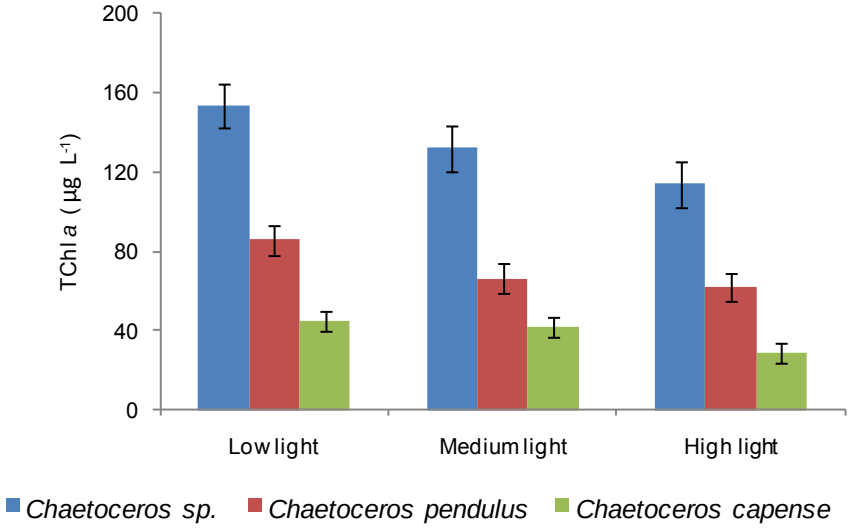

Figure 4.0 (a): Comparison of total Chl *a* concentrations, measured by fluorometry, of the three diatom species acclimated to low medium and high irradiances. Data represent mean values (n=3).
Error bars indicate ± one standard error.

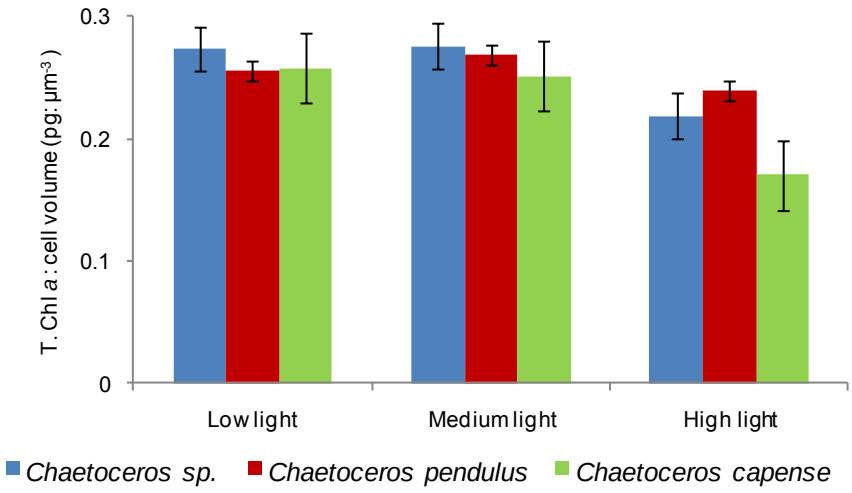

Figure 4.0 (b): Chlorophyll a : cell volume relationship for the three diatom species *Chaetoceros* species (520 µm^3), *Chaetoceros pendulus* (260 µm^3) and *Chaetoceros capense* (170 µm^3).
Error bars indicate ± one standard error.

Differences in Chl *a* readings measured fluorometrically were noted compared to those determined using HPLC analysis (Appendix 4.1). Traces of chlorophyllide *a* were found in the diatom species ranging from 0.5 to 62%. This corresponds to similar percentages of chlorophyllide *a* reported in literature (Suzuki and Fujita 1986; Rodriguez *et al.* 2002) and particularly in the genus *Chaetoceros* (Jeffrey and Hallegraeff 1987). It was reported that certain species of unicellular algae are more vulnerable than others to *e.g*. filtration and centrifugation where chloroplast membranes are damaged easily and activate the conversion to chlorophyllide *a*. In young cultures, even at low suction pressure, filtration can damage thylakoid membranes in certain diatoms but not in others. Extraction in 100% acetone also activates chlorophyllase and the highest activity of the acetone-activated chlorophyllase was found specifically in bloom-forming species such as *Chaetoceros didymus* (< 50%) (Suzuki and Fujita 1986). The differences may reflect inherent (*i.e*. genetic) strain differences or physiological changes in the cells imposed by the insolation and culture conditions. If the final product of chlorophyllide *a* activity is pheophorbide *a*, absorption and fluorescence spectra will be different and Chl *a* could be underestimated (Lorenzen and Jeffrey 1980). Light level had a significant effect on the chlorophyllide *a* percentage of the diatom species ($p = 0.04$). The Chl *a* values measured fluorometrically were used for all standardisation calculations.

4.2 Cell counts

The concentrations of the cells (cells L^{-1}) in the samples used in the experiments, are tabulated in Appendix 4.3. Generally the cell density ranged between 2.8×10^6 to 15.53×10^6 cells L^{-1} for the *Chaetoceros* species with large cell diameters. In the samples from the smaller-celled species, *Chaetoceros* cf. *pendulus* and *Chaetoceros capense*, densities ranged from 11.38×10^6 to 66.00×10^6 cells L^{-1}. In experiment 10, *Chaetoceros capense* density was lower at 7.97×10^6 cells L^{-1}. As with the dinoflagellates, the diatom samples were examined using a microscope and were selected for their cell density and viability.

4.3 Photosynthesis *versus* irradiance curves and photosynthetic parameters

The photosynthesis *versus* irradiance curve (*P vs E*) curve describes the rate of photosynthesis as a function of irradiance (*E*), *P* represents the carbon fixation rate expressed in mg C m^{-3} h^{-1} and *E* the incident irradiance in terms of photosynthetic available radiation (PAR) expressed as µmol quanta m^{-2} s^{-2}. The shape and magnitude of the curve reflects the biological processes that regulate photosynthesis. The rates of gross photosynthesis, as a function of irradiance, were measured in a photosynthetron using the ^{14}C technique in the same way as for the dinoflagellates. The data were fitted to the model of Platt *et al.* (1980), with the fit being forced through zero using a non-linear estimation routine (STATSOFT 2008, Statistica 8). P^*_m increased from low (33 µmol quanta m^{-2} to high light (647 µmol quanta m^{-2} s^{-1}). Photoinhibition was not observed under high irradiance conditions in any of the three diatom species. The photosynthetic parameters showed that diatoms photosynthesise more efficiently in high light conditions compared with the dinoflagellates which are generally more efficient at lower irradiances. Examples of the *P vs E* curves of three different experiments with the three diatom species, *Chaetoceros* sp. (a–c), *Chaetoceros capense*

(d-f) and *Chaetoceros* cf. *pendulus* (g-i), are presented in Figure 4.1 and Table 4.1. The coefficient of determination (R^2) for each curve ranged between 0.95 – 0.99 showing a good fit to the *P vs E* model. The photosynthesis *versus* irradiance curve obtained from *in vitro* incubations of diatoms in the photosynthetron showed a wide variation at the different light levels in subsequent experiments using the same species. As in the dinoflagellates, additional incubations were carried out when sufficient culture material became available which meant a time interval sometimes of several weeks. It is not certain that this fact may contribute to the observed variability as it was noted that, even under controlled laboratory conditions, in respect of nutrients, temperature and light, seasonal differences were apparent in the cultures' growth rates. Scatter of data points along the *P vs E* curve was essentially small in the large-celled species as well as in the smaller-celled species. Approximate volumes for these species were *Chaetoceros* sp.- ~520 μm^3, *Chaetoceros* cf. *pendulus* - ~ 260 μm^3 and *Chaetoceros capense* - ~170 μm^3. Scatter was at its minimum in the smallest-celled species. Tilzer *et al.* (1993) experienced scatter when using small volume incubation vessels and noted that it was accentuated in cultures when larger celled organisms were used.

The initial slope of the light curve of chlorophyll *a*-specific photosynthesis, α*, (alpha – the light utilisation coefficient) varied slightly at all light levels (Table 4.1) generally showing an increase from low light to high light in all resulting data which is in accordance with other researchers' findings (MacIntyre *et al.* 2002). One exception was *Chaetoceros* cf. *pendulus* where the highest value of α* was noted at low irradiance (α* = 0.036 (±SE 0.002) mg C m^{-3} (mg Chl *a*) $^{-1}$ (μmol quanta m^{-2} s^{-1})$^{-1}$). The mean values of α* ranged from 0.015 at LL to 0.036 at HL . Some examples of the *P vs E* curve experiments are presented in Figure 4.1. For the statistical analysis the data were square-root transformed so that the assumptions were met. The two-way fixed effect ANOVA indicated that the responses of the three diatom species to different light levels were significantly different effecting the value per cell of α* ($F = 20.5$, $df = 2$, $p = 2.27E\text{-}05$), light itself was not significant (Appendix 4.3 (a-5)).

In the phytoplankton species *Chaetoceros* sp. representing the largest cell, the initial slope was the steepest of the three species at all light levels demonstrating a more efficient photoacclimation response compared with the other species. The species showed a good correlation between cell volume and light levels as the value of α* decreased with increasing size of the cell (Figure 4.2 (b). In all *Chaetoceros* species α* was higher at high irradiances reflecting the proportionality of α* with the light absorption efficiency per unit chlorophyll of these diatom species.

The light-saturated photosynthesis parameters, P_m* (maximum photosynthetic rate), normalised to chlorophyll *a*, ranged between 1.56 – 13.14 mg C (mg Chl *a*)$^{-1}$ h^{-1}. Variations were observed inter- and intra specifically for the *Chaetoceros* species presented in Figure 4.2. Generally the lowest values of P_m* were measured at low light levels increasing from medium to high light in all three species of *Chaetoceros*. The reverse was noted in the dinoflagellate species where P_m* decreased from LL to HL. One exception was *Chaetoceros* cf. *pendulus* that showed a very slight increase at medium light. The mean maximum

photosynthetic rate in *Chaetoceros capense* was the highest at high irradiances (range from low to high light: 4.0 (±SE 1.2) to 13.0 (±SE 1.4) mg C (mg Chl *a*)$^{-1}$ h^{-1}) compared with the other two species.

The P^*_m values varied systematically with cell size (Figure 4.2(a)) with the large-celled species having a reduced value in all three parameters (P_m^* , α^* and E_k) of *P vs E* curve at high light. This was the not so apparent in the dinoflagellate species. Statistical analysis of the P^*_m data revealed that the inter-specific differences in photosynthetic rate were significant in the *Chaetoceros* species ($F = 6.5$, $df = 2$, $p = 0.0072$), whereas neither light level nor interaction between irradiance level and species had a significant influence on the value of P^*_m (Appendix 4.3 (a-4)).

Table 4.1:Comparison of photosynthetic parameters (normalised to fluorometrically obtained Chl *a*) of different species of diatoms. (*Chaetoceros* sp., *Chaetoceros capense* and *Chaetoceros* cf. *pendulus*).
Sample statistics are mean ± standard error.

		Low light (33 µmol quanta $m^{-2} s^{-1}$)			**Medium light** (178 µmol quanta $m^{-2} s^{-1}$)			**High light** (647 µmol quanta $m^{-2} s^{-1}$)		
Species	**Experiment**	**Maximum Photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} (mg Chl *a*)$^{-1}$ (µmol quanta $m^{-2} s^{-1}$)$^{-1}$	**Light Saturation Parameter** (µmol quanta $m^{-2} s^{-1}$)	**Maximum photosynthetic rate** (mg C m^{-3} (mg Chl a^{-1}) h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} (mg Chl a^{-1}) (µmol quanta $m^{-2} s^{-1}$)$^{-1}$	**Light Saturation Parameter** (µmol quanta $m^{-2} s^{-1}$)	**Maximum photosynthetic Rate** (mg C m^{-3} (mg Chl a^{-1}) h^{-1})	**Maximum light utilization coefficient** (mg C m^{-3} (mg Chl a^{-1}) (µmol quanta $m^{-2} s^{-1}$)$^{-1}$	**Light saturation Parameter** (µmol quanta $m^{-2} s^{-1}$)
		P^*_m	α^*	E_k	P^*_m	α^*	E_k	P^*_m	α^*	E_k
Chaetoceros sp.	2	2.37	0.02	105	5.23	0.04	131	5.40	0.04	138
Chaetoceros sp.	4	1.28	0.01	120	2.01	0.01	146	2.25	0.01	191
Chaetoceros sp.	7	1.79	0.02	87	2.73	0.03	100	2.59	0.02	131
Chaetoceros sp.	13	0.78	0.002	157	2.59	0.01	259	3.18	0.01	268
Mean		**1.56**	**0.01**	**117**	**3.15**	**0.02**	**159**	**3.35**	**0.02**	**182**
± SE		0.9	1.2	1.3	1.2	1.1	1.2	1.3	1.1	1.2
C. capense	5	3.71	0.02	153	5.93	0.02	255	17.0	0.05	356
C. capense	6	0.54	0.01	94	1.31	0.01	130	2.28	0.01	204
C. capense	10	7.90	0.03	234	17.9	0.05	330	20.0	0.05	403
Mean		**4.05**	**0.02**	**160**	**8.40**	**0.03**	**238**	**13.1**	**0.04**	**321**
± SE		1.2	1.3	1.2	1.0	1.0	1.3	1.4	1.4	1.4
C. cf. *pendulus*	10	3.43	0.02	155	5.68	0.02	232	5.17	0.02	314
C. cf. *pendulus*	12	3.76	0.03	147	3.96	0.02	200	3.87	0.01	324
Mean		**3.59**	**0.02**	**151**	**4.82**	**0.02**	**216**	**4.52**	**0.01**	**319**
± SE		0.2	0.002	4.0	0.9	0.002	16.2	0.7	0.002	5.3

Low light (33 µmol quanta m^{-2} s^{-1}	Medium light (178 µmol quanta m^{-2} s^{-1})	High light (647 µmol quanta m^{-2} s^{-1})

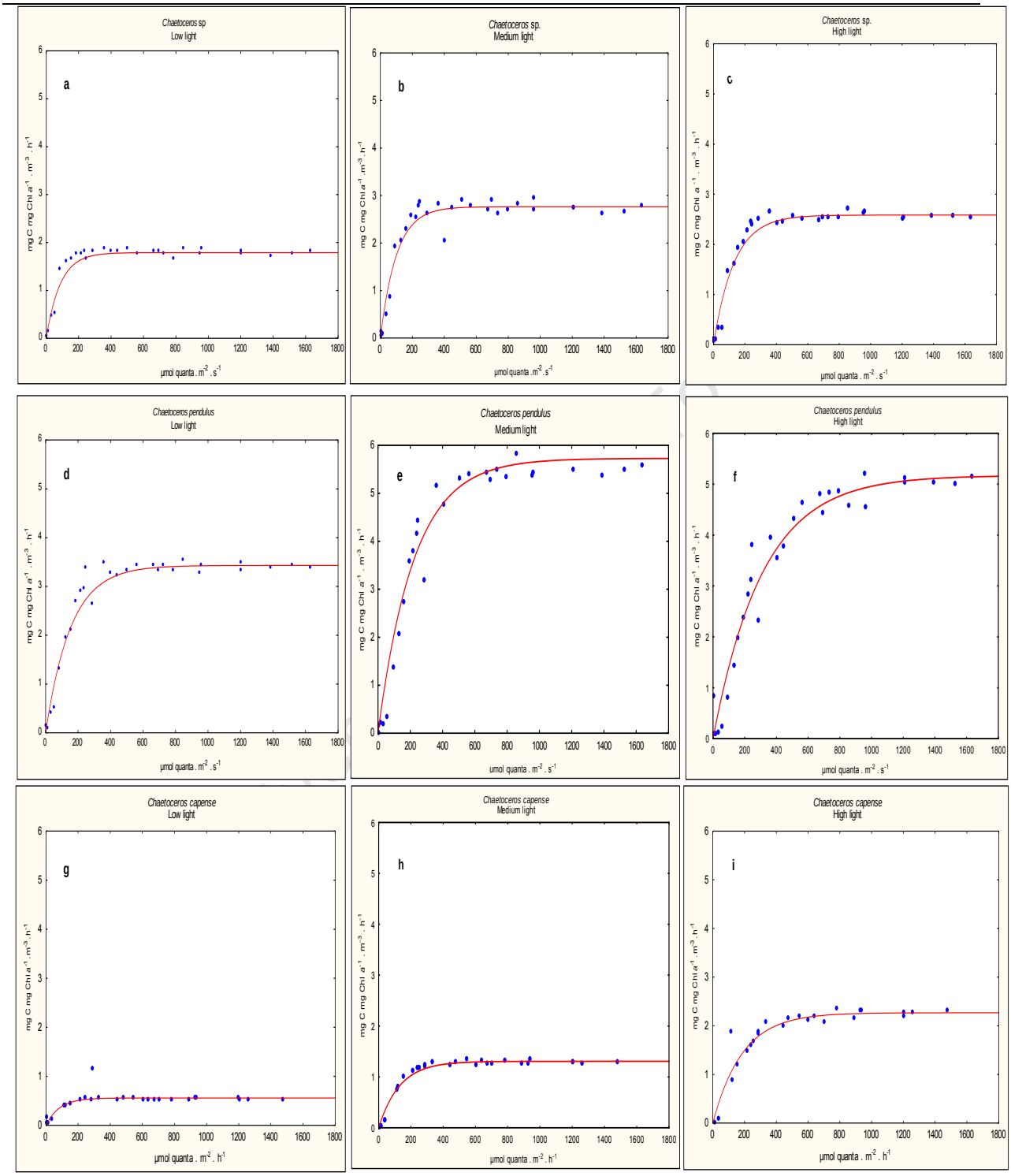

Figure 4.1: Example of *P versus E* curves for diatom species. (*Chaetoceros* sp. (Expt. 7) a – c; *Chaetoceros* cf. *pendulus*, (Expt. 10) d– f; *Chaetoceros capense*, (Expt.6) g – i). The coefficient of determination, R^2, in all cases was 0.95 - 0.99.

The mean irradiance value, E_k (P^*_m / α^*) for the diatom species at which the maximum rate, P_m , would be reached if P were to increase linearly with increased irradiance E, ranged from 117 µmol quanta m^{-2} s^{-1} at low light to 321 µmol quanta m^{-2} s^{-1} at high light levels for the *Chaetoceros* species. E_k for *Chaetoceros capense* was the highest at all irradiances ranging from 160 to 321 µmol quanta m^{-2} s^{-1}. A definite trend was demonstrated here in all three *Chaetoceros* species where the mean light saturation rates, E_k, increased from low light to high light conditions generally in parallel with P^*_m (Figure 4.2 (c)). This rise in E_k therefore is correlated to an increase in the maximum specific photosynthetic rate (P^*_m) with increased irradiances. With more light the rate of operation of the carboxylation system also increases, producing more $NADPH_2$ and ATP to saturate the dark reaction system (Kirk 1996). Statistical analysis of the E_k data showed that both, species and light level, have a significant effect (species – $F = 5.09$, $df = 2$, $p = 0.017$; light level – $F = 6.18$, $df = 2$, $p = 0.009$). There was no interaction between species and light level indicating that all three diatoms respond to light in the same way. *Post hoc* tests indicated that *Chaetoceros capense* was the most efficient at high light conditions (Appendix 4.3 (a-2)).

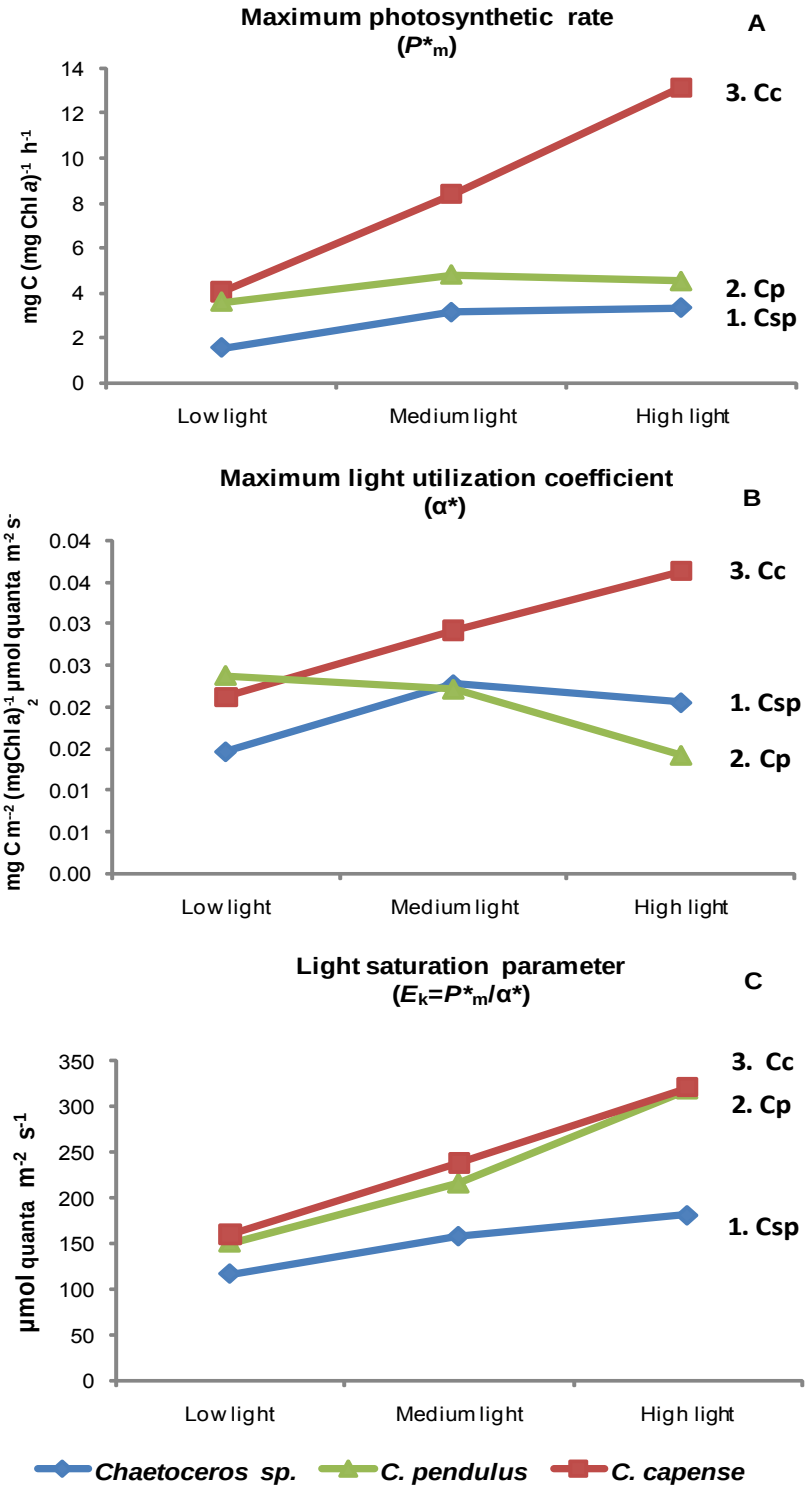

Figure 4.2: Comparison of the mean photosynthetic parameters: (A) Maximum photosynthetic rate (P^*_m), (B) Maximum light utilisation coefficient (α*) and (C) Light saturation parameter (E_k) of the different diatom species (1) *Chaetoceros* sp., (2) *Chaetoceros* cf. *pendulus* and (3) *Chaetoceros capense* .Note that the photosynthetic parameters varied systematically with cell size particularly in P^*_m and E_k.
The approximate cell volumes were: *Chaetoceros* sp. 520 µm^3, *C.* cf. *pendulus* 260 µm^3 and *C. capense* 170 µm^3.

4.4 Pigment composition

The photo-pigment indices were quantified to assess the changing contributions of chlorophylls and carotenoids to the total pigment pool. The diatoms used in the experiments contained the light harvesting pigments chlorophylls *a*, chlorophylls c_1 and c_2, and the photosynthetic carotenoid (PSC) fucoxanthin. *Chaetoceros* cf. *pendulus* also contained chlorophyll c_3. Chlorophyll *a* (Chl *a*) was present at the highest concentrations at low light levels generally decreasing with increasing irradiance in all three diatom species. The Ch *a* content changed with variations in the relative concentrations of accessory pigments. Examples of the accessory photosynthetic pigment ratios per total pigment are shown in Figure 4.3 (a) (Appendix 4.5). It indicated that the chlorophyll *a* proportion to total pigments constituted the highest concentration with very slight variation at low, medium and high light treatments. The ratio from low light to high light decreased by < 0.05 (range 0.55 to 0.47). Similarly, the photosynthetic carotenoid fucoxanthin ratios per total pigment were generally highest at low light levels, decreasing from low light to high light by approximately 0.1 in the largest-celled species *Chaetoceros* sp. and < 0.5 in the smaller-celled species. The opposite occurred in the ratios of chlorophylls c_1 and c_2 which generally increased from low light to high light in all three *Chaetoceros* species by approximately < 0.06. The fucoxanthin concentrations were generally half that of Chl *a* decreasing from low to high light. The two-way fixed effect ANOVA indicates that responses of the PSC concentrations to changing light levels were insignificant (species, $F = 0.62$, $df = 2$, $p = 0.54$), but the effect of light was significant, $F = 3.97$, $df = 2$, $p = 0.03$) (Appendix 4.4 (a -1)). There was no interaction effect between species and light ($p = > 0.05$).

The photoprotective carotenoid (PPC) ratios of the pigments diadinoxanthin, diatoxanthin and *β*-carotene generally increased with increasing light intensities. Diadinoxanthin ratio to total pigment variance was low between the three species (range 0.06 – 0.13). Unlike the dinoflagellates, dinoxanthin was absent in the diatoms (Figure 4.3 (a)). There was a difference in *Chaetoceros* sp., *Chaetoceros* cf. *pendulus* and *Chaetoceros capense* between the ratios of the photoprotective carotenoids (PPC), diadinoxanthin, diatoxanthin, and *β*-carotene to total pigment concentration. Statistically the differences between the ratios were found to be significant using a 2-way fixed effect ANOVA ($F = 0.84$, $df = 2$, $p = 0.44$) (Appendix 4.4 (a-2). The effect of species and light on the ratios between PSP to PPC were found to be insignificant. ($p = > 0.05$).

In the pigment diadinoxanthin the effect of light intensity was shown to be the most significant ($F = 7.4$, $df = 2$, $p = 0.004$), as in diatoxanthin ($F = 6.2$, $df = 2$, $p = 0.008$. In *β*-carotene species had a significant effect ($F = 14.7$, $df = 2$, $p = 0.0001$). There was no interaction effect between any of the species used ($p > 0.05$), Appendices 4.6 (a)).

When comparing the pigment ratios to total pigments (Figure 4.3 (a) with pigment contents per cell (Figure 4.3 (b) a clearer insight is given into the changes that take place at cellular level with changing irradiances. The mechanism of photoacclimation appears to vary depending on the phytoplankton species. The total pigment

pool does not change considerably but the individual intracellular pigments do, either increasing or decreasing depending on the light environment (Figure 4.3 (b)). The Chl *a* content per cell at low light generally decreases by approximately 50% in high light conditions in all three *Chaetoceros* species, but decreases can be as high as 75% as was noted in one of the experiments with *Chaetoceros* sp. and *Chaetoceros capense*. Fucoxanthin followed a similar pattern being reduced to half its former concentration at high light. The Chl c_1 and c_2 content varied mostly in parallel with fucoxanthin and Chl *a* except in *Chaetoceros* sp. where Chl c_1 and c_2 concentrations remained higher at medium light compared with fucoxanthin.

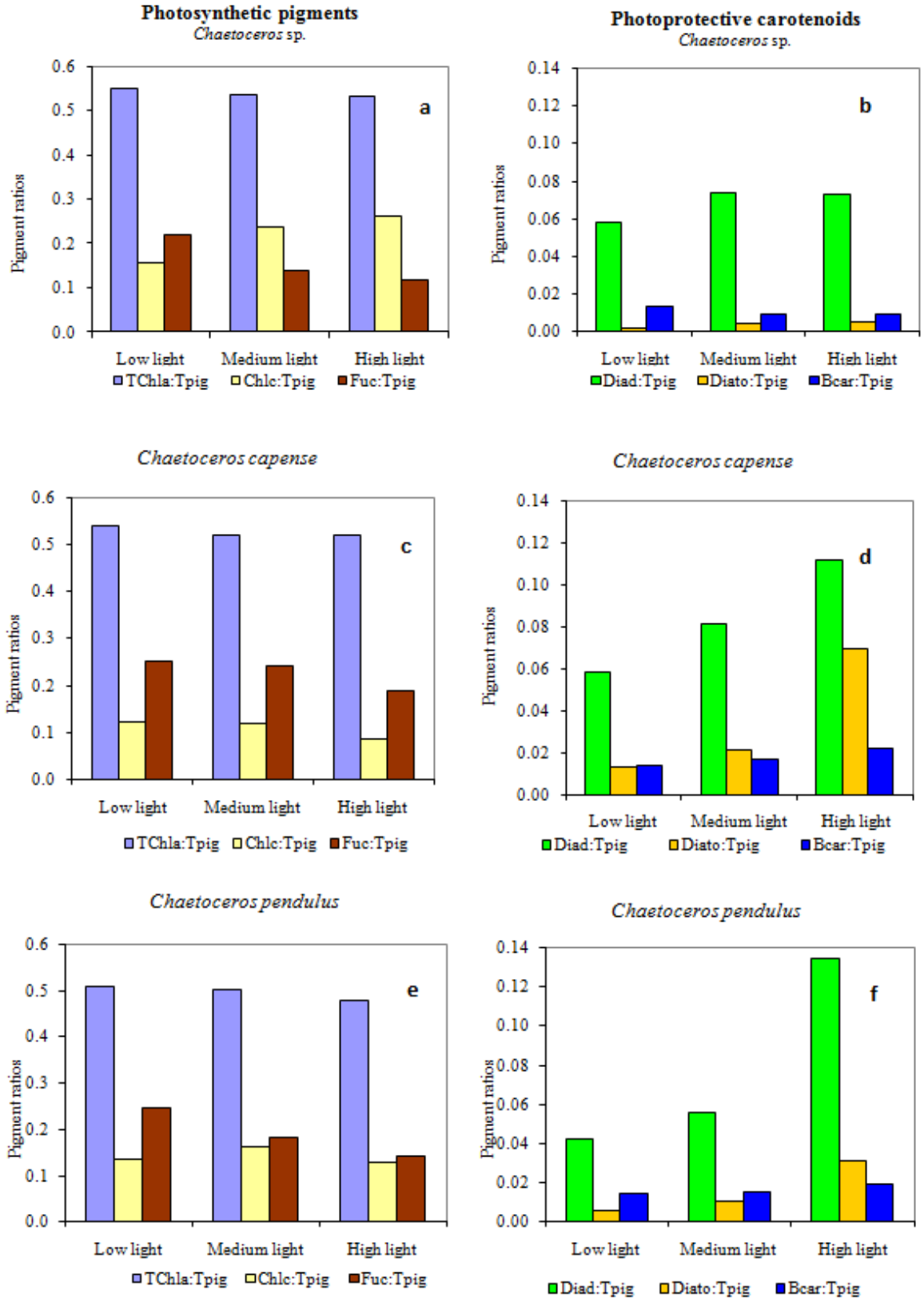

Figure 4.3(a): Examples of pigment ratios to total pigment for the three diatom species incubated under varying irradiance as indicated. *Chaetoceros* sp., a and b; *Chaetoceros capense*, c and d; *Chaetoceros* cf. *pendulus*, e and f.

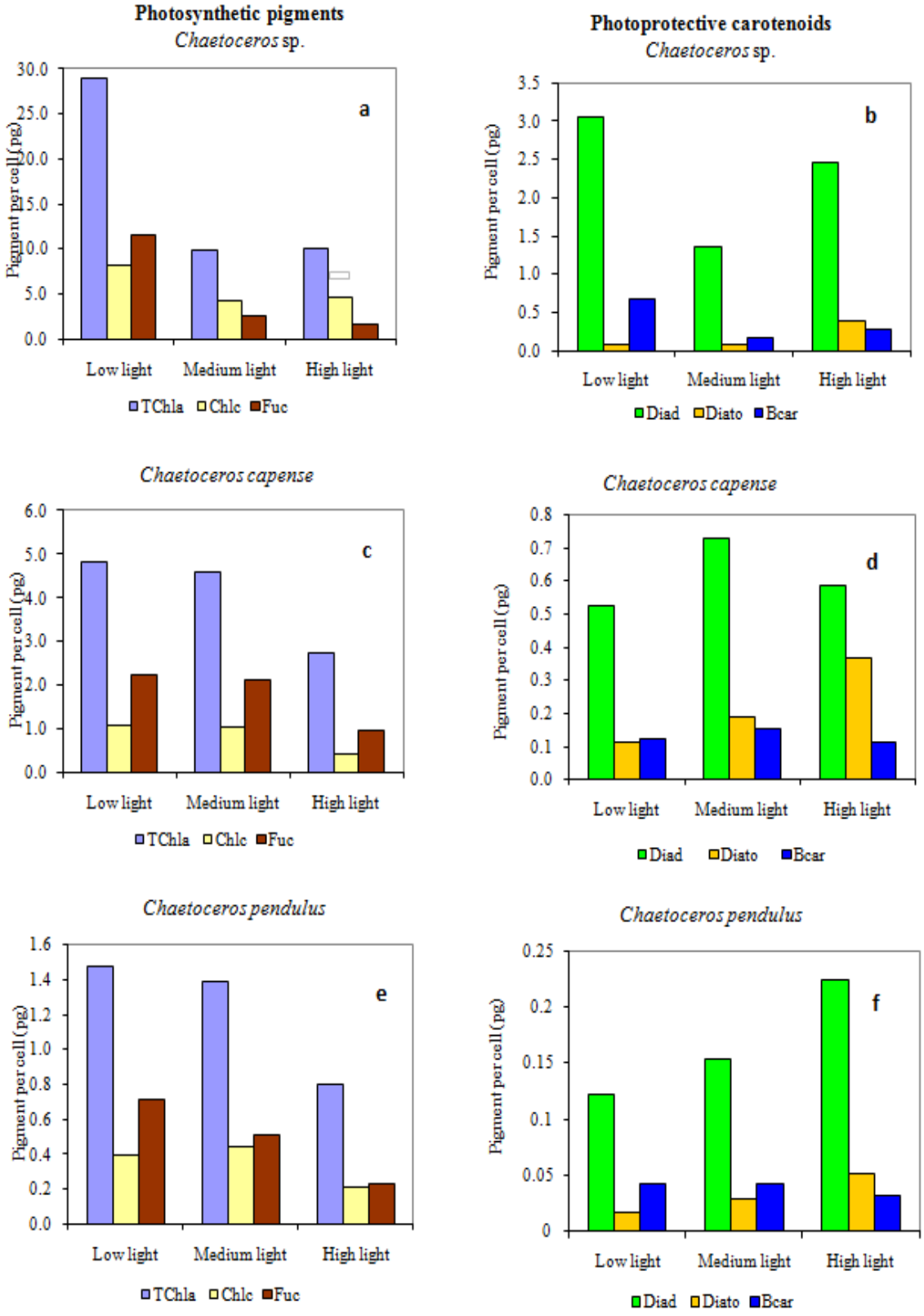

Figure 4.3 (b): Examples of pigments per cell (pg) for the three diatom species incubated under varying irradiances as indicated. *Chaetoceros* sp., a and b; *Chaetoceros capense*, c and d; *Chaetoceros* cf. *pendulus*, e and f. Note the different scales.

As mentioned above, the ratio of fucoxanthin (photosynthetic carotenoid PSC)) to total pigment concentration was highest in the low light acclimated diatom species and decreased at medium and high irradiances. This trend was reported by other researchers (Falkowski and LaRoche 1991; Moisan *et al*. 1998). At low light levels the PSC ratios ranged from 13.7 to 29.5% and high light ratios ranged from 13.6 to 16.2% of total pigments. The reverse was true for the photoprotective carotenoids. Here the percentage of all the low light acclimated species of *Chaetoceros* ranged from 4.0 to 4.8% and increased at high light levels from 7.4 to 13.4% (Appendix 4.6). In the diatom species used, diadinoxanthin made up to 10.3 to 52.1% of total carotenoids (Figure 4.5) increasing from low to high irradiances in all three diatom species. In the dinoflagellates the diadinoxanthin percentage of total carotenoids ranged from 4.1 to 26.7%, which falls within the range reported by Jeffrey *et al*. (1975) for dinoflagellates (Appendix 4.7). In the case of fucoxanthin the variances in the data could not be stabilized for statistical analysis. However the normality assumption was met. The percent diadinoxanthin showed that again only the response to light level was significant ($F = 10.97$, $df = 2$, $p = 0.0006$). There was no difference between species as they responded in a similar manner in respect of the diadinoxanthin concentrations ($p = > 0.05$).

4.4.1 Xanthophyll cycling

Diadinoxanthin is an important photo-protective pigment (Lavaud *et* al. 2004) and in some algae also plays a role as a light-harvesting pigment (Owens *et al.* 1987). In literature it is mentioned that the DD-cycle affords only a moderate short-term adjustment in the effective absorption cross section of PS II. During specific situations, such as during nutrient stress, the DD pool is enlarged (Geider *et al.* 1996, and others). The capacity of thermal dissipation of excitation energy in the antennae may therefore increase. In my experiments the higher concentration of diadinoxanthin recorded in the *Chaetoceros* species compared to the dinoflagellates may represent one of the species' (mixers) strategies to tolerate high-light environments before applying photo-protective mechanisms as is suggested by Richardson *et al.* (1983) (Figure 4.6 and Appendix 4.8).

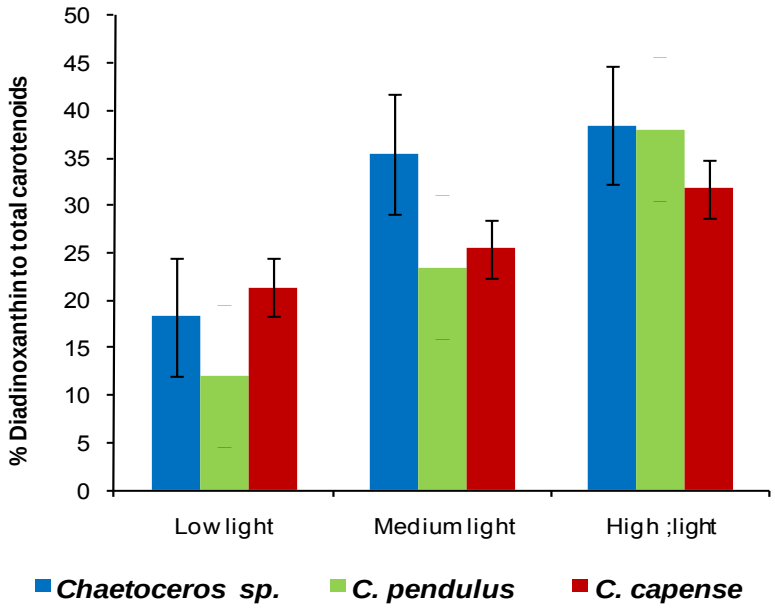

Figure 4.5: Percentage diadinoxanthin to total carotenoids in the three diatom species grown at three different levels of irradiance (low light, medium light and high light.
Error bars indicate ± one standard error.

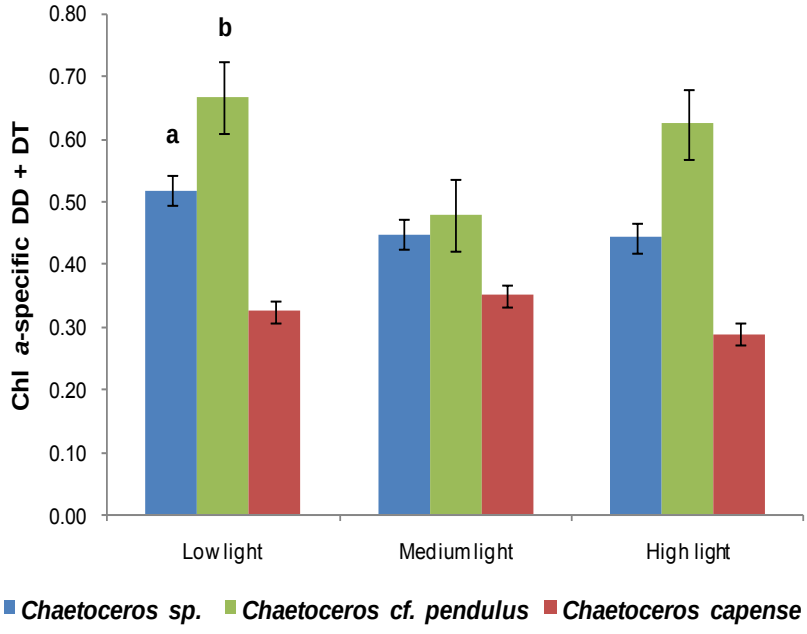

Figure 4.6: Comparison of the chlorophyll *a*-specific xanthophyll pool (DD+DT/Chl *a*) values for the three diatom species acclimated to three different irradiance levels for four days. The mean xanthophyll pool of the two diatoms *Chaetoceros* sp. (a) and *Chaetoceros* cf. *pendulus* (b) reached the highest value at low irradiance levels. The dinoflagellates' xanthophyll pool was the highest at high light. *Error bars indicate ±one standard error.*

Comparing the experiments undertaken with diatoms it was found that the xanthophyll pool data expressed as chlorophyll *a*-specific ((DD + DT)/Chl *a*) was highest in the monoculture of *Chaetoceros* cf. *pendulus* when acclimated to lower light irradiances and decreased at higher irradiance levels. The mean ratio of the xanthophyll pools for the diatom species grown under different light conditions ranged from 0.33 – 0.67 (LL), 0.35 – 0.48 (ML) and 0.29 – 0.62 (HL) (Appendix 4.8). Although the results are in accordance with other studies conducted with monocultures of diatom species such as *Chaetoceros gracilis* and *Thalassiosira weissflogii* (Goericke and Welschmeyer 1992; Fujiki and Taguchi 2001), statistical analyses revealed no significant differences in the values of the xanthophyll pools among the three species ($p > 0.05$). As mentioned in Chapter 3, other researchers recorded (DD + DT) /Chl *a* ratios of 0.0053 – 0.18 in natural phytoplankton assemblages which are much lower than those observed in laboratory experiments with diatom cells.

After experimenting with the diatom *Phaeodactylum tricornutum*, Olaizola *et al.* (1994) suggested that there are two distinct light-induced processes affecting DT: firstly a rapid light-induced conversion of DD to DT; secondly, after a prolonged exposure to high light (approximately 30 minutes), a further DT increase without DD decreasing appreciably. In the first instance, the functional DD pool can be rapidly de-epoxidised. In the second case the DD pool may possibly have a different function and may not be available to the de-epoxidase. A non-epoxidase pool of DD was observed by Olaizola and Yamamoto (1994) in the diatom *Chaetoceros meulleri*. It is thought that DT, a de-epoxidased pigment is synthesised first and accumulates without the need to de-epoxidise DD at higher irradiances. Olaizola *et al.* (1994) found DD increasing at the expense of DT at 415 and 750 µmol **.** m^{-2} **.** s^{-1}. It is thought, therefore, that any accumulation of DT must be the result of *de novo* pigment synthesis triggered by shifts to higher irradiances.

Generally in these experiments the diadinoxanthin (DD) concentrations in the three diatom species decreased from low light to high light with a corresponding increase in diatoxanthin (DT) (Figure 4.7) with the exception of *Chaetoceros c*f. *pendulus* where both DD and DT increased from LL to HL. The mean chlorophyll *a*-specific DD+DT pool of the diatom *Chaetoceros* sp. was the only species however where the pigment pool, after decreasing slightly from LL to ML, remained approximately the same at the other light treatments, suggesting a *de novo* pigment synthesis at higher irradiances (Appendix 4.8). Here again no significant effects were observed for species, light levels and interaction between the two ($p > 0.05$). However testing the individual pigments diadinoxanthin (DD) and diatoxanthin (DT) separately shows that both species and light level have a significant effect, but no interaction effect is apparent on the variable DT (Species $F = 3.7$, $df = 2$, $p = 0.42$; Light level – $F = 12.05$, $df = 2$, $p = 0.0004$). *C.* cf. *pendulus* is here the most significant. For the other variable DD, only species has an effect ($F = 4.64$, $df = 2$, $p = 0.023$), there is no effect of light level and no interaction effect. (Appendices 4.8 (a-1 and a-2)).

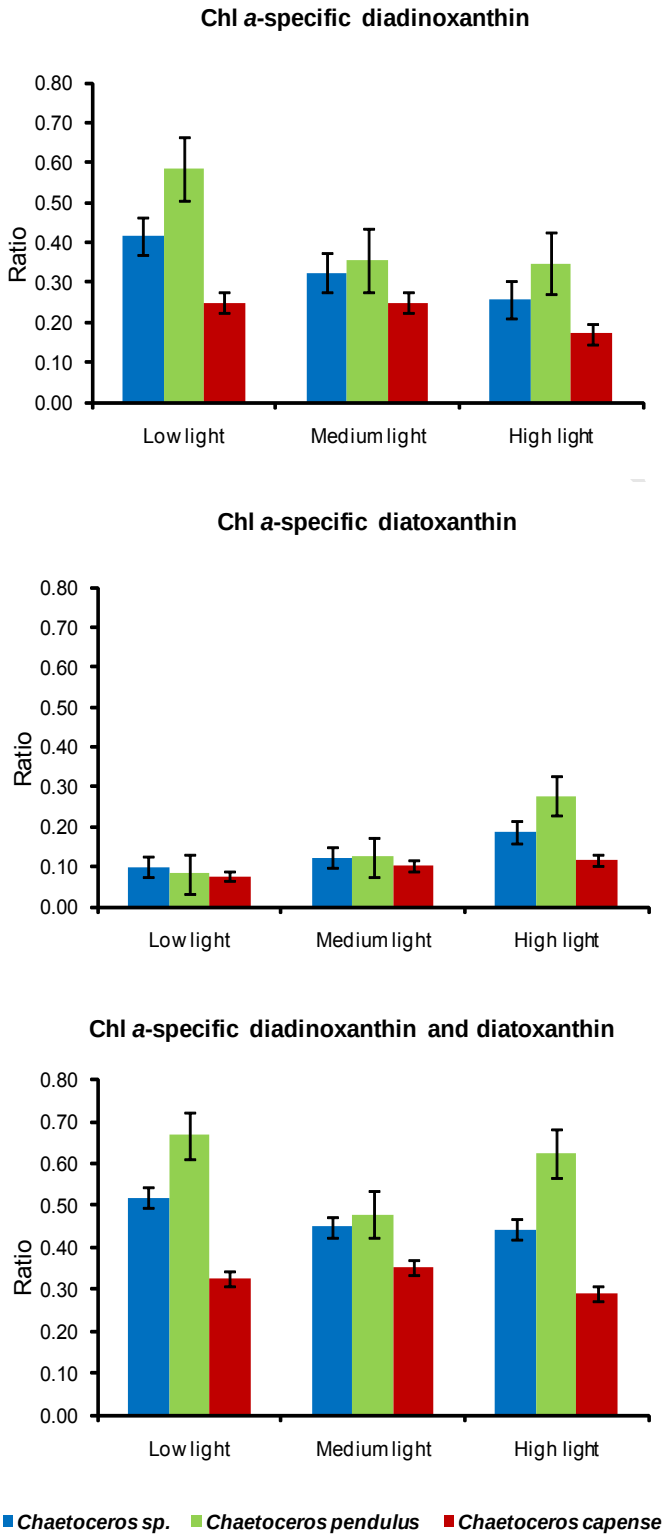

Figure 4.7: Changes in the ratio of diadinoxanthin (DD) and diatoxanthin (DT) as a result of interconversion between DD and DT during xanthophyll cycling in diatoms. A comparison between cultures grown at different light levels. *Error bars indicate ± one standard error.*

4.5 Absorption

Measurement of light absorption by phytoplankton cells is complicated by the fact that at low cell densities absorption is usually low and it is difficult to get an accurate estimation. At high particle densities, gradients of irradiance are produced within the culture vessel as cells both absorb and scatter light. As recommended, therefore, samples used were optically thin to give the least ambiguous estimate of absorption (Kirk 1996).

Subsamples for absorption measurements were taken from the same photoacclimated culture samples on the day the *P vs E* experiments were carried out. The samples were filtered and frozen immediately as with the dinoflagellate experiments and the measurements were normalised to Chl *a* concentrations. The variations in the chlorophyll *a*-specific absorption spectra for the three diatom species grown under different experimental light conditions are shown in Figure 4.8. Absorption increased with increased irradiance in the diatom species between the wavelengths 435 – 440 nm and 671 – 676 nm.

The spectra indicate that Chl *a* has a strong peak in the red region (671-676 nm). Carotenoids (e.g. fucoxanthin) have a weak absorption shoulder between 550 and 650 nm in the green region, the least absorption taking place at approximately 575 nm. The main light absorption band, due to a combination of chlorophylls *a*, $c_{1,2}$ and c_3 and carotenoids, lies in the blue/green band showing the characteristic two peak heights at approximately 430 and 440 nm in the case of species *Chaetoceros* sp. and *Chaetoceros* cf. *pendulus* (Figure 4.8). The differences in the absorption, measured in a spectrophotometer (see Chapter 2) are characteristic of the different species of diatoms and are the result of their cell size and packaging of the internal pigment complements. Statistically it was shown that the differences in absorption are species specific ($p < 0.05$). There was no effect of light level ($p > 0.05$). Examples of the pigment data for the spectra in Figure 4.8 are shown in Table 4.2 (*Chaetoceros* sp. Expt. 4; *Chaetoceros capense*, Expt. 5; *Chaetoceros* cf. *pendulus*, Expt. 10). Chl *a*–specific absorption seems to be lower when the photosynthetic pigments are dense as expected in the larger-celled *Chaetoceros* sp.. This may be due to the packaging effect causing internal self-shading (Dubinsky 1992), an effect which occurs particularly in large-celled microalgae. This packaging effect on absorption is evident at the Chl *a* peak in the red region (674 nm) where absorption by other pigments is virtually non-existent (Figure 4.8). One of the absorption spectra of *Chaetoceros capense* has a high absorption around 410 nm which appears to indicate that there may be some detrital material with mature or scenescent cell present, although this was not apparent when the samples were microscopically examined at the beginning of each experiment. As noted above (Chapter 3.3.1) the *Chaetoceros* species (diatoms) are particularly 'fragile' or 'sensitive' to the type of filtration and extraction methods used causing the conversion of Chl *a* to chlorophyllide *a* (Jeffrey *et al.* 1997) which give erroneous results in the values of biomass and absorption. However, with ratios of 440 nm to 675 nm being in the range 1.0 to 2.0, it is plausible that the cultures were in a healthy state.

At all light levels the chlorophyll *a*-specific absorption values for the *Chaetoceros* species at wavelength 440 nm were up to one magnitude greater then at wavelength 675 nm (Figure 4.9) with *Chaetoceros capense*

increasing the most in high light by 44% at 440 nm and 34% at 675 nm. *Chaetoceros* sp. and *Chaetoceros* cf. *pendulus* increased by 26 and 5%; and 20 and 3% at 440 and 675 nm respectively. The same trend was found in the dinoflagellate species (Figure 3.5). Here again the non-parametric Kuskal-Wallis test confirmed that light level has no effect on Chl *a*-specific absorption ($p > 0.05$) only a species effect ($p = 0.015$).

The chlorophyll *a*-specific mean spectral absorption coefficients at wavelengths 440 and 675 nm are tabulated in Appendix 4.9. Comparing the data of the three diatom species indicates that at 440 nm most absorption takes place, showing increased absorption at higher irradiance levels. The value of the mean spectral chlorophyll *a*-specific absorption coefficient at wavebands 440 and 675 nm of *Chaetoceros capense* was greatest under high light conditions (range at 440 nm 0.046 (LL) to 0.083 (HL) and at 675 nm 0.29 (LL) to 0.44 (HL)). All diatom species, as with dinoflagellate species, showed great variation in absorption (Figure 4.10). The mean spectral absorption value normalised to Chl *a*, measured by fluorometry, at low, medium and high light ranged from 0.019 to 0.046, 0.018 to 0.057 and 0.020 to 0.083 m^2 (mg Chl *a*)$^{-1}$ (Appendix 4.9). This corresponds to a similar variation observed for other diatom species at 440 and 675 nm (Maske and Haardt 1987). The increase in Chl *a*-specific absorption in *Chaetoceros capense* at high light seems to be exceptional and may be an aberration.

The absorption coefficient varied systematically with cell size with the smallest celled species, *Chaetoceros capense* having the highest absorption values and *Chaetoceros* sp. the lowest. This characteristic related to cell size was not as apparent in the dinoflagellate species.

The variation in the absorption spectra of the three diatom species can be attributed to several factors i.e. the different light harvesting complexes (LHC), the presence of different chromoproteins in the cell, the number of PSUs, the packaging effect, cell size, to name but a few, as discussed more fully in Chapter 3.

In Figure 4.11 and Appendix 4.10 the ratios of Chl *a*-specific absorption at 440 nm to that of 675 nm of the three diatom species at the three different light levels are presented. Generally the samples of the diatom cultures show ratios between 1.00 and 2.00 indicating that the cultures were in a healthy state (see Kiefer *et al*. 1979; Yentsch and Phinney 1982) . Similar observations were made for the dinoflagellate species as ratios fell between 1.00 and 2.00, although there were some exceptions *i.e. Alexandrium catenella* (replicate 3) and *Gymnodinium zeta* (replicates 1 and 2) where the ratios were slightly greater then 2.00 (Appendix 3.9). An increase in absorption by degraded pigments due to ageing of the culture such as suggested by Kiefer *et al.* (1979), Yentsch and Phinney (1982) was not apparent from the data. Statistical analysis using a two-way fixed effect ANOVA indicated that there was a significant difference between the three species (Species - $F = 11.09$, $df = 2$, $p = 0.0008$) and their responses to changing light levels (Light level - $F = 7.13$, $df = 2$, $p = 0.0056$). Pairwise comparison of the three species of diatoms indicated significant differences among the species with *C. capense* and high light being the most significant (Appendix 4.10 (a)).

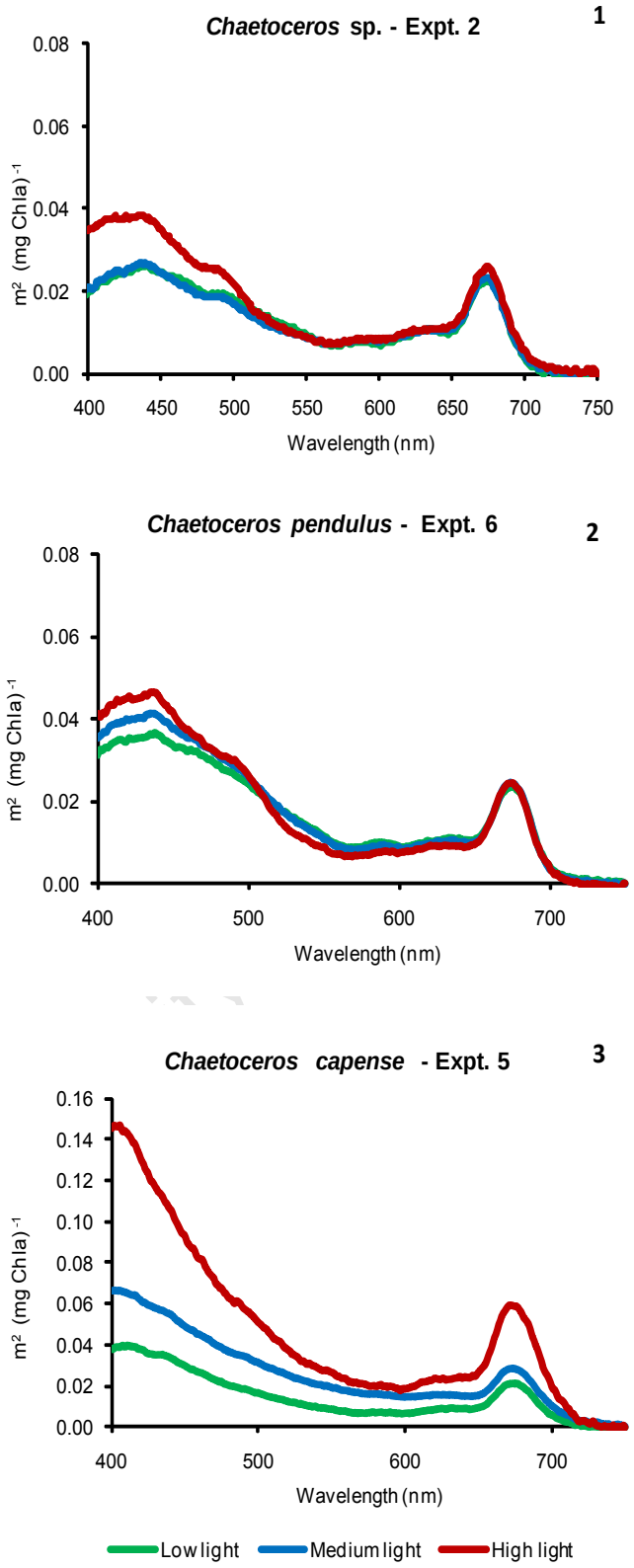

Figure 4.8: Chl *a*-specific absorption spectra for three diatom species grown under different experimental irradiances. (*Chaetoceros* sp. (1), *Chaetoceros* cf. *pendulus* (2), *Chaetoceros capense* (3)). Note different scale in Figure 4.8(3). (Absorption values were normalised using fluorometrically measured Chl *a*).

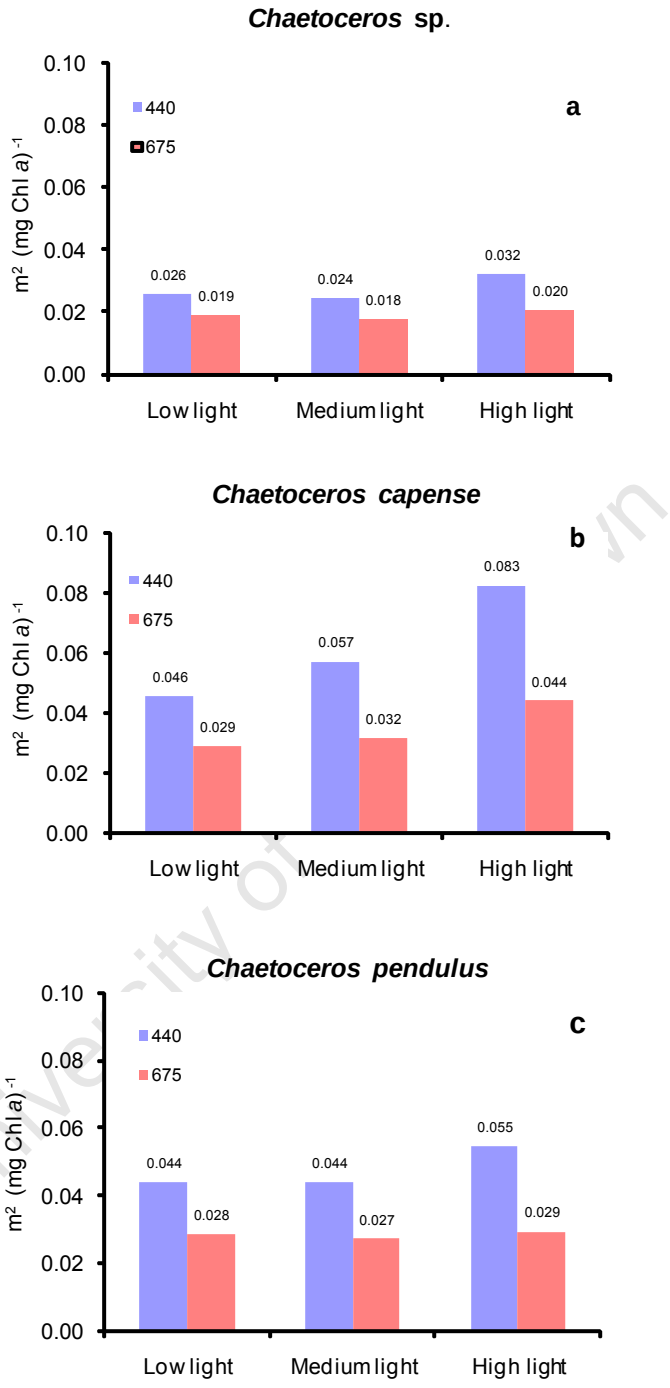

Figure 4.9: Chl *a*-specific absorption coefficient values for three diatom species (*Chaetoceros* sp., *Chaetoceros capense* and *Chaetoceros* cf. *pendulus*) observed at wavelengths 440 and 675 nm. (Absorption values were normalised to fluorometrically measured Chl *a*).

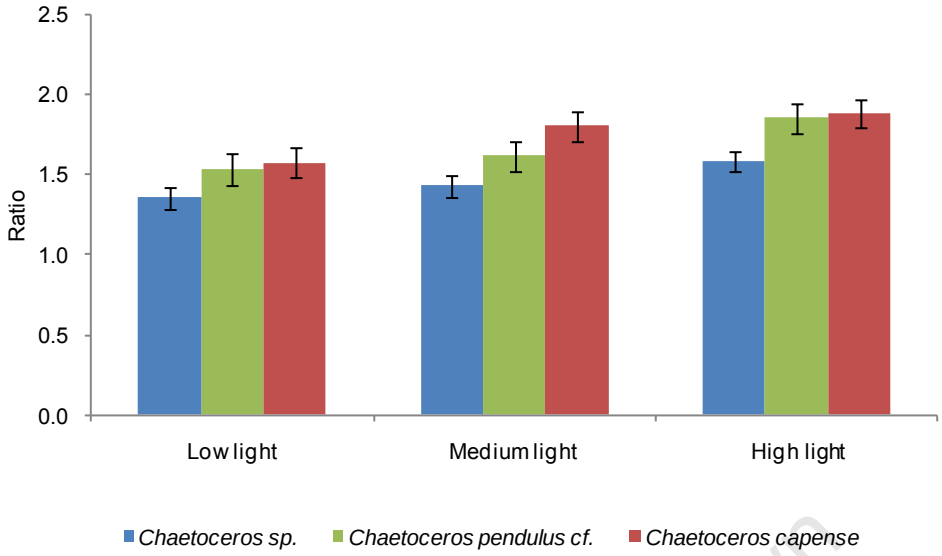

Figure 4.10: The ratio of Chl *a*-specific absorption at 440 nm to that of 675 nm of the three diatom species measured at low, medium and high light conditions. (Chl *a* was measured fluorometrically). *Error bars indicate ± one standard error.*

Table 4.2: Pigment data for the phytoplankton species shown in Figure 4.8. Size is approximate length (µm). Pigment : Chl *a* ratios. Total fluorometrically measured Chl *a* in pg per cell.

		Low light					**Medium light**					**High light**				
Diatom species	**Size µm**	**T.Chl*a* Cell^{-1}**	**Chlc:Chl*a***	**[a]PSC:Chl*a***	**[b]PPC:Chl*a***	**[c]Xanth:Chl*a***	**T.Chl*a* Cell^{-1}**	**Chlc:Chl*a***	**[a]PSC:Chl*a***	**[b]PPC:Chl*a***	**[c]Xanth:Chl*a***	**T.Chl Cell^{-1}**	**Chlc:Chl*a***	**[a]PSC:Chl*a***	**[b]PPC:Chl*a***	**[c]Xanth:Chl*a***
Chaetoceros sp. (Expt. 2)	24 to 53	29.3	0.5	0.5	0.09	0.03	9.0	0.78	0.4	0.1	0.03	10.5	0.9	0.3	0.3	0.04
Chaetoceros capense (Expt. 5)	18 to 37	2.73	0.9	0.1	0.08	0.03	2.6	1.2	0.2	0.1	0.04	1.08	1.2	0.1	0.1	0.03
Chaetoceros cf. *pendulus* (Expt. 10)	24 to 48	2.59	0.6	0.4	0.1	0.02	1.0	0.8	0.3	0.1	0.02	1.2	1.0	0.2	0.3	0.03

Chlc, the sum of all c chlorophylls

PSC, photosynthetic carotenoids

PPC, photoprotective carotenoids

Xanth, xanthophylls

[a,] fucoxanthin

[b,] diadinoxanthin and diatoxanthin

[c,] β-carotene

4.6 Estimated maximum quantum yield

The quantum yield of carbon fixation is an index of the efficiency with which the energy is transferred from the pigments to the electron transport carriers in the phytoplankton cell and an indicator of the photochemical mechanism (Bannister and Weidemann 1983; Kirk 1996 and others). A comparison of the estimated maximum quantum yields (ϕ_m) of photosynthesis of the three different diatom species, derived from the initial slope of α^* of the *P vs E* relationship and the absorption cross section a^* relative to chlorophyll *a* in m^2 (mg Chl *a*)$^{-1}$ is given in Figure 4.11. The maximum quantum yield (ϕ_m) for the three replicates of the diatom species generally showed a higher maximum quantum yield at medium irradiance (range 0.11 – 0.52 mol C (Chl a^{-1}) µmol quanta m^{-2} s^{-1}) (Figure 4.11). At low, medium and high irradiance levels the mean estimated quantum yield for the diatom species ranged between 0.23 to 0.35, 0.31 to 0.33 and 0.22 to 0.27 mol C (Chl a^{-1}) µmol quanta m^{-2} s^{-1} respectively. The largest of the *Chaetoceros* species, *Chaetoceros* sp., recorded the highest estimates of ϕ_m (range 0.14–0.52 mol C (Chl a^{-1}) µmol quanta m^{-2} s^{-1}) at medium light level. The non-parametric test using Kruskal-Wallis rank test indicated that there is a significant effect of species on the maximum quantum yield value ($p < 0.05$), light level however, showed no effect ($p > 0.05$).

The maximum quantum yield (ϕ_m) data showed a similar variability for the repeat experiments of each species as was found in the dinoflagellates although the mean values for the three light levels were essentially the same (range 0.11 – 0.24 mol C (Chl a^{-1}) µmol quanta m^{-2} s^{-1}). The values of the maximum quantum yield in the data set, like those of the dinoflagellates, were fairly high compared to the theoretical maximum value of ϕ_m which plant species in principle might achieve *i.e.* 0.112 to 0.125 mol C fixed mol^{-1} photons absorbed (Kirk 1996; Long *et al*. 1993). Estimated values ranging between 0.033 to 0.101 for field samples (Cleveland *et al.* 1989) and values below 0.016 mol C (mol photons)$^{-1}$ (Lewis *et al.* 1985) were noted by other researchers. Lewis *et al*. (1985) suggests that nitrogen availability affects the maximum quantum yield, lowering ϕ_m values when nitrates are reduced causing a decreased carbon-based photosynthetic rate and *vice versa.* Since the maximum quantum yield is considered to be wavelength dependent (Schofield *et al.* 1996; MacIntyre and Cullen 2005) the variability could possibly be attributed to the species' sensitivity to spectral quality as the incubation light source used was a tungsten halogen lamp which produced a fairly flat spectrum, especially in the blue-green region (Kyewalyanga 1997) where phytoplankton have a major absorption peak. Another factor could be the differences in the species' light harvesting complexes (LHC) that include different concentrations of fucoxanthin (Appendix 4.5) structurally bound to Chl *a* – proteins which may change the individual cells' wavelength dependency (Schofield *et al.* 1996). The concentration of photoprotectant pigments, the non-photosynthetic carotenoids, strongly affects ϕ_m. High concentrations of these pigments reduces the photosynthetic quantum yields as light absorbed by these pigment particles cannot be transferred to the photosynthetic reaction centres

(Marra *et al.* 2000, Cleveland *et al.* 1989). This trend was only evident from the data of two repeat experiments of *Chaetoceros* sp. and of *C.* cf. *pendulus* where the ratios of PSC to PPC dropped from 6.0 LL to 0.6 HL and 7.4 LL to 1.1 HL with corresponding reduction in ϕ_m at high light. Many causes for the variation in maximum quantum yield are discussed in literature (Sosik and Mitchell 1991).

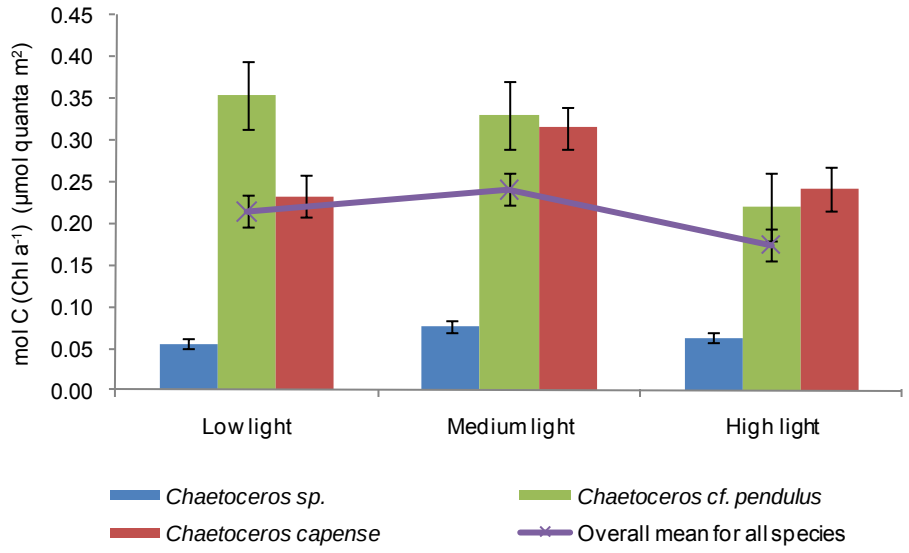

Figure 4.11: Comparison of the estimated maximum quantum yield for the three diatom species acclimated to different irradiances as shown. Overall mean (n= 3) for the diatoms used in the experiments.
Error bars indicate ±one standard error.

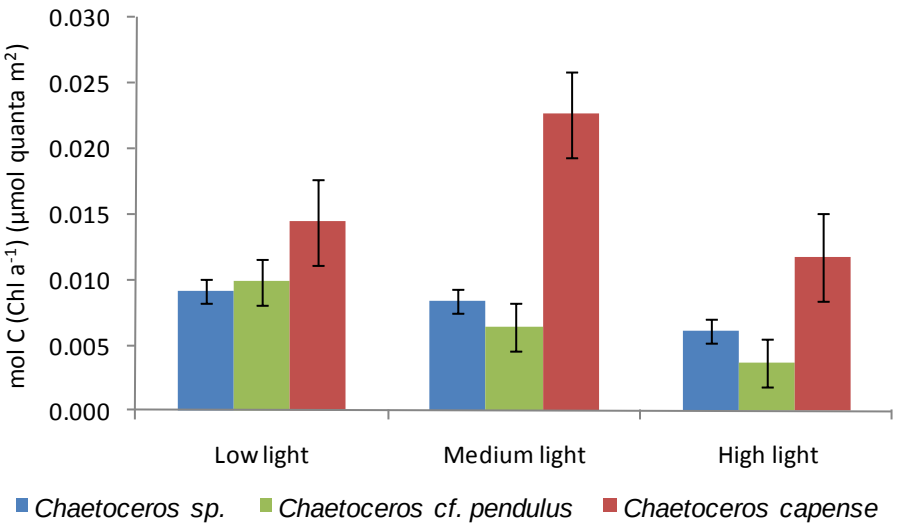

Figure 4.12: Comparison of the estimated maximum quantum yield per cell for the three diatom species acclimated to the different irradiances as shown. Error *bars indicate ± one standard error.*

Comparing the maximum quantum yield per cell of the three diatom species did not follow the same pattern as noted in the dinoflagellate species as here the smallest celled species *Chaetoceros capense* achieved the highest quantum yields and the largest celled species *Chaetoceros* sp. the lowest. The mean maximum quantum yield per cell for these species taken over all light levels was 0.016 and 0.008 mol C (Chl *a*)$^{-1}$ µmol quanta m^{-2} s^{-1} respectively. Statistical analysis showed that the effect of species on the maximum quantum yield value was significant (F = 4.64, df = 2, p = 0.024) (Appendix 4.12 (a-2)).

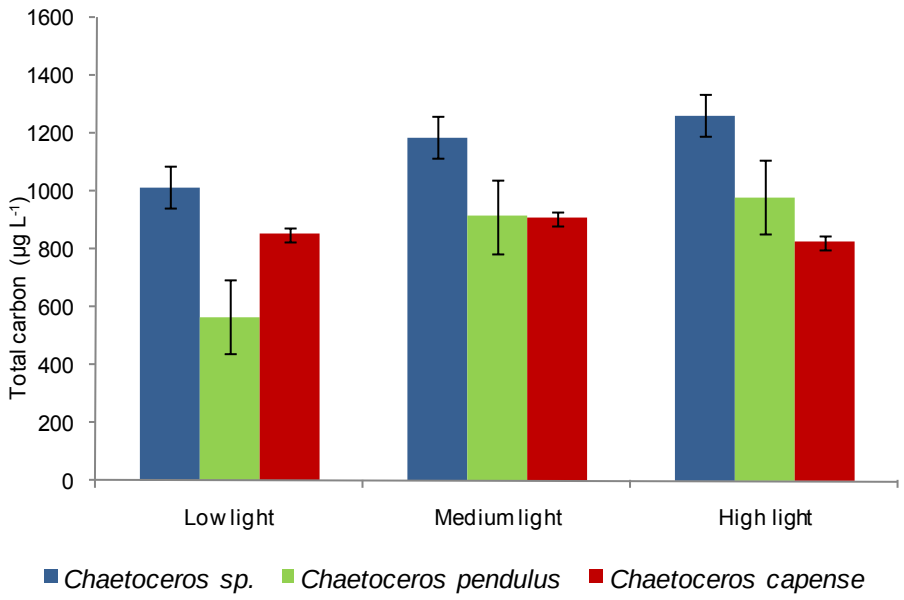

Figure 4.13 (a): Total carbon concentrations of the three diatom species grown under low (~33 µmol quanta m^{-2} s^{-1}), medium (~178 µmol quanta m^{-2} s^{-1}) and high light (~647 µmol quanta m^{-2} s^{-1}) conditions. The data represents the mean of three experiments. *Error bars indicate ± one standard error.*

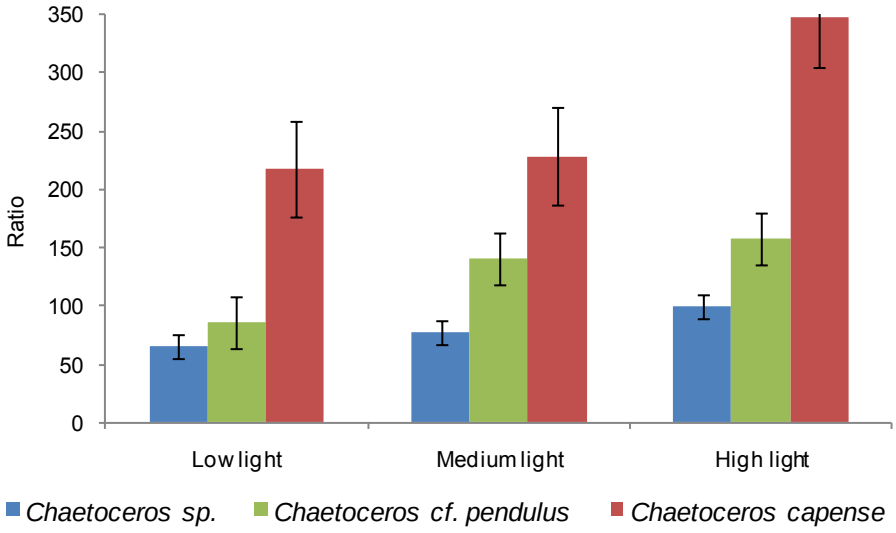

Figure 4.13 (b): Comparison of total carbon to chlorophyll *a* ratios of the three diatom species acclimated to three different irradiance levels as illustrated. The data represents the mean of three experiments.
Error bars indicate ± one standard error.

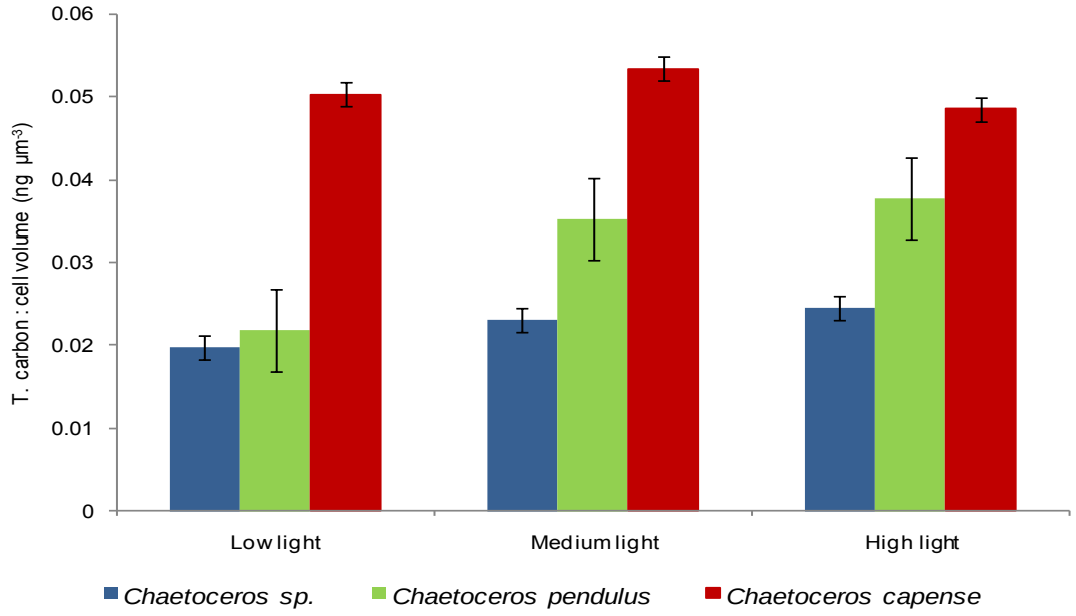

Figure 4.13 (c): Comparison of total carbon to cell volume relationship for the three diatom species grown under low (~33 µmol quanta m^{-2} s^{-1}), medium (~178 µmol quanta m^{-2} s^{-1} and high light conditions (~647 µmol quanta m^{-2} s^{-1}). *Error bars indicate ± one standard error.*

4.7 Carbon to chlorophyll *a* ratio

The differences of the total carbon (TC) concentrations for the cultures of the three diatom species grown under low, medium and high irradiances, although variable, were small (Figure 4.13 (a) and Appendix 4.13). Generally the mean concentrations increased at higher irradiances. The highest value of TC was noted in the culture of the large-celled species *Chaetoceros* sp. (10.7 (LL), 11.9 (ML) and 12.6 mg L^{-1} (HL)), increasing by 20%. In *Chaetoceros pendulus* a sharp rise was noted in TC from low light to high light by 42%. The TC concentration in *Chaetoceros capense* remained approximately constant with little variation between the light levels. (>6%). Statistical analysis showed that the difference between the *Chaetoceros* species in relation to the carbon content and their response to changing light levels was insignificant ($p > 0.05$).

The mean total carbon to chlorophyll a ratios generally increased from low to high light environments. *Chaetoceros* sp., *Chaetoceros capense* and *Chaetoceros* cf. *pendulus* increasing by 35, 37 and 46 respectively (Figure 4.13 (b)). The ratios of the species *Chaetoceros capense* (replicate experiments 1 and 3) were particularly high which corresponded to low Chl *a* L^{-1} content.

The carbon content per cell remained fairly constant in all three diatom species at low, medium and high light levels. The two species *Chaetoceros* sp. and *Chaetoceros capense* showed an increase between 21 to 24% from low light to high light. The exception was *Chaetoceros* cf. *pendulus* with a decrease from low to high light of 21% in cellular carbon content coupled with a 30% increase at medium irradiance. Analyses of the carbon data of the diatoms seemed to indicate that there was no correlation between cell size and TC content. The total C : cell volume, however, showed a significant decrease with increasing cell volume. The variance was highly species-specific (Figure 4.13 (c). A similar trend was demonstrated in the dinoflagellate analyses of the carbon data. One can only surmise that other factors such as physiological responses play a role during photoacclimation to changing irradiances in the unicellular diatom species used in these experiments. As was pointed out by Richardson et al. (1996) intracellular concentrations do not co-vary with cell size.

Generally cultures of the diatom species showed a lower total carbon content than dinoflagellate species. This is in agreement with other reported findings (Geider *et al*. 1996). Statistical analysis using a two-way fixed effect ANOVA showed that the response of the three diatom cultures to changing light levels was not significant ($p = 0.4$), but the effect of species and light on the ratio of total carbon to total chlorophyll *a* ratio was significant (Species – $F = 55.34$, $df = 2$, $p = 1.19E-07$; light level – $F = 11.44$, $df = 2$, $p = 0.0.0009$ (Appendix 4.13 (a-1 to a-3)).

4.8 Summary

The diatoms examined in this thesis exhibited definite general trends characteristic of microalgae adapted to a turbulent environment. Comparing the *P vs E* curves of the cultures of the three diatom species indicated that all three species are more efficient in a higher light environment. Curve fitting was good (R^2 = > 0.95 and < 0.99) and scatter of data points was minimal. The largest-celled species *Chaetoceros* sp. showed a steep initial curve (α*) which seems to indicate a faster photoacclimation response compared with *Chaetoceros capense* and *Chaetoceros* cf. *pendulus.* No photoinhibition was noted (maximum light level in the photosynthetron was 1200 μmol quanta m^{-2} s^{-1}). Although the phytoplankton used for the cultures were of the same genus, each species had a characteristic combination of photoacclimation trends *i.e*. *Chaetoceros capense* photosynthesized most efficiently in high light (mean range 4.0 (LL) to 13.0 mg C m^{-3} (mg $Chla^{-1}$) h^{-1} (HL)) followed by *Chaetoceros* cf. *pendulus* (mean range 3.5 (LL) to 4.5 mg C m^{-3} (mg $Chla^{-1}$) h^{-1} (HL)) and lowest efficiency was noted in *Chaetoceros* sp. (mean range 1.5 (LL) to 3.3 mg C m^{-3} (mg $Chla^{-1}$) h^{-1} (HL)). As in the analysis of the photosynthetic parameters in the dinoflagellates (Chapter 3), analysis of the parameters of the diatom cultures emphasised the importance of cell size in α*, P^*_m and E_k as the data varied systematically with cell size from low to high light, the highest value representing the smallest species. This trend was particularly apparent in the parameters representing the maximum photosynthetic rate (P_m*) and the light saturation parameter (E_k).

All photosynthetic pigment concentrations (PSP) decreased from low to high light in all three *Chaetoceros* species. The decreases ranged from 15 to 50% depending on the species and also varied between the different experiments which were conducted when sufficient culture material became available at different times throughout the year. The photoprotective carotenoids (PPC) followed a different pattern increasing from LL to HL by up to 65% as was noted in the experiments with *Chaetoceros* cf. *pendulus.*

As expected, all photoprotective pigment ratios to total pigments in species of *Chaetoceros* showed definite increases from LL to HL (range 4.5 to 13.5). The highest increase was noted in *Chaetoceros* cf. *pendulus* where diadinoxanthin increased from LL to HL by 70%. Lutz *et al.* (2003) observed elevated proportions of PSPs at low light with an increase in PPCs at high light. MacIntyre *et al.* (2002) also reported a decline in accessory light harvesting pigment in response to increased irradiance between 50 – 80%, which is in agreement with this study. The variability in accessory pigments provides an insight into the species-specific, photoacclimation processes to changing light conditions. The ratios of Chl *a* to accessory pigments, therefore, should show little variation with increased irradiance (Dubinsky *et al.*1986). Aiken *et al.* (2004) found contrasting results in natural populations.

A suite of factors influence the optical properties of phytoplankton for instance cell size (Ciotti *et al* 2002). The shape of the absorption spectra in this study seems to vary systematically with cell size which would

explain the variability observed in the shape of the absorption spectra between 400 to 675 nm. The systematic variability could be related to the dominant cell size in the *Chaetoceros* cultures as well as the packaging and composition of accessory pigments. Up to more than 80% variability has been observed by Ciotti *et al.* (2002). Pigment packaging (or self-shading) is a well documented source of variability for phytoplankton absorption and is a positive function in both cell size and intracellular concentration of pigments (Sathyendranath *et al.* 1987). For monospecific laboratory cultures grown in a nutrient replete medium Agusti (1991) states that cell diameter correlates negatively with concentration of intracellular pigments and helps to minimise self-shading.

Generally the value of the mean spectral chlorophyll *a*-specific absorption of the three diatom species was greater with increased irradiances, with the greatest absorption taking place in the wavebands 435 - 440 nm (blue region) and 671 – 676 nm (red region). There was one exception in one experiment with *Chaetoceros capense* which showed absorption at 410 nm at HL which may be an aberration.

The maximum quantum yield (ϕ_m) of the diatom species generally showed a higher ϕ_m at medium irradiances with the largest-celled species *Chaetoceros* sp. showing the highest estimates (range 0.11–0.24 mol C (Chl a^{-1}) µmol quanta m^{-2} $s^{-1)}$.

The total carbon (TC) content did not differ greatly between the different species all showing a similar percentage increase at higher light levels by approximately 25%. Increases of up to 42% from low light to high light have been noted for one species *i.e. Chaetoceros* cf. *pendulus*. The total carbon content in the diatoms was lower than in the dinoflagellates. This can be partly attributed to morphological aspects of the diatoms such as the silica outer cover (frustule) and a large cell vacuole for *i.e.* buoyancy control.

CHAPTER 5. LIGHT SHIFT RESPONSES IN A DINOFLAGELLATE AND A DIATOM: COMPARISON AND EVALUATION

5.1 Introduction

An understanding of phototrophic microalgae is imperative because of their effect on the global environment, on oxygen production, carbon cycling and their role in marine food webs. The microalgal species used in these experiments grow in a very dynamic environment as a result of hydrodynamic processes. They are exposed to large fluctuations in light intensities over a wide range of time scales from seconds to hours (Demers *et al.* 1986). The physiological responses of microalgae serve an adaptive mechanism to the physical and other fluctuations of the environment. The physiological responses to environmental fluctuations involve several mechanisms such as size and density of the photosynthetic units, the composition and distribution of pigments (Falkowski and Owens 1980, Sukenik *et al.* 1987) as well as the activity levels of photosynthetic enzymes (Falkowski and Wirick 1981). Harris (1980) pointed out that all these mechanisms respond at different rates, some at very rapid time scales of 10 to 1000 seconds and others such as pigment syntheses, at time scales between hours and days. Sakshaug *et al.* (1987) demonstrated that rapid (in the order of minutes) and reversible changes occurred in *in vivo* fluorescence per cell in diatoms when transferred from low to high light environments. Demers *et al.* (1991) established that these changes in phytoplankton were associated with rapid and reversible changes in the concentration of photosynthetic pigments and in particular carotenoids (diadinoxanthin and diatoxanthin) taking part in the xanthophyll cycle. This cycle involves the light-driven reactions which transform epoxy-containing xanthophylls (oxy-derivatives of carotenes) into epoxy-free pigments. The goal of these experiments was to analyse and compare the photoacclimation responses of the dinoflagellate *Prorocentrum triestinum* and the diatom *Chaetoceros capense* in low and high light environments to better understand how these organisms maximise the available irradiance under controlled laboratory conditions. The exposure to the two light treatments provides evidence of the species photoacclimation to their new environment within the limits of their genetic potential.

5.1.1 Experimental method

Two phytoplankton species, the dinoflagellate *Prorocentrum triestinum* (70 μm^3) and diatom *Chaetoceros capense* (170 μm^3), were cultured to investigate and compare the extent of photoacclimation responses to light shifts from low to high irradiances and *vice versa.* The purpose was to establish whether it was possible to measure those changes that take place after a half hour and an hour exposure to different light intensities. The changes to be measured were the photosynthesis *versus* irradiance (*P vs E*) parameters, pigment concentrations, particularly chlorophyll *a* content, and the concentration of xanthophyll cycle pigments, absorption coefficient values and carbon assimilation. Monocultures of the dinoflagellate and

diatom species were exposed to low light and high light treatments to allow inter specific comparisons of the physiological properties which underlie the photosynthesis *versus* irradiance (*P vs E)* relationship. These two species were chosen as sufficient stock culture for experimentation was available at the time.

Under nutrient replete and temperature controlled conditions the monocultures that were used were initially acclimated to a low light environment (40 µmol quanta m^{-2} s^{-1}) for four days. The cultures were then transferred into a temperature (17 ºC) controlled water bath at a lower light intensity (33 µmol quanta m^{-2} s-1) for 0.5 h. Thereafter the cultures were shifted to high light (HL, 647 µmol quanta m^{-2} s^{-1}) for 2 h, and again shifted to low light (LL, 33 µmol quanta m^{-2} s^{-1}) for another 2 h, ending in a shift to HL for 0.5 h. During the five hour duration of the experiment, sufficient subsamples of the two monocultures were taken at either half hourly or hourly intervals, as indicated in Figure 5.2, for the various measurements as listed above.

5.2 Chlorophyll *a* measurements

All measurements of chlorophyll *a* (Chl *a*) were made using the standard fluorometric technique according to Welschmeyer (1994). During the half hourly and hourly transfers from LL to HL and *vice versa* using *Chaetoceros capense* and *Prorocentrum triestinum*, differences were noted in the photoacclimation responses between the diatom and the dinoflagellate species. In *Chaetoceros capense* the total chlorophyll *a* (TChl *a*) concentration increased after every change in light level after the initial 0.5 h. Thereafter it remained fairly constant. In *Prorocentrum triestinum* a decrease took place in Chl *a* following transfer to a different light intensity followed by a slight increase (Figure 5.1; Appendix 5.1). The variations, however, appeared to be minimal and did not constitute a clear pattern.

Prorocentrum triestinum responded to the light change from LL to HL with an initial 4.0 µg Chl *a* L^{-1} increase which was the reverse to that noted in the diatom culture. At transfer to LL a decrease occurred of ± 2 µg Chl *a* L^{-1}, then increasing again rapidly and peaking at 2.5 h in HL. A sharp decline occurred again at transfer to LL by approximately 7 µg Chl *a* L^{-1} within 2.5 h remaining fairly constant showing an increase after 4 h. After transfer to HL a decrease was noted of 3.1 µg Chl *a* L^{-1}. The changes in the Chl *a* concentration in the dinoflagellate culture were generally an order of magnitude greater in response to light changes compared to those in the diatom culture. The time span in which changes took place however, appeared to be similar, but the nature of the experiments only allowed estimates within half hour time spans. It cannot therefore be established whether the responses of the two species were exactly the same or that photoacclimation takes place within a shorter time interval. Overall the Chl *a* concentration changes that occurred due to the transfer to different irradiance intensities were greatest (mean 9%) in the dinoflagellate species (range 0.08 – 7.67 µg Chl *a* L^{-1}) compared with the diatom species (range 0.01 – 1.84 µg Chl *a* L^{-1}). Also, after five hours the overall Chl *a* concentration showed a significant increase which was not the case in the *Chaetoceros* culture.

The chlorophyllide *a* values recorded for the light shift experiments with the dinoflagellate *Prorocentrum triestinum* (< 2%) and the diatom *Chaetoceros capense* (< 60%) fell in the same range as reported in Chapter 3 and 4. These data supports the findings of other researchers (e.g. Suzuki and Fujita 1986; Sigleo *et al.* 2000; Rodriguez *et al.* 2002 and others). Jeffrey and Hallegraeff (1987) reported chlorophyllide *a* formation in many of the *Chaetoceros* species of up to 100% which suggests that these species are particularly "fragile" and susceptible to degradation of Chl *a* during experimental analyses.

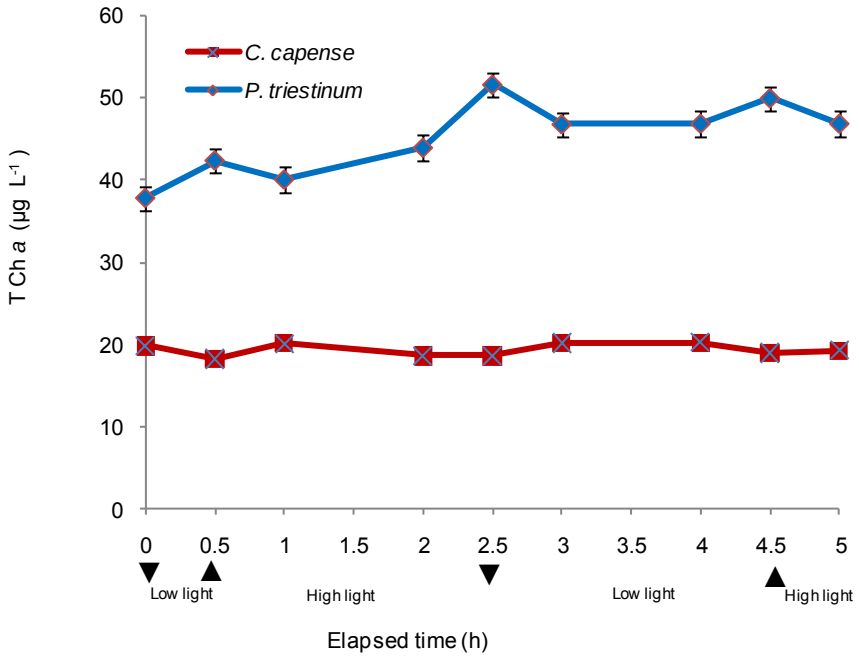

Figure 5.1: Changes in Total Chl *a* concentrations, measured fluorometrically at half hourly and hourly intervals in the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* when shifted from low light (▼) to high light (▲) and *vice versa. Error bars indicate ±one standard error.*

5.3 Cell counts

The concentrations of cells (cells L^{-1}) in the samples used in the experiments are shown in Figure 5.2. Initially the samples were selected for their viability and cell density which was 9 x 10^6 for the *Chaetoceros capense* and 8 x 10^6 cells L^{-1} for *Prorocentrum triestinum.* For both the diatom and the dinoflagellate species cell numbers in the sample counts increased under high light conditions to approximately 19 x 10^6 cells L^{-1} and 15 x 10^6, respectively. At the end of a five hour period the counts

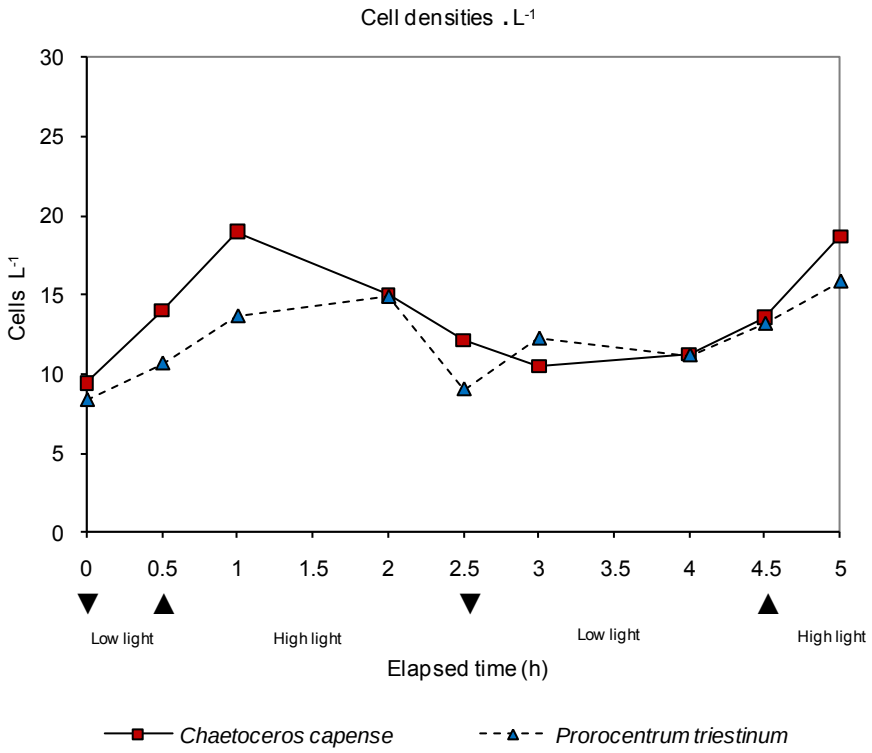

Figure 5.2: Variations in cell densities of the two species *C. capense and P. triestinum* during the time series with shifts from low light (▼) to high light (▲) and *vice versa* over the period of 5 h. The symbols indicate the time samples were transferred. (Cell number x 10^{-6})

were in HL 19 x 10^{6} and 16 x 10^{6} cells L^{-1} for *Chaetoceros capense* and *Prorocentrum triestinum*, respectively.

Thompson *et al.* (1991) reported that cell division continued in both HL to LL and LL to HL transfers in cultures of the diatom *Thalassiosira pseudonana*. The short-term declines in cell numbers appear to be an experimental artefact, possibly due to clumping. The cell numbers recorded in this study remained fairly constant at low light treatments, peaking at high light. It is however difficult to count cells accurately microscopically, using the Utermöll (1958) technique, particularly when chains of cells aggregate and form clumps as is the case in the chain-forming diatom species like *Chaetoceros capense.* Yacobi and Zohary (2010) found that uncertainties derived from 10 to 20% measurement error in the determination of phytoplankton cells abundance using microscope counts are inherent with the method and cannot be avoided. The short-term variations, although relatively minor, can therefore be attributed to experimental error. For this reason I have not presented per cell results in this chapter.

5.4 Photosynthesis *versus* irradiance curves and photosynthetic parameters

The biological processes that regulate the photoacclimation processes, and therefore photosynthesis, are reflected in the magnitude and shape of the curve of photosynthesis *versus* irradiance (*P vs E*). Figure 5.3 presents the *P vs E* curves for the diatom *Chaetoceros capense.* The photosynthetic parameter value, P^*_m, increased over the duration of the experiment from the initial 9.0 to 13.4 mg C (mg Chl *a*)$^{-1}$ h^{-1} at 5 h (mean 10.3±SE 1.7). In low light the P^*_m values ranged from 9.0 to 11.7 mg C (mg Chl *a*)$^{-1}$ h^{-1} and at high light P^*_m values ranged from 9.0 to 13.4 mg C (mg Chl *a*)$^{-1}$ h^{-1} (mean 10.9±SE 1.04). No photoinhibition was apparent in *Chaetoceros capense.*

The *P vs E* curves of the dinoflagellate *Prorocentrum triestinum* are presented in Figure 5.4. The P^*_m values increased minimally from the initial 7.7 mg C (mg Chl *a*)$^{-1}$ h^{-1} at the beginning of the experiment to 8.3 mg C (mg Chl *a*)$^{-1}$ h^{-1} after 5 h which may reflect that no photoacclimation took place during the light-shift experiment. The P^*_m values in low light (33 µmol quanta m^{-2} s^{-1}) ranged from 6.7 to 8.0 mg C (mg Chl *a*)$^{-1}$ h^{-1} (mean 7.4±SE 0.9) and at high light (647 µmol quanta m^{-2} s^{-1}) P^*_m values ranged from 6.7 to 8.3 mg C (mg Chl *a*)$^{-1}$ h^{-1} (mean 7.7±SE 0.9). Similarly no photoinhibition was observed in the *Prorocentrum triestinum* culture during the transfer to high light levels as the value of P^*_m did not show an appreciable decrease at high light levels. These results may indicate, as they do in the *Chaetoceros* species, that the intensity of the irradiance did not reach a level where the photoprotective pigment responses were activated.

Statistical analysis using a nonlinear regression routine (Statistica 7) for the curves, presented in Figure 5.3 and Figure 5.4, shows that R^2 ranged between 0.98 – 0.99 which indicated a good fit to the *P vs E* curves model.

5.4.1. Maximum photosynthetic rate

Figure 5.5(1) presents the changes in the maximum photosynthetic parameters in the two cultures. It shows that an increase in the P^*_m values of *Prorocentrum triestinum* occurred at each transfer to a different light level ranging in high light between 0.5 to 1.8 mg C (mg Chl *a*)$^{-1}$ h^{-1} followed by an increase in low light between 0.5 to 1.6 mg C (mg Chl *a*)$^{-1}$ h^{-1} . The values P^*_m of the diatom *Chaetoceros capense* followed a different pattern, remaining fairly constant on the initial transfer, followed by a decline on transfer from high light to low light (0.7 mg C (mg Chl *a*)$^{-1}$ h^{-1}) at 2 h after which a steady increase was noted (from 12.0 to 13.4 mg C (mg Chl *a*)$^{-1}$ h^{-1}) first in low light following in high light up to the end of the time series after 5 h. Statistical analysis using a two-way fixed effect ANOVA showed that there is a significant difference between the diatom and dinoflagellate species with the diatom *Chaetoceros capense* having a significantly higher P^*_m rate than the dinoflagellate *Prorocentrum triestinum*. (F = 35.48, df = 1,14, p = 0.00003) (Appendix 5.3 (a-1)).

Chaetoceros capense

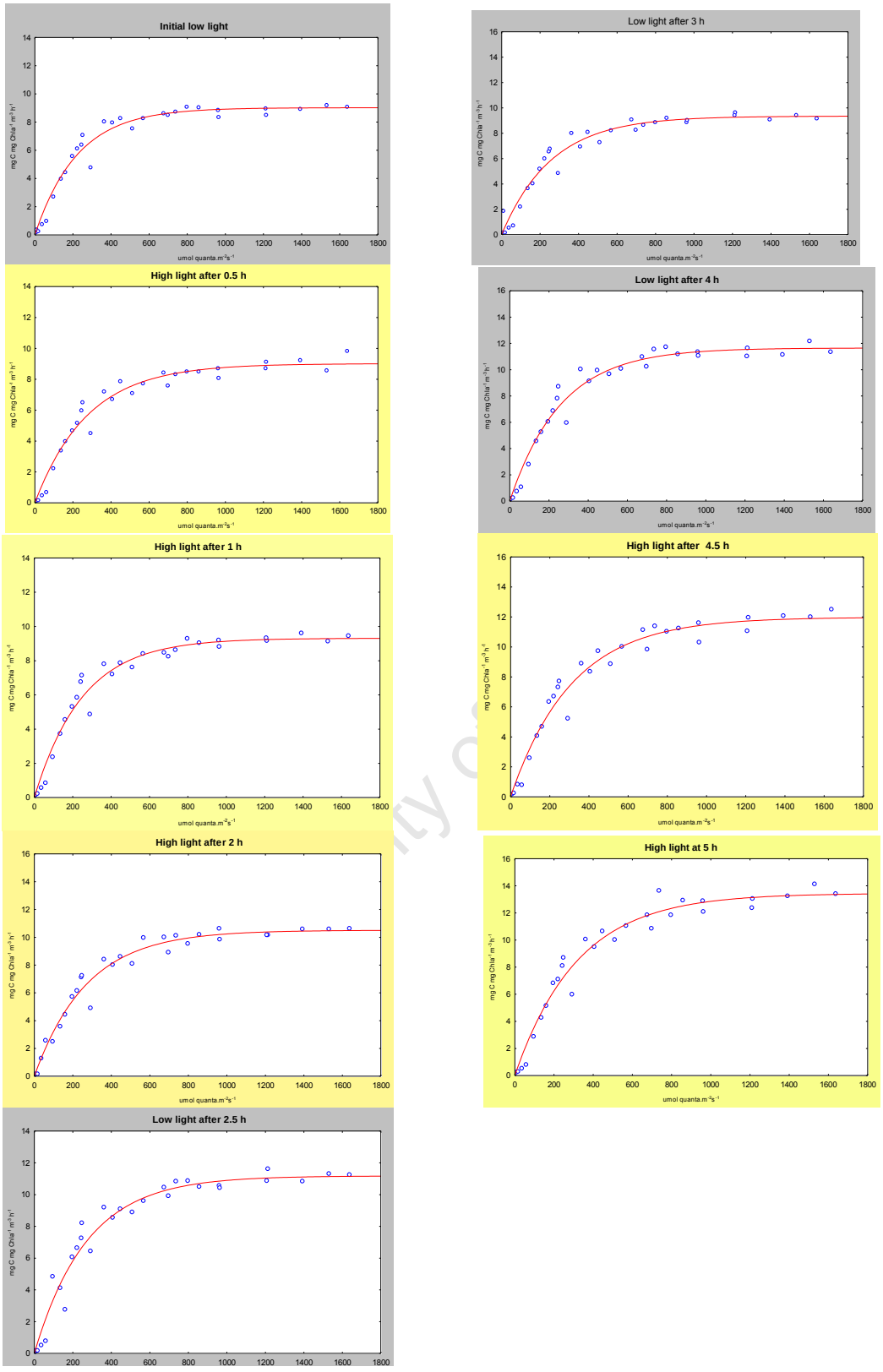

Figure 5.3: Time series of *P vs E* curves for the diatom *Chaetoceros capense* during transfers from low to high irradiances and *vice versa* over a time span of 5 h. (*Low light conditions are indicated by figures with a grey border and yellow borders indicate that cultures were exposed to high light*). (Low light = 33 and high light = 647 µmol quanta m^{-2} s^{-1}).

Prorocentrum triestinum

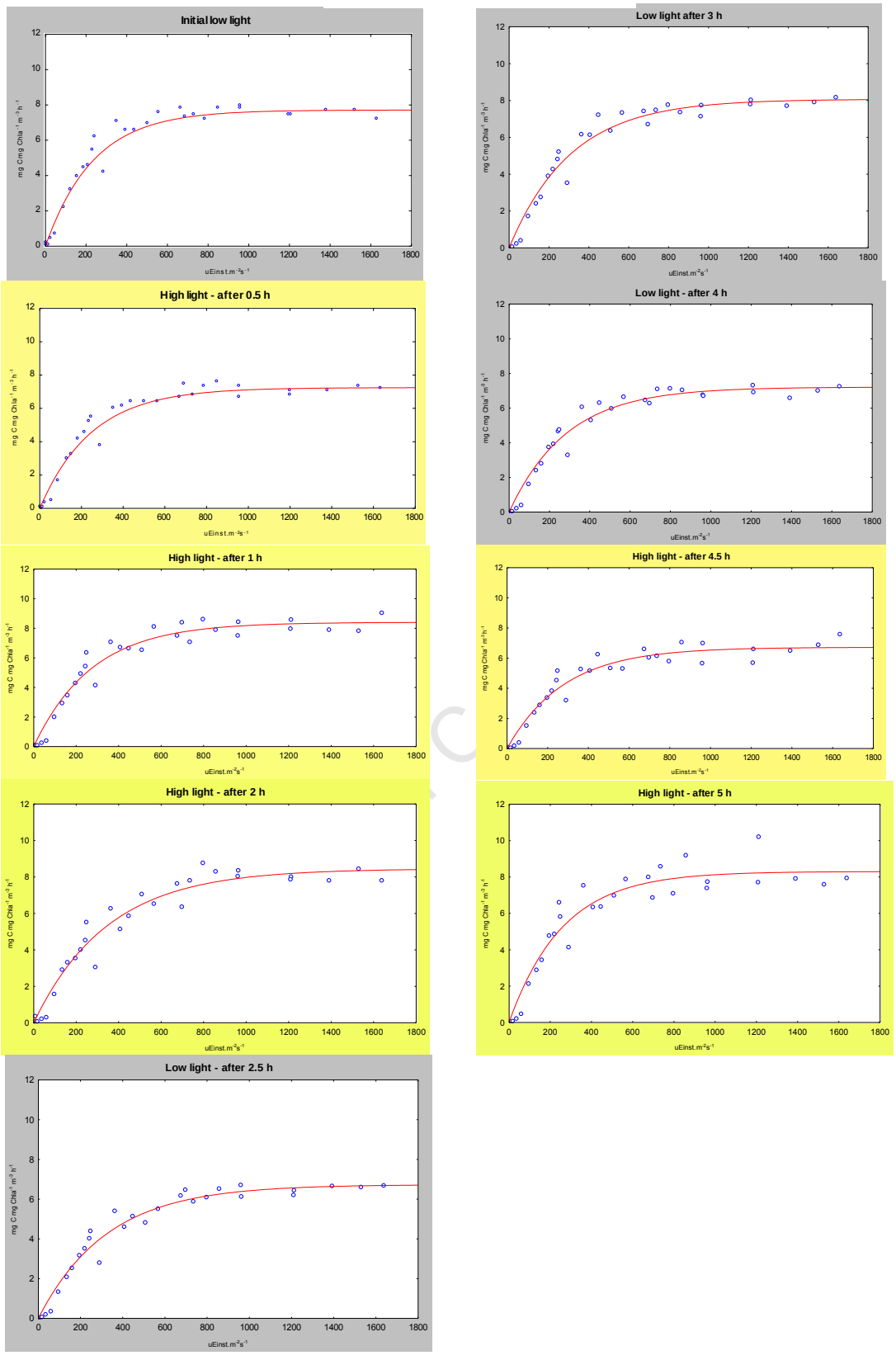

Figure 5.4: Time series of *P vs E* curves for the dinoflagellate *Prorocentrum triestinum* during transfers from low to high irradiances and *vice versa* over a time span of 5 h. (*Low light conditions* are *indicated by figures with a grey border and yellow borders indicate that cultures were exposed to high irradiance*). (Low light = 33 and high light = 647 µmol quanta m^{-2} s^{-1}).

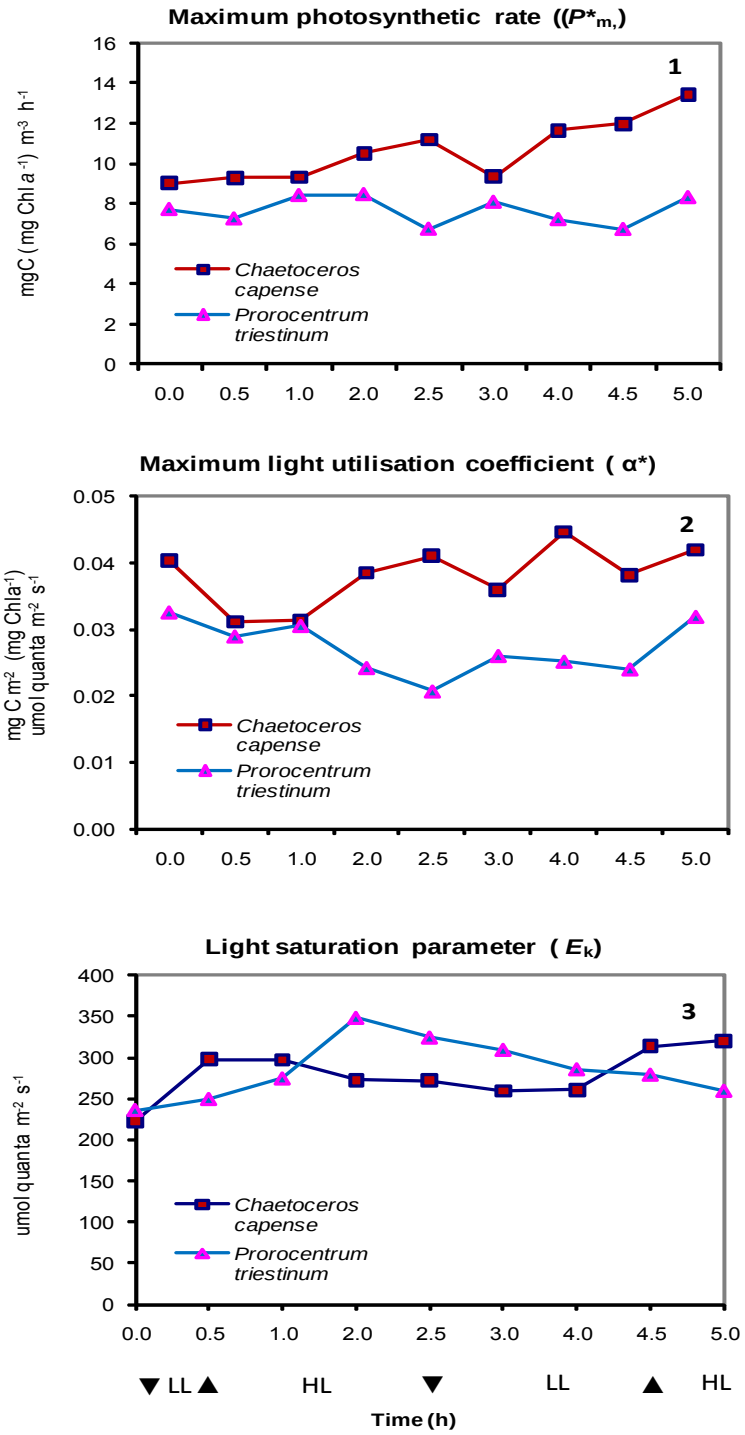

Figure 5.5: Changes in the photosynthetic parameters (*P vs E* curves), P^*_m, α* and E_k, of *Chaetoceros capense* and *Prorocentrum triestinum* during transfers from low (LL) to high (HL) irradiances and *vice versa* as observed during the time series experiments.(*Symbols represent* ▼ LL 0 – 0.5 h; ▲ HL 0.5 – 2.5 h;▼ LL – 2.5 – 4.5 h; ▲ H: - 4.5 – 5 h)..

Depending on the species used it has been reported that photosynthesis can decline by 87% and is accompanied by cell volume increases of up to 22% in less than 12 h following a change from high light to low light. On the other hand, a high rate of photosynthesis was shown to occur within 10 min after transfer from low to high light (Post *et al.* 1985). In contrast, the data of this study showed an initial increase from low light to high light of up to 19% in the P^*_m value followed with a decrease of 16% and increase of 19% in low light. Thereafter, from low to high light, a 30% increase was noted. The P^*_m value of *Prorocentrum triestinum* recorded a lower increase of approximately 7% on each transfer from low to high light and *vice versa*. Unfortunately, although a fairly constant rate of photosynthesis was noted, only changes during these experiments which occurred after 0.5 h could be observed, as measurements at shorter time intervals were not practical.

5.4.2. Maximum light utilisation coefficient

Figure 5.5(2) shows that the maximum light utilisation coefficient, α*, for the diatom *Chaetoceros capense* initially decreased from 0.040 to 0.031 mg C m^{-3} (mg Chl a^{-1}) µmol quanta m^{-2} s^{-1} during low light conditions. After transfer from low light to high light, at 0.5 h, an increase occurred within half an hour to 0.039 mg C m^{-3} (mg Chl a^{-1}) µmol quanta m^{-2} s^{-1}. Thereafter α* fluctuated between 0.036 and 0.042 mg C m^{-3} (mg Chl a^{-1}) µmol quanta m^{-2} s^{-1}. After 0.5 h, after the transfer from high light to low light, the culture's maximum light utilization coefficient, α*, as expected for diatoms in low light, increased to 0.045 mg C m^{-3} (mg Chl a^{-1}) µmol quanta m^{-2} s^{-1} remaining constant for 0.5 h. With the transfer from low to high light the recorded value of α* decreased to 0.042 mg C m^{-3} mg Chl a^{-1} µmol quanta m^{-2} s^{-1} (Table 5.1). The increase recorded which took place in low light and high light for the diatom cultures was 0.76 mg C m^{-3} mg Chl a^{-1} µmol quanta m^{-2} s^{-1} h^{-1}.

The differences between α* recorded for the *Prorocentrum triestinum* were lower. Initially α* decreased from 0.033 to 0.029 mg C m^{-3} (mg Chl a^{-1}) µmol quanta m^{-2} s^{-1} from low to high light, decreasing to 0.024 mg C m^{-2} (mg Chl a^{-1}) µmol quanta m^{-2} s^{-1} for the duration in a high light environment. Culture samples 6 to 9 showed variable α* for low light to high light of 0.026 to 0.032 mg C m^{-3} (mg Chl a^{-1}) µmol quanta m^{-2} s^{-1} indicative of reduced photoacclimation capabilities during the last 2 h of the experiment compared with the diatom species used in the experiment. The mean rate of decline recorded for the dinoflagellate cultures was -0.06 mg C m^{-3} mg Chl a^{-1} µmol quanta m^{-2} h^{-1} over the whole experiment.

Since α* represents the rate of photosynthesis per unit biomass per unit of incident irradiance, the recorded data indicated that generally *Chaetoceros capense* was more efficient in utilising light at high intensities in fixing CO_2 than *Prorocentrum triestinum*. Statistical analyses confirm that there was a significant difference between the photoacclimation responses of the diatom and dinoflagellate species ($F = 49.36$, $df = 1,14$, $p = 0.000006$).

5.4.3. Light saturation parameter

Figure 5.5 (3) shows the irradiance values, E_k (P^*_m / α*) for the diatom *Chaetoceros capense* ranged from 224 µmol quanta m^{-2} to 272 µmol quanta m^{-2} s^{-1} (mean 254 ±SE 5.5) at low light. At high light levels E_k values were higher ranging from 250 to 320 µmol quanta m^{-2} s^{-1} (mean 287 ±SE 14.9). In *Prorocentrum triestinum* E_k values at low light ranged from 236 to 324 µmol quanta m^{-2} s^{-1} (mean 289 ±SE 3.5) and at high light ranged from 250 to 348 µmol quanta m^{-2} s^{-1} (mean 280 ±SE 5.6).

A definite trend was demonstrated here in *Chaetoceros capense* and *Prorocentrum triestinum*. The mean rate of increase in the light saturation parameter, E_k, for *Chaetoceros capense* for the duration of the experiment of 5 h was 8.33 µmol quanta m^{-2} h^{-1} and for *Prorocentrum triestinum* the increase in E_k was 4.51 µmol quanta m^{-2} h^{-1} . The rise and decline of the mean value of E_k in these species paralleled the mean maximum Chl *a*-specific photosynthetic rate (P^*_m) with changing irradiances showing an increased value after 5 h (Figure 5.5 (3)). Generally, with a decline in the P^*_m values an increased value of E_k was recorded as a result of lower values of α*. Statistically there was no significant difference in the E_k data between the diatom and dinoflagellate ($p > 0.05$) (not shown).

Comparing the photosynthetic parameters, P^*_m and E_k (Figure 5.5) of the diatom *Chaetoceros capense* with those of the dinoflagellate *Prorocentrum triestinum*, suggest that the larger celled *Chaetoceros capense* (mixer) had a greater photoacclimation response at high irradiances unlike the smaller celled *Prorocentrum triestinum* (migrator). This may be related to an adaptation in the diatom to a generally turbulent environment with short exposures to increased irradiance levels in the ocean's surface layers.

It is reported that the rate of photoacclimation is generally slower on transfer from high light to low light than from low light to high light as noticed in the diatom *Thalassiosira* spp. (Thompson *et al.* 1991 and others). This has an ecological significance specifically in turbulent waters as brief exposure to high irradiances outweighs much longer exposures to low irradiances (Geider *et al*. 1996). The results of this experiment support these findings and this was most noticeable in the *Chaetoceros capense* culture. The P^*_m value increased over a period of 2 h by 1.04 mg C m^{-3} mg Chl a^{-1} µmol quanta m^{-2} s^{-1} on transfer from high to low light compared to 1.49 mg C m^{-3} mg Chl a^{-1} µmol quanta m^{-2} s^{-1} when transferred from low to high light. This same trend was not true for the dinoflagellate species as P^*_m decreased from high to low light by 1.26 mg C m^{-3} mg Chl a^{-1} µmol quanta m^{-2} s^{-1}, and on transfer from low to high light only showed a slight increase over a two hour period of 0.74 mg C m^{-3} mg Chl a^{-1} µmol quanta m^{-2} s^{-1}.

5.5 Pigment composition

Figure 5.6 shows a slight decrease of 0.33 µg L^{-1} in the total pigment concentration (including photosynthetic and photoprotective pigments) observed in low light in *Chaetoceros capense* at 0.5 h, followed by an increase of 6.37 µg L^{-1} in high light within the second 0.5 h. After the second transfer from high to low light an increase of 3.37 µg L^{-1} occurred within 0.5 h. The highest concentration of total

pigments, 59 µg L^{-1}, was recorded in low light after 4.0 h representing an increase of 5.5 µg L^{-1}. At the end of the experiment the concentration of total pigment showed a slight overall increase of 2.6 µg L^{-1} in high light compared with the initial concentration. The increases and decreases were minimal and were always < 7.0 µg L^{-1}, and may be attributable to experimental/measurement error.

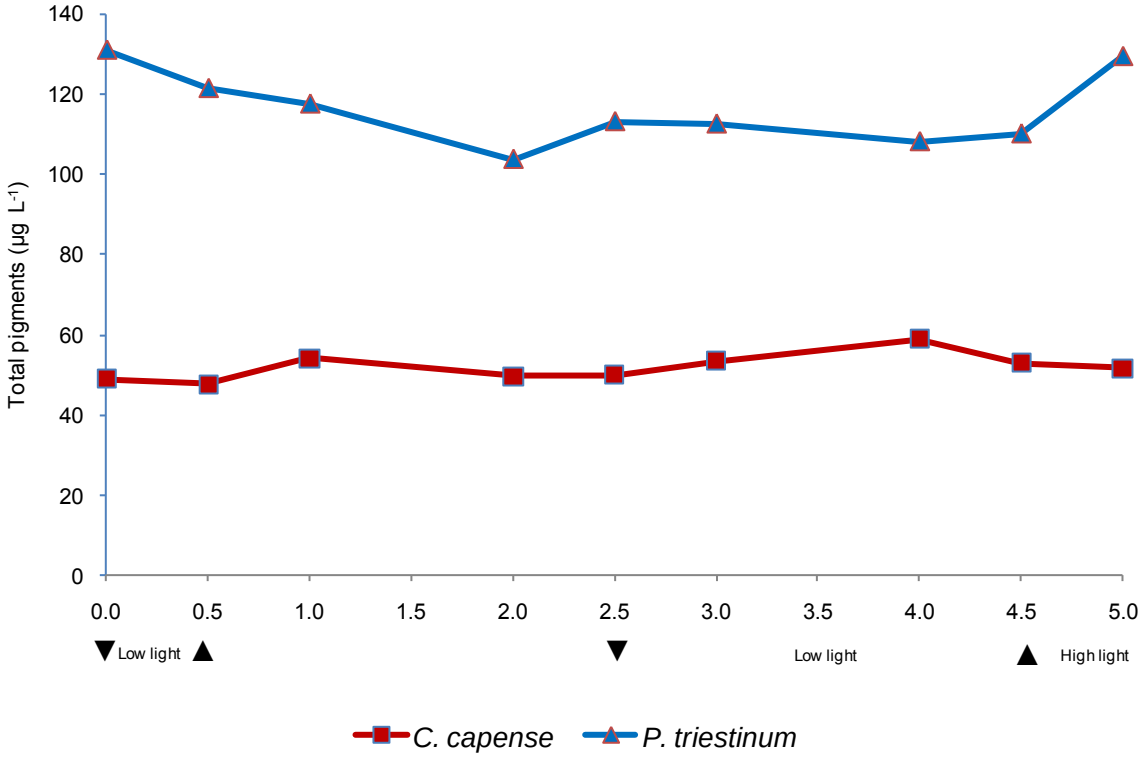

Figure 5.6: A comparison of the changes of total pigment concentrations of the diatom *Chaetoceros capense* and the dinoflagellate *Prorocentrum triestinum* when subjected to three light shifts over a period of 5 h. The symbols (▼ and ▲) indicate low light and high light treatments

In the dinoflagellate *Prorocentrum triestinum*, during the first 2 h, the total pigment concentration measured at half hourly intervals decreased by 9.6, 3.9 and 13.9 µg L^{-1}, followed after 2 h by an increase of 9.5 µg L^{-1} in high light. The greatest increases took place between 2 and 2.5 h and 4.5 to 5 h in high light treatments ending with approximately the same concentration as at the beginning of the experiment. The rate of 0.5 h increases and decreases in total pigment concentrations were never greater than 10 µg L^{-1} which constituted some 25% change.

Figure 5.7 compares the photoacclimation responses in the diatom *Chaetoceros capense* and the dinoflagellate *Prorocentrum triestinum* it was noted that initially the xanthophyll pool (diadinoxanthin (DD) + diatoxanthin (DT)) expressed as chlorophyll *a*-specific was highest (0.08 µg L^{-1} (Chl *a*)$^{-1}$) during high light conditions in *Chaetoceros capense*, showing a decrease during the second half hour, declining to 0.07 µg L^{-1} (Chl *a*)$^{-1}$ after 1.5 h in a high light environment. The xanthophyll pool reached its highest level

after 4 h at low irradiance (0.09 µg L^{-1} (Chl *a*)$^{-1}$). *Prorocentrum triestinum* showed a similar pattern as *Chaetoceros capense* with an increase of the xanthophyll pool at 1 h of 0.27 µg L^{-1} (Chl *a*)$^{-1}$ in high light decreasing steadily from 0.27 to 0.17 increasing at 5 h to 0.25 µg L^{-1} (Chl *a*)$^{-1}$. For both species the last two readings may reflect a stress condition caused by sudden and constant transfers to greater differences in irradiance levels than phytoplankton would generally experience in natural environments and may bear an influence on the results (Appendix 5.5).

Generally, in both species, it was noted that when diadinoxanthin (DD) showed increases in concentration, diatoxanthin (DT) decreased and *vice versa*. This was also reflected in the ratios recorded over the 5 h period as the ratios decreased during high light treatments and increased during low light treatments. However, the changes were very slight and it may not be pertinent to acknowledge the outcome as a positive photoacclimation response. It is suggested by Olaizola *et al.* (1994) that there are possibly two distinct light-induced processes affecting DT: firstly, a rapid light-induced conversion of DD to DT; secondly, after a prolonged exposure to high light (approximately 30 min), a further DT increase without DD decreasing appreciably. In the diatom species *Chaetoceros capense*, the experimental data showed an initial increase in DD with a decrease in DT concentration on transfer from low to high light at 0.5 h (ratio DD :DT = 7.8). The pattern was repeated at 2.5 h on transfer from high to low light (ratio DD : DT = 9.9). This reflects a DD increase within 0.5 h. However, the data for the dinoflagellate species, *Prorocentrum triestinum*, do not follow a similar pattern as on each transfer to a different light intensity the ratio of DD : DT declined in high light following an increase when exposed to a low irradiance between 2.5 and 4 h, decreasing again when the culture was transferred to high light. On two occasions, at 0.5 and 4.5 h the DD concentration declined with a corresponding increase in DT which would support Olaizola *et al.*'s (1994) findings (Appendix 5.5).

A comparison of all pigments during transient light conditions in the diatom and dinoflagellate during the time course of 5 h showed that the pigment concentrations declined within the first 0.5 h in low light except fucoxanthin in the diatom. In *Chaetoceros capense* the pigment concentrations increased within the second 0.5 h, remaining fairly constant for the next 2 h. Thereafter the pigment concentrations increased in low light over a time period of 1 h with a decrease after 4 h (Figure 5.7 (a)).

In the *Prorocentrum triestinum* culture the pigment concentrations showed a decrease for the first 2 h with the exception of Chl *a*. Thereafter increases were noted at 2 and 4.5 h at high irradiances. During the five hour light shift experiment the pigment concentrations had fluctuated but had not increased (Figure 5.7 (b)). The data of the dinoflagellate *Prorocentrum triestinum* and the diatom *Chaetoceros capense* both showed similar pattern with the pigment concentrations reaching an approximate initial concentration after 5 h.

Comparing the changes, at half hourly intervals, of increases and decreases of pigment concentrations per µg L^{-1} that occurred in the diatom and dinoflagellate cultures, it was noted that the changes in the diatom were less than those in the dinoflagellate. The exception was Chl *a* (4.3 µg L^{-1}, range 0.3 to 11.0 µg L^{-1})

which was slightly greater in the diatom compared with the dinoflagellate (change 3.2 µg L^{-1}, range 0 to 4.8 µg L^{-1}) (Appendix 5.7 (a)). In the diatom the photosynthetic pigments chlorophyll c_1 and c_2 and fucoxanthin the changes were 1.04 and 0.7 µg L^{-1} respectively compared with the dinoflagellate. The changes in concentration of the photosynthetic pigments Chl c_1,c_2 and total peridinin were 5 and 4.6 µg L^{-1}.

In both species the photoprotective pigments diadinoxanthin and diatoxanthin showed variations in concentrations at different light levels. Generally diadinoxanthin showed an increase in low light with a

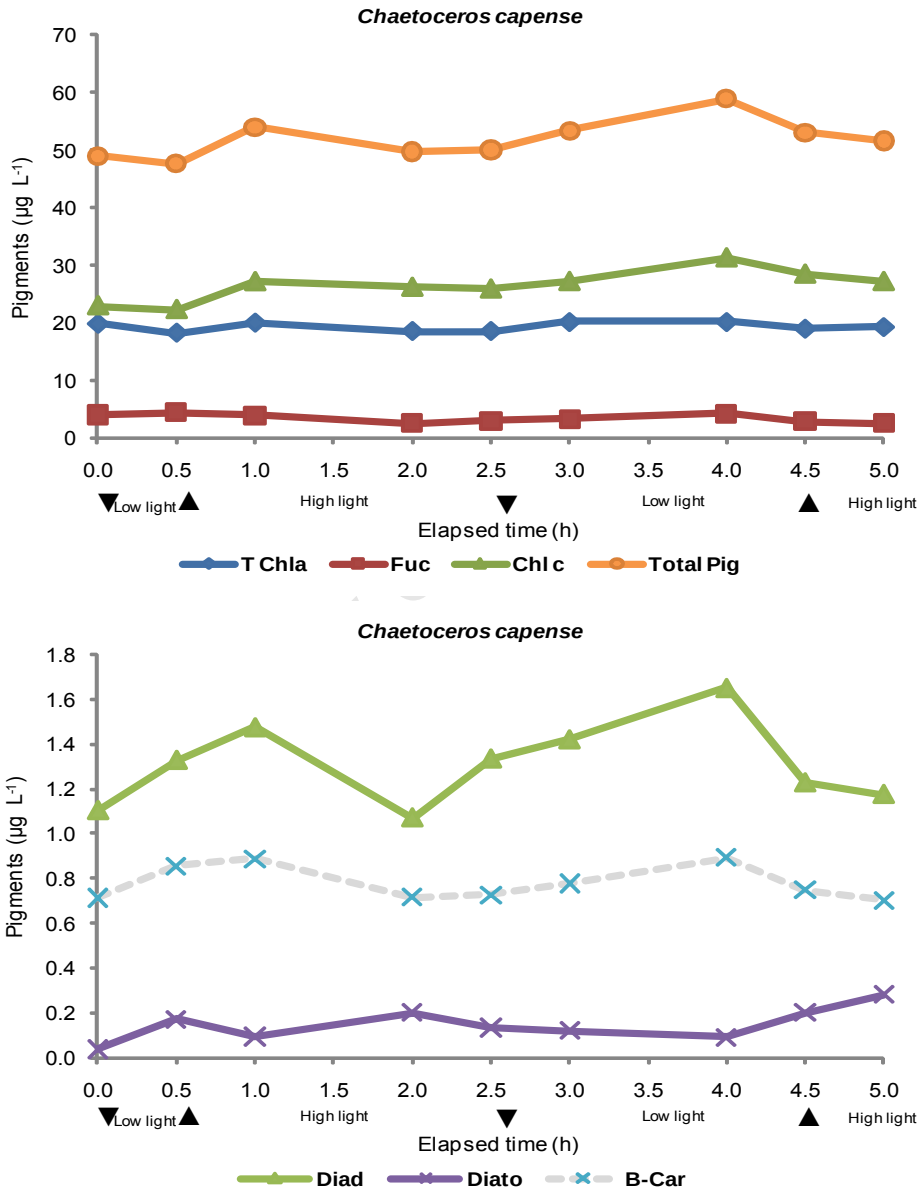

Figure 5.7 (a): Comparison of all pigments during transient light conditions in the diatom *C. capense* during the time course of 5 h. (Photosynthetic pigments (PSP are T. Chl *a* - total chlorophyll *a*, Fuc. - total fucoxanthin, Chl c – chlorophylls c_1 and c_2; Photoprotective pigments (PPC) are Diad. - diadinoxanthin, Diat. - diatoxanthin, B-car.- β carotene, T. pig. - total pigment).

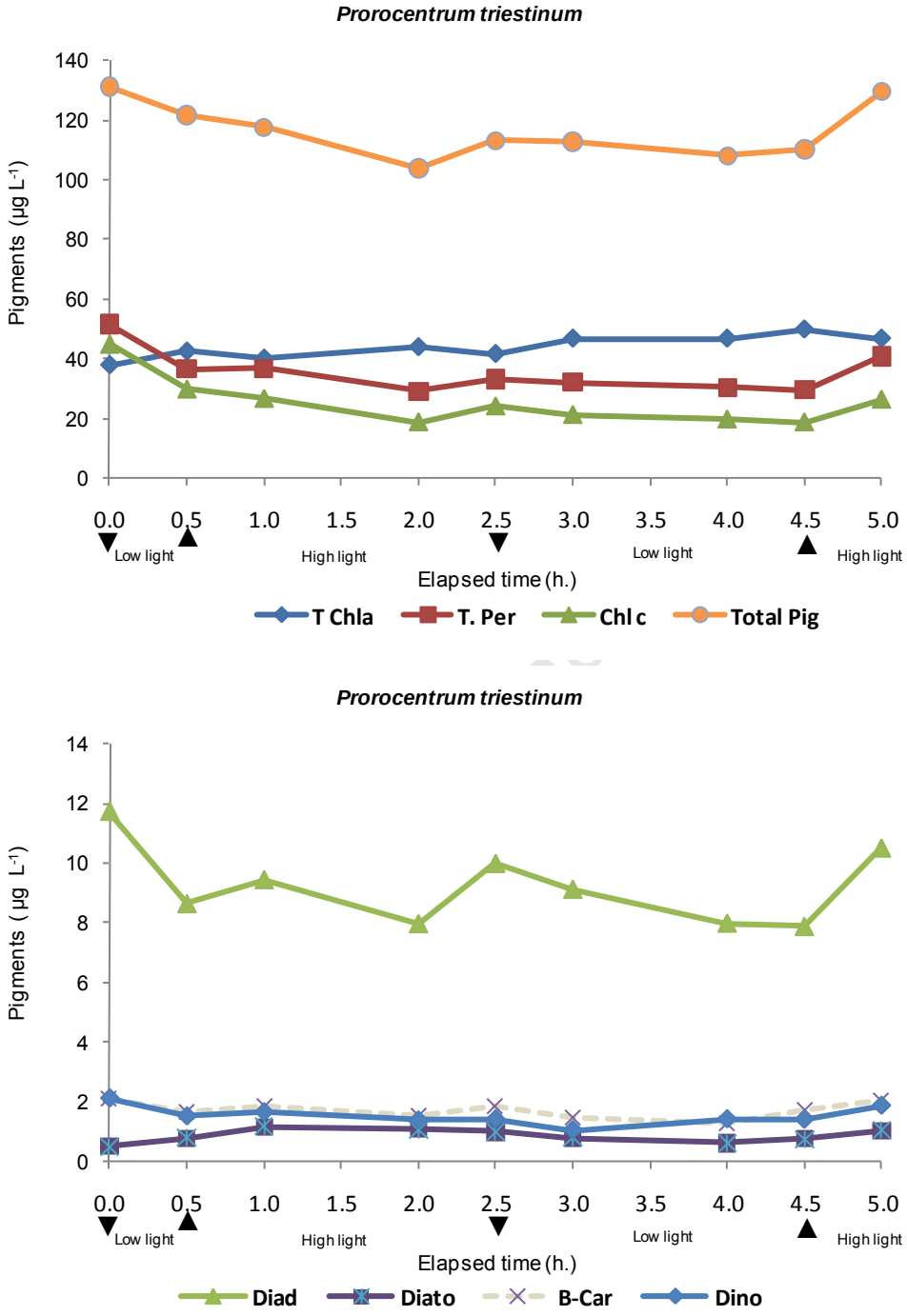

Figure 5.7 (b): Comparison of all pigments during transient light conditions in the dinoflagellate *P. triestinum* during the time course of 5 h. (Photosynthetic pigments (PSP) are T. Chl *a* - total chlorophyll *a*, T. Per. – total peridinin, Chl c_1 c_2 – chlorophylls c_1 and c_2; Photoprotective pigments (PPC) are Diad. - diadinoxanthin, Diat. - diatoxanthin, B-car. - β carotene, Dino - dinoxanthin, T. pig. - total pigment).

corresponding decrease in diatoxanthin with the reverse taking place in high light conditions. The mean rate of increase of diadinoxanthin in *Chaetoceros capense* was 0.026 μg L^{-1} h^{-1} and in *Prorocentrum triestinum* a decrease was noted of -0.19 μg L^{-1} h^{-1}. The increase in the photoprotective pigment diatoxanthin in the diatom culture was 0.024 μg L^{-1} h^{-1} but did not change in the dinoflagellate culture. The observed small changes in the concentration of the photoprotective pigment, particularly of diadinoxanthin, seem to represent a photoacclimation response to different irradiance levels.

The photoacclimation responses in the PSP seems to be faster in the smaller celled *Prorocentrum triestinum* than in the larger celled *Chaetoceros capense* although the initial increased level of concentration, unlike that of *Chaetoceros capense*, is not maintained after 0.5 h as indicated in Figure 5.7 where a decline takes place between 2.5 to 3 h with a small increase over a period of 1 h (up to 4 h). The PSP in *Prorocentrum triestinum* vary in unison but in *Chaetoceros capense* this is not the case with Chl *a* declining at 4 h where fucoxanthin still showed an increase (Appendix 5.6). The rate of increases of Chl *a* in the diatom was 0.03 and in the dinoflagellate was 1.9 μg L^{-1} h^{-1}.

Generally the light harvesting pigment chlorophyll *a*, which paralleled the total pigment concentration, was present at the highest concentrations at low light levels declining with increasing irradiance in both species. Figure 5.8(a) shows that the ratios, expressed as a percentage, of the photosynthetic pigments (PSP) to chlorophyll *a* in *Chaetoceros capense* declined in high light during the first hour, remaining fairly constant up to 4 h after which a slight increase was apparent. The ratio of chlorophyll *a* to photoprotective carotenoids (PPC) represented a mirror image of PSP to Chl *a*. The ratio of PSP : PPC showed minimal variation, remaining almost constant for the 5 h period (Appendix 5.6).

The ratio of PSP to Chl *a* for *Prorocentrum triestinum* showed an initial increase in low light. The overall increase continued for the following 4.5 h, first in high light then low light, ending with a decrease in high light after 5 h. The ratio of Chl *a* to PPC pattern was virtually a mirror image of the last mentioned ratio. This pattern was also noted in the *Chaetoceros capense* but far less distinct. The changes that took place in the ratios of PSP to Chl *a* ranged from 0.3 to 11% and Chl *a* to PPC ranged from 0.3 to 14%. They were however more pronounced than observed in the diatom culture indicating a degree of photoacclimation having taken place in the dinoflagellate culture over the 5.0 hour period (Figure 5.8). As in *Chaetoceros capense* the ratio of PSP to PPC showed slight fluctuations over time ending with a minimal increase in the ratio (1%) after 5 h.

Interestingly a difference was noted in the PSP to Chl *a* ratios (expressed as a percentage) in the two species. In the diatom the ratios decreased from the initial 42 % over the 5.0 h duration of the time series to 39%, the lowest ratio (36%) was reached in low light at 4 h. The exact reverse was noted in the Chl *a* to PPC ratios. In the dinoflagellate the Chl *a* ratios increased from the initial 26 to 41%, the highest being at 4 h with the opposite taking place in the Chl *a* to PPC ratios. In both species the ratios for total pigments to PSP and PSP to PPC remained fairly constant throughout the experiment indicating that the pigment pool

does not vary significantly but the individual pigments show a positive photoacclimation response to changing light intensities during light shifts.

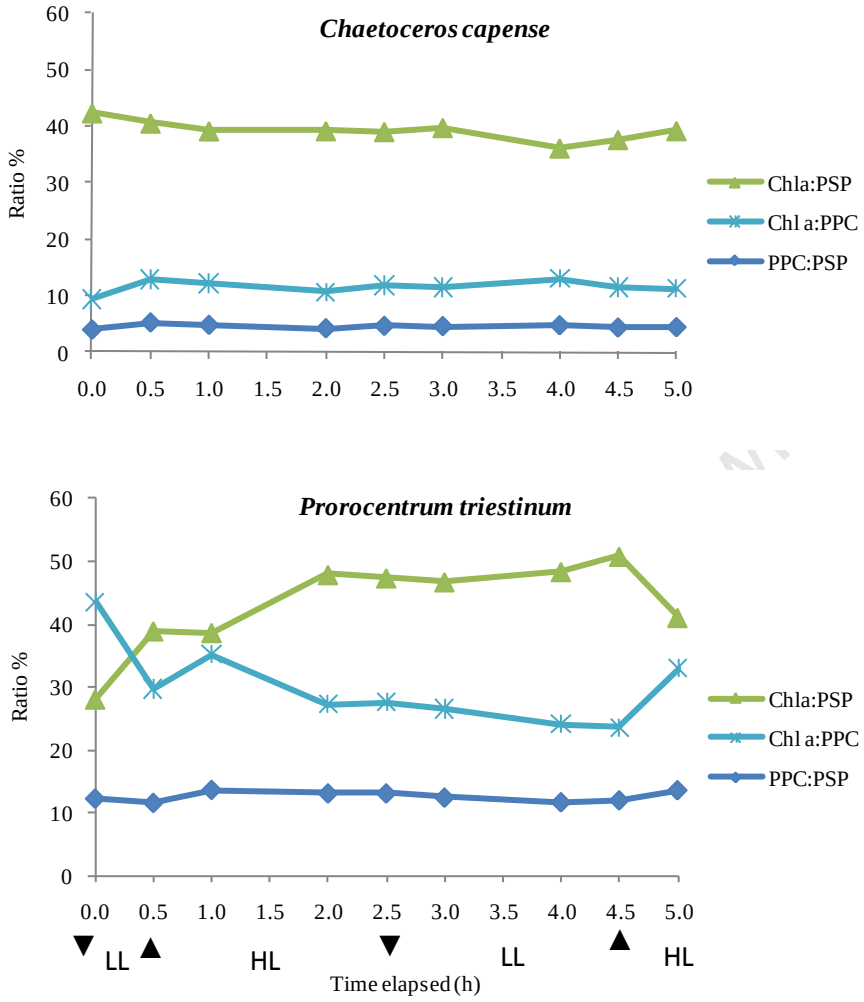

Figure 5.8: A comparison of the changes in the ratios of photosynthetic pigments (PSP) and photoprotective carotenoids (PPC) of the diatom *Chaetoceros capense* and the dinoflagellate *Prorocentrum triestinum* when subjected to three different irradiance levels over a period of 5 h.
(PSP = chlorophyll c_1 and c_2, fucoxanthin (in diatoms), peridinin (in dinoflagellates), Chl *a* = chlorophyll *a*.
PPC = diadinoxanthin, diatoxanthin, *β*-carotene).
The symbols (▼ and ▲) indicate low light and high light treatments

5.6 Absorption

The variations in the chlorophyll *a*-specific absorption spectra for the diatom and dinoflagellate species grown under different experimental light conditions are shown in Figures 5.8 and 5.9 respectively. Most apparent are the changing shapes of the spectra, with a flattening during low light treatment noticeable in *Chaetoceros capense*, and indicating increased absorption in the blue-green and red regions on transfer to high light levels. The spectrum of *Chaetoceros capense* at high light has a very characteristic shape

between wavelengths 400 nm and 445 nm showing a distinct absorption peak. A similar 'noisiness' at low wavelengths was apparent in *Chaetoceros capense* as described in Chapter 4 (see Figure 4.8.3). Absorption increased at all wavelengths for the diatom species after transfer to high light levels, decreasing considerably on transfer to a low light environment. The dinoflagellate *Prorocentrum triestinum* followed a fairly similar pattern (Figure 5.9).

Figures 5.9 and 5.10 show that, as noted in Chapter 3 and 4, the spectra indicated a strong peak of Chl *a* absorption in the red region (673-675 nm) with fucoxanthin and peridinin having a weaker absorption shoulder between 550 and 650 nm in the green region. The main light absorption band, a combination of chlorophylls $c_{1,\ 2}$ and $_3$ and carotenoids (425 to 525 nm) showed the characteristic two peak shoulder at approximately 430 and 440 nm which was not observed in the dinoflagellate culture.

Figure 5.11 shows that in the first 0.5 h the chlorophyll *a*-specific absorption values for *Chaetoceros capense* at the major absorption peaks (wavelength 440 and 675 nm) decreased slightly in low light after which they increased in a high light environment. A change took place after 2.0 h with a slight decline in low light until 4 h when an increase of 34% was noted at both wavelengths. The absorption values at all wavelengths generally increased on transfer from low to high light by approximately 13 to 34%. The declines from high to low light represented up to 11% loss in absorption. The Chl *a*-specific absorption values for wavelengths 440, 460, 490 and 675 nm showed slight variation, all following a similar pattern. The least effective absorption took place at wavelengths 550 and 660 nm (Appendix 5.8).

A similar pattern was observed in the dinoflagellate cultures with the absorption coefficients at 440 nm. The chlorophyll *a*-specific absorption values for *Prorocentrum triestinum* increased initially within the first 0.5 h in a low light environment in 440 nm waveband. Absorption did not increase significantly between 0.5 and 1 h when placed in a high light environment remaining fairly constant until 2 h. Between 2 and 3 h absorption fluctuated showing firstly a decline in the 440 nm waveband of 21% and in the 675 nm waveband of 22%. Thereafter an increase at 440 nm of 15% and at 675 nm of 16.5% was noted. After 4.5 h an increase occurred of 12 and 11% in wavebands 440 and 675 nm, respectively. As was noted in the diatom, the absorption values increased on transfer from low to high light by approximately 22% which was less than the value recorded for the diatom. Similarly the Chl *a*-specific absorption values decreased on transfer from high to low light in both the diatom and dinoflagellate (Table 5.2). Interesting to note was that Chl *a*-specific absorption in the dinoflagellate *Prorocentrum triestinum* at wavelength 440 nm as at wavelength 675 nm generally co-varied in both low and high light conditions. The same was observed for wavelengths 490 nm where the absorption values were approximately the same over the 5 h time span (Appendix 5.8). This was not the case in the diatom *Chaetoceros capense* as the absorption in the 440 nm wavelength increased minimally between 0.5 and 2 h compared with the absorption in the 675 nm wavelength in high light (Figure 5.11). This variation of the total absorption with wavelengths in the two species gives an insight into the evolutionary adaptation and ecological niche preferences of the diatom compared to the dinoflagellate.

Figure 5.12. shows the ratio of phytoplankton absorption at wavelengths 440 and 675 nm. The ratios ranged between 1.3 and 1.5 in low light and 1.4 to 1.5 in high light for *Chaetoceros capense*. In *Prorocentrum triestinum* the ratios ranged from 1.1 to 1.3 in low light and 1.2 to 1.3 in high light (Table 5.2). The recorded range in this study is in agreement with Kiefer *et al.* (1979) (1.0 to 2.0 ratio) and is an indication that the cultures are in a healthy state Kiefer *et al.* (1979). Although the ratios varied minimally there was a difference in the response to changes of light level in both species. *Chaetoceros capense* showed an immediate increase in absorption on changes to a different light level, noticeable at 0.5 h and 2 h. This was not the case in *Prorocentrum triestinum.* Initially the ratio increased from 1.1 to 1.3 in low to high light up to 1 h after which the ratio remained virtually constant for the remaining 4 h. Phytoplankton absorption is largely dependent on the light harvesting pigment (LHC) complexes present. However, in this study, there did not seem to be an apparent correlation to LHC as no clear pattern was exhibited during the light-shift experiment.

Table 5.2. Comparison of peak height values and ratios, normalised to fluorometrically measured chlorophyll *a*, at 440 and 675 nm in the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* cultures during the light shift experiment. (Low light – LL, High light – HL).

		Chaetoceros capense			***Prorocentrum triestinum***		
Light condition	Time (h)	440 nm	675 nm	440: 675nm ratio	440 nm	675 nm	440: 675nm Ratio
LL	0.0	0.181	0.128	1.42	0.139	0.123	1.13
HL	0.5	0.172	0.124	1.39	0.140	0.111	1.26
HL	1.0	0.199	0.132	1.50	0.150	0.116	1.30
	1.5						
HL	2.0	0.205	0.143	1.43	0.148	0.114	1.30
LL	2.5	0.168	0.127	1.33	0.117	0.088	1.32
LL	3.0	0.187	0.129	1.44	0.138	0.106	1.30
	3.5						
LL	4.0	0.157	0.106	1.48	0.138	0.107	1.29
HL	4.5	0.242	0.165	1.46	0.133	0.102	1.30
HL	5.0	0.186	0.132	1.41	0.151	0.115	1.32

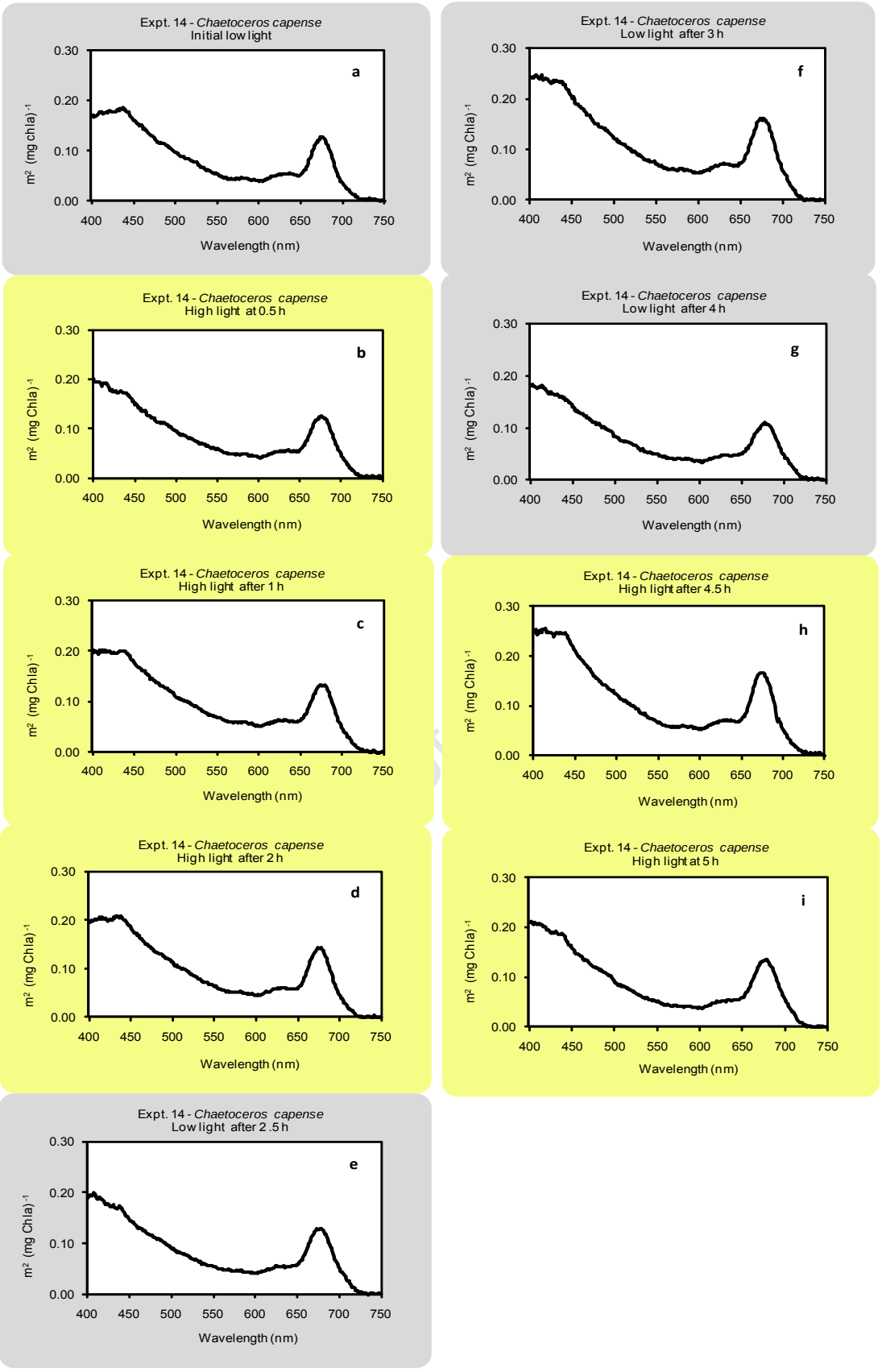

Figure 5.9: Chl *a* –specific absorption coefficient spectra during half hourly and hourly light shifts of the diatom *Chaetoceros capense*. (*Low light is indicated by figures with grey borders and high light with yellow borders*).

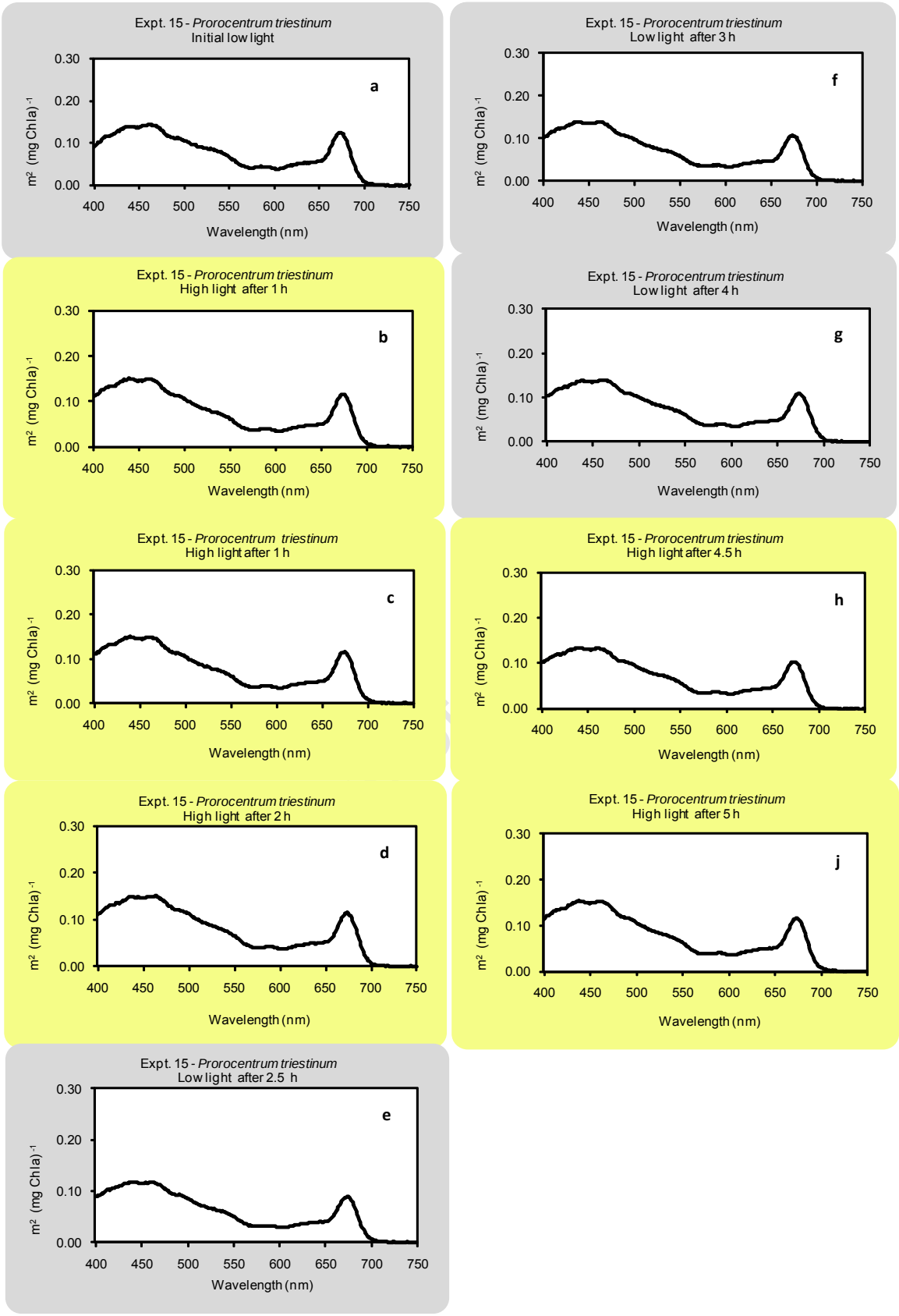

Figure 5.10: Chl *a* –specific absorption coefficient spectra during half hourly and hourly light shifts of the dinoflagellate *Prorocentrum triestinum.* (*Low light is indicated by figures with grey borders and high light with yellow borders*).

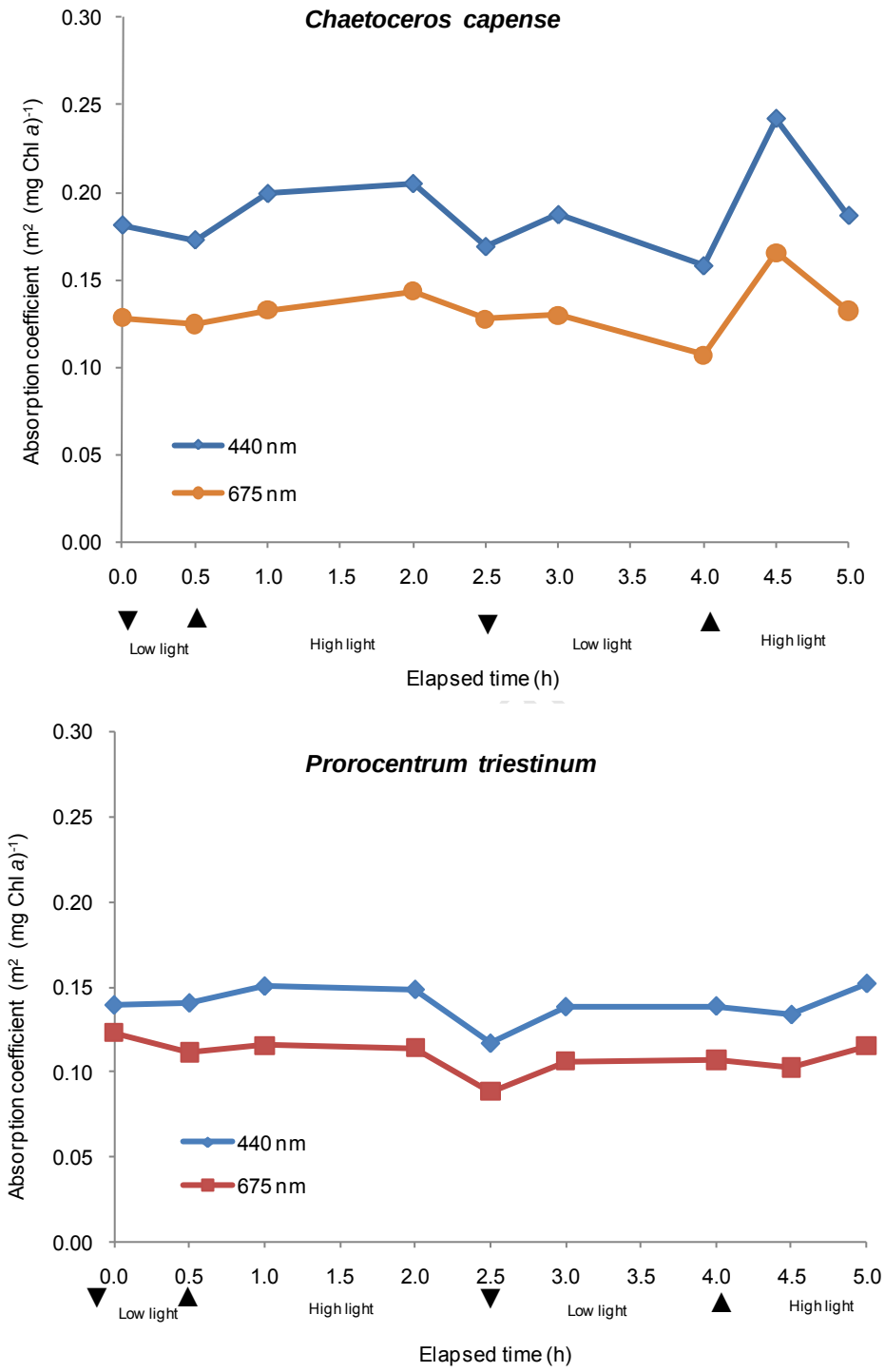

Figure 5.11: Graph showing the changes in the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* of Chl *a*- specific absorption coefficient values after 0.5 and 1 h interval shifts from low to high light and *vice versa*. (Symbols ▼ and ▲ represent low and high light treatments respectively). (Absorption values were normalised to fluorometrically measured Chl *a*).

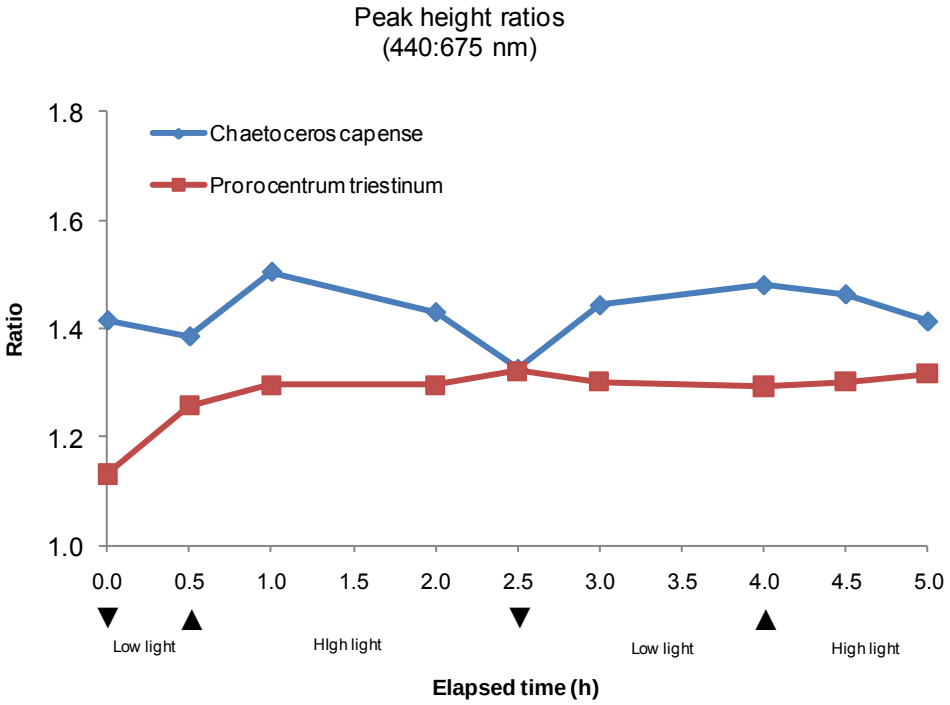

Figure 5.12: Comparison of peak height ratios at 440 to 675 nm of the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* over a time period of 5 h during light shifts from low to high light and *vice versa*.

5.7 Estimated maximum quantum yield

A comparison of the estimated maximum quantum yields (ϕ_m) of the diatom and the dinoflagellate cultures, derived from the initial slope of α* of the photosynthesis *versus* irradiance (*P vs E*) relationship and the absorption cross section a* relative to chlorophyll *a* in m^{-1} (mg Chl *a*)$^{-1}$, is presented in Figure 5.13. Within the first 0.5 h, when the *Chaetoceros capense* culture was transferred to high irradiance, the quantum yield (ϕ_m) decreased from 0.10 to 0.08 mol C (µmol quanta m^{-2} s^{-1}). On transfer to a low irradiance the ϕ_m increased as is shown after 2.5 h, to 0.09 mol C (µmol quanta m^{-2} s^{-1}) (see Appendix 5.9). The value of ϕ_m fluctuated between 0.07 in a high light environment to 0.10 mol C (µmol quanta m^{-2} s^{-1}) in low light. This trend of reduced ϕ_m occurring at high irradiances and increasing in low light has been reported by other researchers (Cleveland *et al.* 1989; Kirk 1996). The dinoflagellate *Prorocentrum triestinum* showed a similar ϕ_m trend over the 5 h period decreasing from the initial 0.09 mol C (µmol quanta m^{-2} s^{-1}) at low light to 0.06 mol C (µmol quanta m^{-2} s^{-1}) at 2 h during high light treatment (Figure 5.13) after which the value increased to 0.08 mol C (µmol quanta m^{-2} s^{-1}) during low light. *Prorocentrum triestinum* seemed to recover in high light over the 5 h time period to the initial value as was also the case in *Chaetoceros capense*. The overall mean for the diatom *Chaetoceros capense* over the 5 h time series was slightly higher than was recorded for the dinoflagellate *Prorocentrum triestinum* (0.09 and 0.08 mol C (µmol quanta m^{-2} s^{-}

^{1}) respectively (Figure 5.13). Langdon (1988) in his review indicated a similar trend with higher values of ϕ_m for diatoms compared to dinoflagellates. The values of ϕ_m obtained during the previous experiments with cultures of diatom and dinoflagellate species compared to the time series results were in some instances higher (dinoflagellates ranged from 0.02 – 0.4 and diatoms 0.05 – 0.35) (see figure 3.9(a), Chapter 3 and figure 4.12, Chapter 4).

Schofield *et al.* (1996) mention that different concentrations of the photosynthetic carotenoid fucoxanthin structurally bound to Chl *a* – proteins and other photoprotectant pigments (e.g. peridinin) may affect the individual cells' wavelengths dependency and therefore ϕ_m. High concentrations of these pigments are found to reduce the photosynthetic quantum yields (Marra *et al.* 2000). This tendency was evident from the pigment data of the two cultures which followed a similar pattern during the time series. The reduction in pigment concentrations (see Figure 5.8 (a)) shows a corresponding reduction in ϕ_m at high light and *vice versa* (Figure 5.13). It was noted that the maximum quantum yields for the dinoflagellate and diatom species were fairly similar ranging between 0.08 – 0.10 and 0.06 – 0.09 mol C (µmol quanta m^{-2} s^{-1}) respectively. At the end of the time series the ϕ_m value for the diatom and the dinoflagellate species was slightly higher than the initial value, and was still rising, which seemed to indicate that in both species effective photoacclimation responses had taken place during the 0.5 and 1 h intervals. The pigment quota per cell, the state of the culture and the efficiency of absorption of available irradiance are some of the important factors influencing the efficiency of the photosynthetic apparatus. Many factors are therefore the cause of variability in the values of ϕ_m in diatoms and dinoflagellates at the different light intensities.

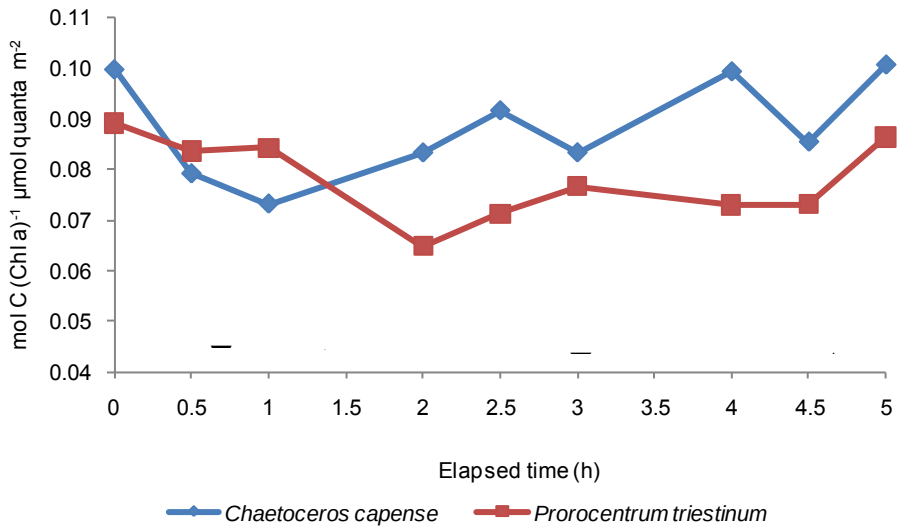

Figure 5.13: Comparison of estimated maximum quantum (ϕ_m) yield of the two phytoplankton species, *Chaetoceros capense* (diatom) and *Prorocentrum triestinum* (dinoflagellate) during transient irradiances over a period of 5 h. (Symbol ▼ indicates low light, ▲ indicates high light environment).

5.8 Carbon to chlorophyll *a* ratio

Figure 5.14 shows that the total carbon (TC, organic + inorganic carbon), nitrogen (N) and chlorophyll *a* (Chl *a*) contents for the diatom and dinoflagellate cultures, when shifted from low to high irradiances and *vice versa* during a 5 h time series, fluctuated minimally in both species (Appendix 5.10). In the diatom *Chaetoceros capense* the total carbon content remained fairly stable, showing an increased content by 0.37 mg L^{-1} after 5 h. The nitrogen content remained virtually steady throughout and paralleled the nitrogen content in the dinoflagellate. The total carbon content for *Prorocentrum triestinum* showed greater fluctuations increasing in high light, particularly between 1.0 and 2.5 h. The increase here approximately doubled and was greater than in the diatom culture (1.35 mg L^{-1}) which is in agreement with other reported findings (Figure 5.14).

Figure 5.15 shows that, as noted in Chapters 3 and 4, the diatom species showed a lower C to Chl *a* ratio than dinoflagellate species. This is in agreement with other reported findings (Geider *et al.* 1996). The ratio (expressed as a percentage) of total carbon to total chlorophyll *a* fluctuated marginally in the diatom showing a decrease within the first 0.5 h during low light treatment from 0.6 – 0.49% and after 2 h the ratio showed an increase to 0.56% in high light. Thereafter the ratio fluctuated minimally between 0.60 to 0.52% in low light and 0.55 to 0.53% in high light. The T Chl *a* to nitrogen ratio for *C. capense* followed a similar pattern ranging from 2.5 to 2.9 in low light and 2.4 to 2.8 in high light (Appendix 5.11). The ratios of TC : Chl *a* in *Prorocentrum triestinum* was approximately similar to that of the diatom. The exception was the N : Chl *a* ratio which showed an initial increase in low light in the first hour similar to that of the absorption ratios which reflects the increases noted in Chl *a* concentration during low irradiance levels (see Figure 5.12).

Taylor *et al.* (1997) are of the opinion that irradiance levels are the major factors regulating cellular C : Chl ratios. Prézelin (1976) also refers to increases in irradiance causing an increase in C : Chl *a* ratio as was observed in both the diatom and dinoflagellate cultures in this study (Appendix 5.11). Differences in C to Chl *a* ratios are thought to relate to cell structure as diatoms generally have large centrally situated vacuoles which can take up to 60% of the cell's total volume. Dinoflagellates on the other hand, have much smaller vacuoles which account for only 10% of total cell volume. This would explain the higher TC per cell data recorded for the dinoflagellate species used, compared to the diatom cultures, as diatoms would have a lower biomass to cell volume ratios. Chan (1980) states that diatoms have a higher photosynthetic capacity to biomass ratios than dinoflagellates. This is related to the diatom's higher chlorophyll *a* to cell protein ratio in comparison to dinoflagellates. Generally, under favourable conditions, diatoms will have a greater growth rate then dinoflagellates. It is expected therefore that they will outcompete dinoflagellates in their preferred environment. The ratio C : N indicates the physiological state of the phytoplankton cultures (Kirk

1996). The ratio in both the diatom and dinoflagellate cultures decreased initially by 2% when transferred to a high light environment (Appendix 5.12). The ratio fluctuated in both species by < 2%.

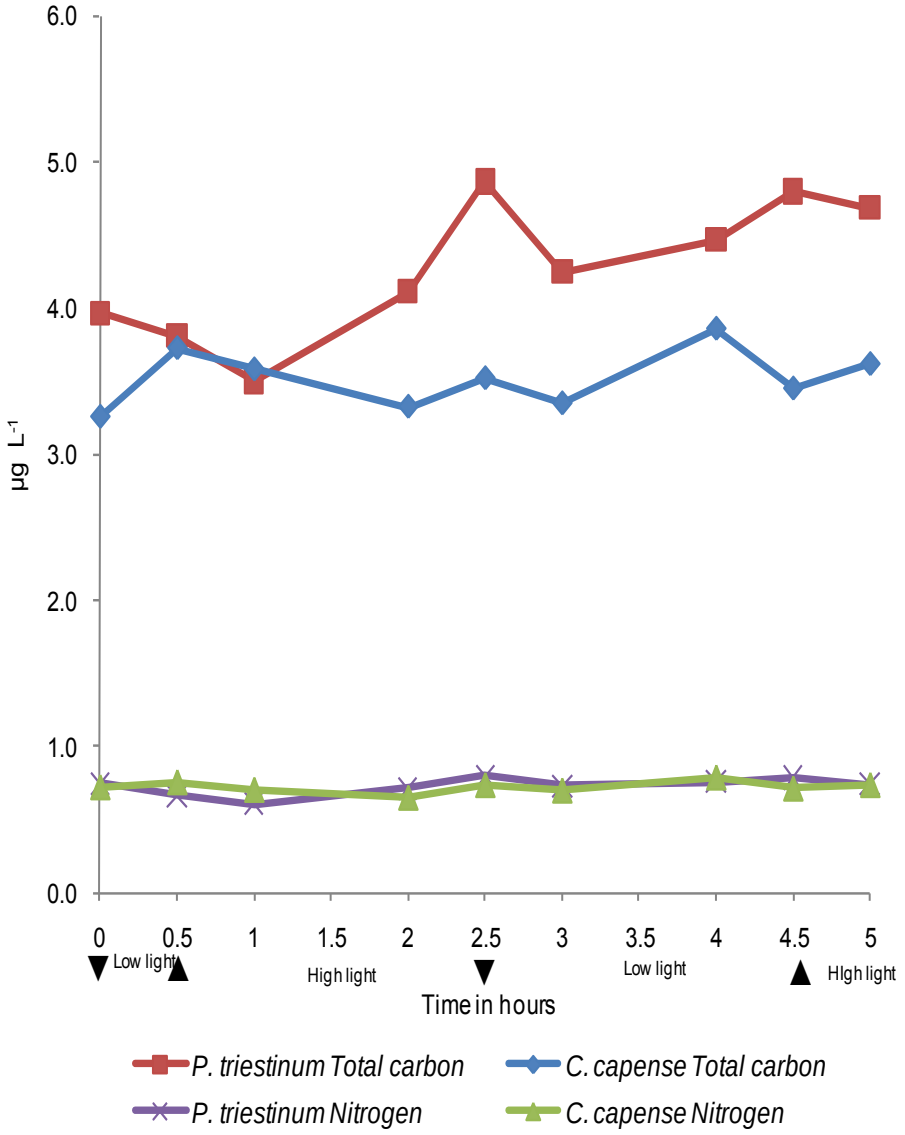

Figure 5.14: Comparison of changes taking place in total carbon and nitrogen concentrations in the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* during transient light conditions. (Symbol ▼ indicates low light and ▲ indicates high light environment).

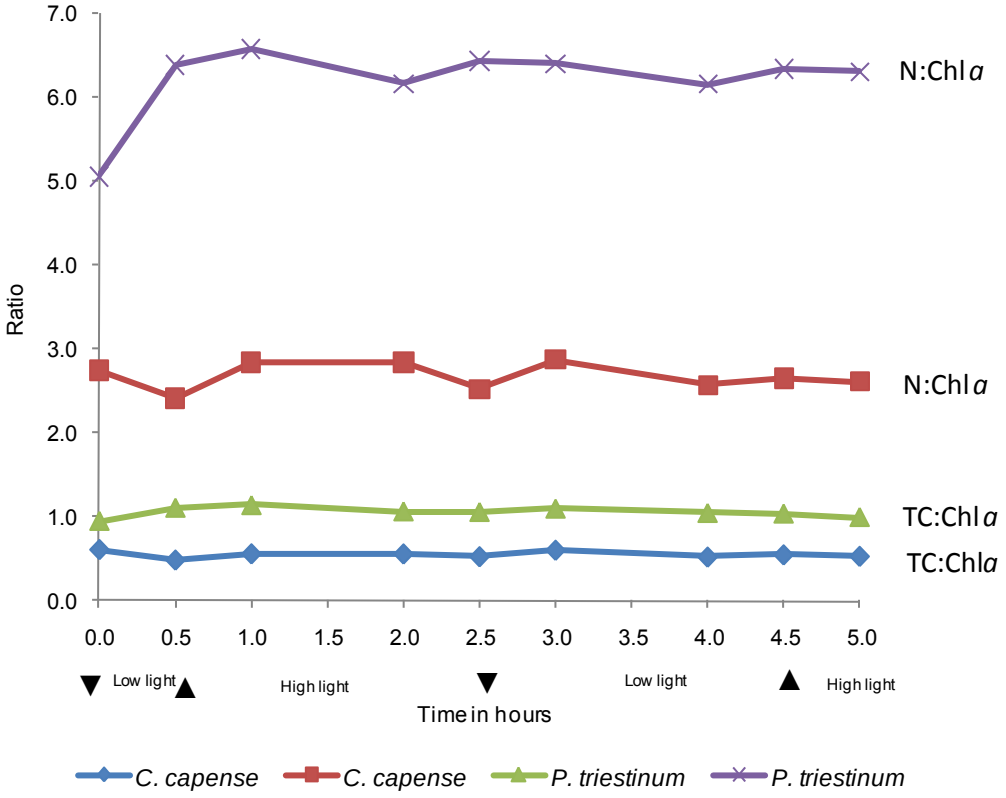

Figure 5.15: Ratios expressed as a percentage of total carbon to chlorophyll *a* (fluorometrically measured) and nitrogen to chlorophyll *a* in *Chaetoceros capense* and *Prorocentrum triestinum* during transient light conditions. (Symbol ▼ indicates low light and ▲ indicates high light).

5.9 Summary

Short-term changes in the photosynthetic mechanism as a result of photoacclimation responses to light intensity were investigated during this study in two different species belonging to two different groups of phytoplankton, *i.e. Chaetoceros capense* (diatom) and *Prorocentrum triestinum* (dinoflagellate). Two processes are involved in regulating the photosynthetic reactions which include maintaining maximum electron flow through photosystem II, the light harvesting complex II state transitions and photosystem II repair cycle. (These are preconditions to a subsequent long term mechanism of photoacclimation). The short-term changes in the cultures of the two species in the time trials showed species-specific but small adaptive trends. Usually changes were noted in the first 0.5 h of exposure to low light. Of particular interest in the diatom was the increase in total Chl *a* concentration, in P^*_m values which stabilized after 2.5 h, the α* value and E_k values which only showed an increase after 4 h, that is 2 h in low light and 2 h in high light. The fluctuating diadinoxanthin and diatoxanthin ratios with the xanthophyll pool remaining almost

constant throughout. *Chaetoceros capense* showed an effective adaptation to variable light conditions and seemed to acclimate well. These trends also noted in Chapter 4, were typical of this group's capacity to photoacclimate to variable light conditions and represent an ecological adaptation to a mixing environment. The measured photosynthetic parameters also showed that the diatoms was more adapted to high light than to low light as they had an increased photosynthetic rate in a high light environment (P^*_m – range 9.0 (LL) to 13.4 (HL) mg C m^{-3} (mg $Chla^{-1}$) h^{-1} during the course of the light shift experiment.

The dinoflagellate, on the other hand, was generally more adapted to low light and photosynthesised more efficiently at lower irradiances (P^*_m – range 7.3 (LL) to 8.5 mg C m^{-3} (mg $Chla^{-1}$) h^{-1} (HL)) with the highest photosynthetic rate at 3 h at low light of 8.1 mg C m^{-3} (mg $Chla^{-1}$) h^{-1} (Table 5.1). Interestingly *Prorocentrum triestinum* showed an initial decline in P^*_m value at each transfer to a different light level ranging from 6 to 21% whilst the diatom *Chaetoceros capense* was able to handle light transfers more efficiently with a 2.5 to 6% decline in P^*_m values.

The rate of change in the dinoflagellate seemed to be slower. For instance, increases in pigment content only took place after 2 h. P^*_m values increased only slightly when in a low light environment. E_k values dipped sharply after 2.5 h. The slower adaptation noted in this group possibly relates to a behavioural strategy since migrators can regulate their depths to an optimum irradiance level by swimming to a particular niche preference in the water column, albeit only during stratified conditions.

Significant changes were observed for both species in their cellular pigment composition as well as in their Chl *a*-specific light absorption ($\alpha^*(\lambda)$). Both species photoacclimated to increasing irradiance by reducing their intracellular Chl *a* content and increasing their carotenoid : Chl *a* ratio resulting in a higher Chl *a*-specific absorption coefficient (α^*). Significant interspecies differences were apparent which were probably due to the differences in cell size/volume, which co-varied with pigment composition. Staehr *et al*. (2002) reports that cell size has a greater influence on the variability in absorption coefficients than the changing pigment composition due to prevailing light conditions.

Interestingly a difference was noted in the photosynthetic pigments (PSP) to Chl *a* ratios in the two species. In the diatom the ratio decreased from the initial 42% over the 5 h duration of the time series to 39%, the lowest ratio (36%) was reached in low light at 4 h. The exact reverse was noted in the Chl *a* to photoprotective pigments (PPC) ratios (Figure 5.7). In the dinoflagellate the PSP to Chl *a* ratios increased from the initial 26 to 41%, the highest being at 4 h with the opposite taking place in the Chl *a* to PPC ratios. In both species the ratios for total pigments to PSP and PSP to PPC remained fairly constant throughout the experiment indicating that the pigment pool did not vary significantly but the individual pigments show a definite photoacclimation responds to changing light intensities. The 4 h period seems to be a significant time frame in respect of photoacclimation responses of α^* and E_k as well as pigment adjustments in both species.

The results in this study point to a species-specific trend and suggest that the diatom *Chaetoceros capense* is capable of making more effective use of high light conditions compared to the dinoflagellate *Prorocentrum triestinum.* The absorption values in both species generally increased on transfer from low light to high light, decreasing from transfer from high light to low light at all wavelengths. However, in the case of the diatom the increases were approximately 34% whilst in the dinoflagellate the increase was significantly lower at approximately 25%. The rate of photoacclimation in the PSPCs seems to be faster in the smaller celled *Prorocentrum triestinum* than in the larger celled *Chaetoceros capense* although the initial increased level of concentration, unlike that of *Chaetoceros capense*, is not maintained after 0.5 h as indicated in Figure 5.9 where a decline takes place between 2.5 to 3 h with a small increase over a period of 1 h (up to 4 h). The PSCs in *Prorocentrum triestinum* vary in unison but in *Chaetoceros capense* this is not the case with Chl *a* declining at 4 h where fucoxanthin still showed an increase.

Similarly the Chl *a*-specific absorption values decreased on transfer from high light to low light in both the diatom and dinoflagellate. This variation of the total absorption with wavelengths in the two species gives an insight into the evolutionary adaptation and ecological niche preferences of the diatom compared to the dinoflagellate.

The value of maximum quantum yields ($\phi_{m)}$ fluctuated between 0.07 in a high light environment to 0.10 mol C (µmol quanta m^{-2} s^{-1}) in low light. This trend of reduced ϕ_m occurring at high irradiances and increasing in low light has been reported by other researchers (Cleveland *et al.* 1989, Kirk 1996). The dinoflagellate *Prorocentrum triestinum* showed a similar ϕ_m trend (see Figure 5.13). Langdon (1988) in his review indicated a similar trend with higher values of ϕ_m for diatoms compared to dinoflagellates. High concentrations of PPCs are found to reduce the photosynthetic quantum yields (Marra *et al.* 2000). This tendency of decreasing pigment concentrations in high light was evident from the pigment data of the two cultures which followed a similar pattern during the time series. The reduction in pigment concentrations showed a corresponding reduction in ϕ_m at high light and *vice versa* (Appendix 5.12).

It was apparent that in the diatom the C to Chl *a* ratio was lower than in the dinoflagellate. This is in agreement with other reported findings (Geider *et al.* 1996). Irradiance level is the major factor regulating cellular C : Chl ratios. Prézelin (1976) also refers to increases in irradiance causing an increase in C : Chl *a* ratio as was observed in both the diatom and dinoflagellate cultures in this study. Differences in C to Chl *a* ratios of different species are thought to relate to cell structure. The protoplast in the diatom cell is contained within a rigid siliceous cell wall (frustule) unlike the dinoflagellates. The frustule allows the diatoms to have large centrally situated vacuoles which can take up to 61% of the cell's total volume. Dinoflagellates on the other hand, have much smaller vacuoles which account for only 10% of total cell volume (Round *et al.* 2000).

CHAPTER 6. COMPARISON AND EVALUATION OF PHOTO ADAPTIVE RESPONSES

6.1 Physiological acclimation

Photoacclimation is a universal feature of algal physiology. The success of phytoplankton in a marine environment depends on achieving a positive balance between cell growth/division and cell death. Photoacclimation enhances cell division and therefore contributes to this balance.

In their natural environment, phytoplankton are often exposed to fluctuations in incident irradiance due to vertical displacements in the water column induced by turbulent fluid motion. The processes of physiological acclimation to these fluctuations result in variation in a number of measurable quantities which can be expressed at time scales from seconds to days. In the light shift experiments in this study the shortest period of time in which measurable changes took place were noted at half hourly and hourly intervals over a 5 h period.

Variation in the parameters of the photoacclimation responses in the cultures of the five species of dinoflagellates (*Alexandrium catenella, Protoceratium reticulatum, Prorocentrum micans, Prorocentrum triestinum, Gymnodinium zeta*) and three diatom species (*Chaetoceros* sp., *Chaetoceros capense, Chaetoceros* cf. *pendulus*) was found to be significant. In the long-term photoacclimation experiments described in Chapters 3 and 4, definite differences within the two groups, diatoms and dinoflagellates, and significant species-specific trends were revealed. These are supported by statistical analyses with respect to the parameters of *P versus E* curves (P^*_m, α* and E_k), chemical composition (C and N), photosynthetic capacity, pigment ratios, maximum quantum yield and carbon to chlorophyll ratio. No photoinhibition was apparent in either of the two groups of phytoplankton under the experimental conditions of this study. This may be partly due to factors such as the length of time the monocultures were previously acclimated to low, medium and high light intensities before experimentation and the short exposure time to relatively low irradiances during the incubation experiments. The short 20 min incubations may be too short for photoinhibition changes to become apparent. The highest light intensity in the photosynthetron was approximately 1212 µmol quanta m^{-2} s^{-1} which is lower than sea surface irradiances reported in the Benguela Ecosystem during late summer (approximately PAR 1500 µmol m^{-2} s^{-1}) (Mitchell-Innes *et al.* 2000). For diatoms saturation light intensities were noted to be < 1000 µmol m^{-2} s^{-1} and < 750 µmol m^{-2} s^{-1} for dinoflagellates. In both groups an active xanthophyll cycle, represented by photoprotective pigments diatoxanthin and diadinoxanthin, was noted which would have prevented photo-damage to occur in such a short exposure time to a high light environment.

6.2 Chlorophyll *a* concentrations

In the representative species of the two groups of phytoplankton, dinoflagellates (class Dinophyceae) (Chapter 3) and diatoms (class Bacillariophyceae) (Chapter 4), the total chlorophyll *a* concentrations measured by fluorometry showed a steady increase during low light treatments decreasing at high irradiances. This is in agreement with other published findings. The percentage decrease from low light to high light was less in the dinoflagellate species (up to 29%) compared with the diatom species (up to 34%). The total chlorophyll *a* (T. Chl *a*) concentrations corresponded well to cell size as they varied systematically with cell volume. The changes in the Chl *a* cell quota decreased similarly from low to high light in all species ranging from 60 to 63% in the dinoflagellate and diatom, respectively. Generally, however, the increases and decreases are smaller in large-celled species compared to small-celled species. The differences in Chl *a* cell quota are not only reflected in the cell volumes but are also dependent on the species' shape, packaging effect (particularly the effect on absorption (Stuart *et al.* 1998) and other morphological differences as pointed out by Morel and Bricaud 1981, Kirk (1996) and others.

A comparison of total Chl *a* concentrations per cell volume of the five dinoflagellate species corresponded well to the estimated cell volume of the species used as the Chl *a* concentration varied systematically with cell volume (Chapter 3, Figure 3.0 (a)). The smallest-celled *Gymnodinium* cf. *zeta* showed the highest concentration of Chl *a* at all light levels increasing from LL to HL by 32%. The largest-celled *Prorocentrum micans* had the least concentration of Chl *a* in relation to cell volume showing a smaller increase from LL to HL of 6%.

In the diatoms the relationship between Chl *a* and cell volume did not seem to be as sensitive. The distinction was not as apparent as observed in the dinoflagellates. The Chl *a* concentrations per cell volume at LL and ML in two species (*Chaetoceros* sp. and *C. pendulus*) were similar and contrary to expectations the *Chaetoceros* spp. showed a decrease in the Chl *a* concentration from LL to HL by > 2% with the exception of *C. capense* which showed a decrease from LL to HL by 34% (Chapter 4, Figure 4.0 (b). The difference may be attributable to the fact that *Chaetoceros* sp. and *C. capense* are both chain-forming species unlike *C. pendulus* which is single-celled.

6.3 Photosynthetic parameters of *P versus E* curves

The photosynthesis *versus* irradiance (*P vs E*) data resulting from the experiments described in this thesis show that the physiological acclimation of the photosynthetic apparatus, during short (20 min) incubations and exposures to different light intensities, were species-specific (Chapter 3 and 4).

The changes during short-term photoacclimation varied, albeit marginally, between the diatom and dinoflagellate species as recorded in the light shift experiments (Chapter 5). For instance changes in the photosynthetic parameters, P^*_m and α^*, were noted in the first 0.5 h in both the diatom and dinoflagellate peaking much earlier in the dinoflagellate *Prorocentrum triestinum* (after 2 h) than in the diatom

Chaetoceros capense (2 h) (Table 6.1). Changes in the pigment concentrations were apparent within the first 0.5 h in both diatom and dinoflagellate species.

In the dinoflagellates the mean light utilisation coefficient, α*, was highest at low light in all species of dinoflagellates, decreasing in high light. *Alexandrium catenella* recorded the highest value of 0.05 mg C m^{-3} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$ at low light (Chapter 3). The reverse was noted for the diatom species where the highest value for α* were observed in high light conditions with *Chaetoceros capense* recording the highest value (0.04 mg C m^{-3} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$) at high light (Chapter 4). The data of the diatom species possibly indicates a trend to increased efficiency in utilising light at higher intensities compared to the dinoflagellate species. This was particularly noted in *Chaetoceros capense* where the value of α* virtually doubled from low light (0.02 mg C m^{-3} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$) to high light (0.04 mg C m^{-3} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$). Overall the different responses to changing light levels ranged from 0.05 in low light to 0.01 mg C m^{-3} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$ in high light in the dinoflagellate species and from 0.02 to 0.04 mg C m^{-3} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$ in the diatoms which was the opposite.

Table 6.1: Comparison of photosynthetic parameters, normalised to fluorometrically measured Chl *a*, between the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* after transfer from low to high irradiance levels and *vice versa* at 0.5 h and 1 h intervals during short-term photoacclimation experiments.

	Sample		Time (h)	Maximum photosynthetic Rate (mg C (mg Chl *a*$^{-1}$) m^{-3} hr^{-1}) P^*_m	Maximum light utilization Coefficient (mg C m^{-3} (mg Chl *a*$^{-1}$) µmol quanta m^{-2} s^{-1}) α*	Light Saturation Parameter (µmol quanta m^{-2} s^{-1}) E_k
Chaetoceros capense	1	Low light	0	9.02	0.040	223
	2	High light	0.5	9.29	0.031	297
	3	High light	1	9.31	0.031	297
	4	High light	2	10.51	0.039	272
	5	Low light	2.5	11.18	0.041	271
	6	Low light	3	9.35	0.036	259
	7	Low light	4	11.65	0.045	260
	8	High light	4.5	11.98	0.038	313
	9	High light	5	13.45	0.042	320
Prorocentrum triestinum	1	Low light	0	7.71	0.033	236
	2	High light	0.5	7.25	0.029	249
	3	High light	1	8.41	0.031	274
	4	High light	2	8.45	0.024	347
	5	Low light	2.5	6.73	0.021	324
	6	Low light	3	8.07	0.026	309
	7	Low light	4	7.21	0.025	285
	8	High light	4.5	6.72	0.024	279
	9	High light	5	8.30	0.032	259

The inter-specific and intra-specific variations in the maximum photosynthetic rate normalised to fluorometrically measured chlorophyll *a* (P^*_m) in the dinoflagellates were influenced by the light absorption properties of the pigment complexes of the algae. The P^*_m values at different irradiance levels were not significantly different statistically in any of the dinoflagellate species. Generally P^*_m decreased during high light conditions. *Alexandrium catenella* proved to be the most efficient light harvesting species of the dinoflagellates tested at all light levels with a mean value range of 9.3 to 5.8 mg C (mg Chl *a*)$^{-1}$ h^{-1} followed by *Protoceratium reticulatum* (range 7.4 to 5.6 mg C (mg Chl *a*)$^{-1}$ h^{-1}). The smallest-celled species, *Gymnodinium zeta* recorded the lowest photosynthetic rate (range from high to low light 2.0 to 1.7 mg C m^{-3} (mg Chl *a*) $^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$) (Chapter 3).

Of the diatoms, *Chaetoceros capense* reached the highest maximum photosynthetic rate (range from low to high light was 4.0 to 13.1 mg C (mg Chl *a*)$^{-1}$ h^{-1}), showing a three-fold increase. The P^*_m value of this smallest-celled, chain-forming diatom used in the experiments, *Chaetoceros capense*, was approximately twice as high as that of the single cell dinoflagellate *Prorocentrum triestinum* 5.1 (low light) to 5.2 mg C (mg Chl *a*)$^{-1}$ h^{-1} (high light) illustrating the diatom's increased efficiency in light harvesting capacity compared to the dinoflagellate (Chapter 4). This was also evident in the light shift experiment where *Chaetoceros capense*, after the first 0.5 h, demonstrated a greater and almost continuous increase in P^*_m values with a corresponding increase in E_k (Table 6.1). The decreases of the P^*_m values were minimal and generally seem to take place in low light.

The mean value of the parameter corresponding to the onset of light saturation of photosynthesis, E_k, for all five dinoflagellate species gradually increased when exposed to low, medium and high light levels. *Prorocentrum triestinum* at its maximum value was 388 µmol quanta m^{-2} s^{-1}. *Prorocentrum micans* recorded the lowest value at medium light of 124 µmol quanta m^{-2} s^{-1}. Similarly the mean light saturation rates for all three diatom species increased with increasing irradiances (range 117 at low light to 321 µmol quanta m^{-2} s^{-1}) at high light. Generally this rise correlated to a greater increase in P^*_m value and a lesser increase in α*. In both the dinoflagellates and diatom species different irradiance levels have a significant effect on the E_k values ($p = 0.006$). Also important is the effect of cell size particularly on the values of E_k and P^*_m. Small-celled diatom and dinoflagellate species showed the highest values at medium and high light for both E_k and P^*_m parameters for the culture samples used in the experiments. The largest species, *Chaetoceros* sp. and *Prorocentrum micans*, showed the lowest mean values of E_k (117 (low light) to 182 µmol quanta m^{-2} s^{-1} (high light) and 163 (low light) to 180 µmol quanta m^{-2} s^{-1} (high light) respectively) clearly illustrating the effect of cell size on photosynthetic parameters. In this study, when the cultures were grown in a nutrient-sufficient environment, it has been possible to give support to the hypothesis that small cell size is advantageous for phytoplankton living in a turbulent environment (Montecino and Quiroz 2000; Montecino *et al.* 1996).

A great deal of species-specific variation is ascribable to cell shape, structure and physiological history. The intrinsic variations in P^*_m and α*, and therefore the value of E_k, in this data set, seem to reflect the

differences in cell volume of the various species used in my experiments. This assumption is well supported at medium and high light with respect to cell size (Figure 3.2 (c) and Figure 4.2 (c)) and demonstrates that small-celled species have a physiological advantage over large-celled species resulting from the combined effects of a group of variables. For instance, with an increased area to volume ratio, a small cell has an increased light absorption efficiency and reduced intracellular shading from a lower pigment concentration. A small cell also has a thinner diffusion boundary layer which aids the fluxes of solutes (nutrients) between cell surface and cell contents, important for cells in limited nutrient conditions in the water column. Cell size also affects sinking speed and the energy cost of flagellar motility (Raven and Kübler 2002). These attributes aid small dinoflagellates to photosynthesise efficiently at lower light levels in the water column. The effect of cell size on E_k is also mentioned by Tilzer (1989), as well as the changes in chlorophyll *a* concentrations, due to photoacclimation, as discussed by Tilzer *et al.* (1993), Platt *et al.* (1992), Prézelin *et al.* (1991) and others. The photosynthetic parameters indicated that generally the dinoflagellates photosynthesise most efficiently in a low light environment, whilst diatoms are most efficient at high irradiances. Overall the data illustrate that relative growth rate and biomass-specific photosynthetic rates increase with decreasing cell size.

A decrease from P^*_m at high light, *i.e.* photoinhibition, which usually occurs after light saturation of photosynthesis, was not observed under high irradiance conditions in any of the dinoflagellate and diatom species. This may be due to the relatively low light levels available in the photosynthetron (< 1200 µmol quanta m^{-3} hr^{-1}) as compared to the natural environment at latitude 34° S and possibly to the short 20 min incubation time.

Comparing the photosynthetic parameters of dinoflagellate and diatom species with natural populations in the southern part of the Benguela Ecosystem similar values for P_m*, α* and E_k were recorded for inshore stations 1 and 2 and offshore stations 7 and 8 (Balarin 2000, (author) unpublished data). The *P vs E* relationships were investigated by means of the classic ^{14}C technique using the photosynthetron (Figure 2.1). Surface samples were taken along an offshore transect consisting of 12 to 15 stations covering a maximum distance of 221 km (depth approximately 200 m) from the coast (Appendix 3.4 (b)). On board incubations were terminated after 20 min as was done with the laboratory culture samples. Variations in the P_m* values recorded for cruises Algoa 79 and 83 showed a definitive trend which could be correlated with the distance offshore where the samples were taken. Low values were recorded close to shore, increasing midway over the shelf and decreasing rapidly in the open ocean beyond the shelf edge (200 m isobath). For Cruise 79 the values for inshore, midway and open ocean were 5.05 , 18.56 and 4.49 mg C (mg Chl *a*)$^{-1}$ h^{-1}) respectively and for Cruise 83 the values were 3.06 , 20.32 and 0.55 mg C (mg Chl *a*)$^{-1}$ h^{-1}) (Appendix 3.4 (b)). The high values at the midway stations (approximately 100 km offshore) corresponded with high total chlorophyll *a* (T. Chl *a*): total pigment (T. pig.) readings. In this region the ratio of T. Chl *a* to T. pig ranged between 0.6 to 0.8. The mean values for P_m* for both cruises were virtually identical (8.9 and 9.1 mg C mg Chl *a*)$^{-1}$ h^{-1}).

The α* parameters of the natural phytoplankton populations (range 0.007 to 0.115, mean 0.032 to 0.041 mg C (mg Chl *a*)$^{-1}$ h^{-1}) corresponded well with the values observed in the laboratory cultures of the dinoflagellates and diatoms species (range 0.006 to 0.103, mean 0.005 to 0.048 mg C (mg Chl *a*)$^{-1}$ h^{-1}) particularly in the inshore and far offshore stations.

The values of the parameter E_k were in a similar range as recorded for the cultured species and followed a similar trend as mentioned above with high values for the mid stations 4 and 5 during August 2000.

An investigation conducted during February/March 1996 at the Lambert's Bay monitoring stations at the west coast of South Africa produced a similar range of photosynthetic parameters from *P vs E* studies for surface samples as mentioned in my study above (Mitchell-Innes *et al.* (2000)). Interestingly the highest values for P_m* were recorded for samples collected at approximately midday between 12.5 and 14.5 h (range 1.86 to 5.69 mg C Chl^{-1} h^{-1}), which demonstrates the cyclic and temporal nature of photosynthesis. The α* values ranged from 0.014 to 0.025 mg C Chl^{-1} h^{-1} (μmol m^{-2} s^{-1})$^{-1}$. Calculated values for E_k ranged from 102 to 285 μmol m^{-2} s^{-1}. Thus overall the photosynthetic parameters observed in natural assemblages at sea agree well with the laboratory studies of cell cultures used in this thesis.

6.4 Pigment composition

The pigment concentration, the photosynthetic pigments (PSP) and photoprotective carotenoids (PPC), changed under different light intensities demonstrating a definite species-specific trend in all cultures of dinoflagellates and diatoms, the magnitude of change being related to the specific species concerned. Generally the PSP concentrations decreased with increasing light intensities and increased under low light conditions. The increase in PSP from LL to HL as much as 58% was observed in the pigment concentration in the dinoflagellate *Protoceratium reticulatum.* Similar increases in cultures grown under LL and HL conditions were noted in all three *Chaetoceros* species (diatoms) ranging from 20 to 40%. Although the PSP concentrations in the dinoflagellates showed similar trends to those found in the diatom species, the increases and decreases were greater and showed more variation in the diatom species, where the changes were of a similar order of magnitude, implying a closer relationship among the diatom species, or a smaller physiological need. At cellular level the Chl *a* quota showed a slight decline from low to high light of up to 34%. This is in agreement with models that describe photoacclimation of cell pigment content in terms of a dynamic energy balance (Kana *et al.* 1997). On the other hand, the photosynthetic pigments, peridinin and fucoxanthin, showed a similar but lesser decline in concentration by 10% from low to high light, compared with Chl *a*. The genus *Prorocentrum* demonstrated a definite decline in peridinin at high light (range 58 to 73%) which was coupled with a substantial decline in the photoprotective pigment diadinoxanthin from low to high light which was not paralleled in the other three dinoflagellate species. Thus PSP concentrations in both groups, diatoms and dinoflagellates, generally increase in low light and decrease in high light environments. In this study, however, the responses to light intensities did not always follow this

pattern as exceptions were noted *i.e.* in the genus *Prorocentrum*. The amount of change were also shown to be particularly species-specific and were, to a great extend, seen to be size related.

The PPC concentrations generally increased with increased light levels and *vice versa* in both dinoflagellate and diatom species, diadinoxanthin being the most important photo-protective pigment present (Chapters 3 and 4). The percent diadinoxanthin (a photoprotective carotenoid) to total carotenoids present in dinoflagellate species is, however, less than was observed in the diatom species demonstrating the diatoms' physiological adaptation to survive in higher light irradiances in the sea surface layers. The percent diadinoxanthin of dinoflagellates showed changes ranging from 7% at low light to 13% at high light levels compared to the diatom species' (10% (LL) to 52% (HL). Statistically, it is noted that this effect was related to light level in the case of diatoms ($p < 0.05$). In the dinoflagellates the different responses of the different species to changing light intensities were significant ($p < 0.05$). A comparison of pigment concentrations in the different species of diatoms and dinoflagellates illustrated clearly that the PSPs generally decreased at high light with a corresponding increase in the PPCs (Chapter 3 and 4). In the photosynthetic pigment analysis of the diatom and dinoflagellate species it was demonstrated that the pigment pool did not change appreciably but the individual pigments within the cell did either increase or decrease depending on the light intensity the cultures were exposed to. Pigment concentrations per cell gave a clearer indication of the pigment concentration changes in the individual cells.

Diadinoxanthin, the most important photoprotective pigment present (up to 27% of total carotenoids in dinoflagellates and up to 52% of total carotenoids in diatoms, showed a significant increase at high light in most species. In contrast a considerable decrease from low light to high light in the diadinoxanthin concentration was noted in the genus *Prorocentrum*, represented by the species *Prorocentrum triestinum* and *Prorocentrum micans*, by 56 and 49% respectively, with Chl *a* cell quotas decreasing by a similar percentage (up to 50%). This seemed to be a characteristic of these species. The concentrations of the photoprotective pigments (PPC), diadinoxanthin (Diad) and diatoxanthin (Diat) varied in their response to light levels showing an increase of 0.026 μg L^{-1} h^{-1} in the diatom and a decrease of 0.19 μg L^{-1} h^{-1} in the dinoflagellate over the duration of the light-shift experiment. Generally when diadinoxanthin showed an increase, diatoxanthin decreased in tandem and *vice versa*. In few instances both PPCs may increase together as was shown in the light-shift experiment (Chapter 5). This type of response as seen, depends a great deal on the intra-cellular ratio of diadinoxanthin and diatoxanthin present at the time since the (de)-epoxidase fine-tunes the Diad : Diat ratio continually (Beer *et al.* 2006).

6.5 Absorption spectra

The differences in the absorption spectra, measured in a spectrophotometer, are characteristic of the different species of dinoflagellates and diatoms. The variations and magnitude of the photoacclimation responses to irradiance levels are a reflection of the packaging effect which is related to their cell size, cell shape and intracellular concentration of pigments (Sathyendranath *et al*. 1987; Kirk 1994; Raven and

Kübler 2002; Bricaud *et al.* 2004). In this study the chlorophyll *a*-specific absorption coefficient a* (λ) at 675 nm showed a significant relationship with cell size in the diatom and dinoflagellate species at all three light levels decreasing significantly with increasing cell size. In the dinoflagellates the range recorded for the largest-celled (*Prorocentrum micans*) to the smallest-celled species (*Gymnodinium* cf. *zeta*) in low and high light was 0.033 to 0.034 and 0.039 to 0.048 m^{-2} (mg Chl *a*)$^{-1}$. For the diatoms *Chaetoceros* sp. and *Chaetoceros capense* the range of a* (λ) was 0.019 to 0.020 and 0.029 to 0.044 m^{-2} (mg Chl *a*)$^{-1}$ in low light and high light respectively. These data illustrated the reduced absorption coefficients in larger cells as compared to smaller-celled species and underlined the importance of cell size. At 440 nm the relationship between cell size and a* (λ) was apparent at all light levels. In the dinoflagellate species the relationship between cell size and Chl a-specific absorption coefficients showed more variation compared with the diatoms. Similar patterns were reported in literature indicating the size-dependence absorption characteristic of phytoplankton (Fujiki and Taguchi 2002; Bouman *et al*. 2003). In this study it was shown statistically that the differences in absorption were definitely species-specific ($p < 0.05$) (Chapter 3 and 4).

The shape and magnitude of the spectra of the different species are important and are dependent on the complements of photosynthetic carotenoids (PSC) and photoprotective carotenoids (PPC) present. Absorption was highest at wavelengths 436 – 443 nm and 672 – 676 nm, the optimum wavelengths being dependent on the species. A general trend, although mostly species-specific, was apparent in the absorption capabilities of the dinoflagellates and the diatom species increasing with higher light conditions, with a few exceptions. Although species showed a wide variation in absorption the results of this study corresponded with similar results observed for Chl *a*-specific absorption of phytoplankton at 440 nm (Hoepfner and Sathyendranath 1993) and at 440 and 675 nm (Maske and Haardt 1987). *Alexandrium catenella* was a typical example where the range from low to high light (0.017 to 0.024 m^{-2} (mg Chl *a*)$^{-1}$) fell close within the range of the theoretical maximum of 0.024 m^{-2} (mg Chl *a*)$^{-1}$ at 675 nm as mentioned by Kirk (1996) and Nelson *et al.* (1993).

The ratios of Chl *a*-specific absorption at 440 to that at 675 nm (range of mean ratios 1.3 to 1.9) indicated highest ratios at high light in the diatom species. In the dinoflagellates (range 1.53 (LL) to 2.36 (HL) the ratios were greater also increasing in high light. An exception was *Prorocentrum micans* which had the highest ratio at low light (mean 1.76). Here again the Chl *a*-specific absorption coefficients ratios of 440:675 nm increases corresponded closely to cell size with the highest increases recorded in the smallest-celled species at all light levels.

Looking at the individual experimental data for each diatom species, it is evident that there is a marked difference between the diatoms and the dinoflagellates absorption at 440 nm, in the blue-green range of the spectrum, and at 675 nm in the red part of the spectrum. At the different light levels the disparity ranged from 51 – 88% in the diatom cultures and in the dinoflagellates from 34 – 73% indicating changes in the proportion of pigments of the cell. On the basis of theoretical explanation one might assume that with increasing depth and therefore increasing blue-green/red ratio in the underwater light field, the

photosynthetic carotenoids/Chl *a* ratio would ultimately increase to achieve a balanced excitation state of photosystem II and I to facilitate harvesting of all available quanta at light-limited depths. This shows that the dinoflagellates have a far greater absorption in the blue waveband than the red waveband compared to the diatoms, which explains the greater density of dinoflagellate cells with increasing depth (Chapter 3).

The observed response trends of the different species of dinoflagellates and diatoms, in this study suggest that the dinoflagellates as a group, are phylo-genetically adapted to the chromatic changes in the underwater light field in such a way that this group can harvest light efficiently at greater depths compared with the diatoms. Also the dinoflagellates generally being a motile group can adjust their vertical depths by migrating to their optimum light level in the water-column. Diatoms on the other hand do well at high irradiance levels as indicated by the three diatom species' photoacclimation responses to high light and the nature of their pigment complexes. This illustrates a more efficient light harvesting capability in the sea surface layers where they are subjected to constant turbulence, mixing and intermittent exposure to high light conditions.

6.6 Maximum quantum yield

The maximum quantum yield data were very variable for the five dinoflagellate and three diatom species. However, a definite trend emerged. The value of ϕ_m was also relatively low in this data set compared to the theoretical maximum value (0.112 to 0.125 mol C fixed mol^{-1} photons absorbed) (Chapter 3 and 4). The sensitivity of the species to the spectral quality of the incubation light source (tungsten-halogen lamp) (Kyewalyanga *et al.* 1997) could possibly be the cause as well as the species-specific concentrations of PSC and PPC pigments. This factor reduces not only the photosynthetic quantum yield (Marra 2000) but also other complex biochemical processes. The emission spectrum of the tungsten-halogen lamp used in the incubator is not neutral either and could be modified through absorption by the water samples and walls of the incubator bottles (Chapter 2). No correction factor, such as proposed by Kyewalyanga (1997) was used to correct for this bias introduced by the shape of the spectrum of the tungsten-halogen lamp (see Figure 2.2, Chapter 2).

The general trend noted in the dinoflagellate cultures was an increase in ϕ_m during low light treatment followed by a decrease at medium and high irradiances. The overall mean estimated maximum quantum yield for the dinoflagellate species during the light-shift experiment (Chapter 5) (ranged from 0.024 (LL) to 0.011 (HL) mol C (µmol quanta m^{-2} s^{1}). A similar trend was observed in the three diatom species, although not as clearly, where the mean estimated maximum quantum yield value for the three experiments was highest in cultures grown at low irradiance levels and decreasing at high irradiances. This corresponds to other reported findings (Senger and Fleischhacker 1978; Clevelands *et al.*1989; Cleveland and Perry 1994; Singsaas *et al.* 2001 and others).

The mean overall ϕ_m value for the diatoms ranged from 0.021 (LL) to 0.017 (HL) mol C (µmol quanta m^{-2} s^{1}) with the highest mean value recorded for *Chaetoceros pendulus* (0.036 mol C (µmol quanta m^{-2} s^{1}) grown in a low light environment (Chapter 4). In this study the recorded mean value of ϕ_m was generally higher in diatoms compared with dinoflagellates. There was no evidence of correlation to cell size. Statistical analysis indicated that there was a significant difference between the dinoflagellate and diatom species with their responses to changing light conditions with *Chaetoceros* spp. showing a greater efficiency with which the photosynthetic apparatus converts absorbed electromagnetic energy to chemical energy at different light levels. This may imply that diatoms, compared with dinoflagellates, are more suited to a rapid changing light environment to which they are intermittently exposed in the surface layers

6.7 Carbon to chlorophyll *a* ratio

Generally the dinoflagellate species showed a greater total carbon content to a similar sized diatom cells. Generally the total carbon content in the diatoms (mean total carbon content – 0.17 ng $cell^{-1}$) was lower than in the dinoflagellates (mean total carbon content – 4.0 ng $cell^{-1}$). This is in agreement with findings reported by Geider *et al*. (1996). The difference in the carbon content can partly be attributed to morphological aspects of the diatoms such as the silica outer cell wall (frustule) and a large cell vacuole for *i.e*. buoyancy control (Round *et al.* 2000).

The general trend showed that the mean ratio of TC : TChl *a* increased from low light (LL) to high light (HL) in the five dinoflagellate species (mean TC : TChl *a* range 39 (LL) to 346 (HL)) with a corresponding decrease in TChl *a* content. The cultures of the diatom species showed a similar trend with an overall mean increase (range 65 (LL) to 347 (HL)) with increasing light. The increases noted ranged from 25 to 38% in dinoflagellates and 35 to 46% in the diatom species. In some cases the ratio doubled as observed in the small-celled *Gymnodinium zeta*. High ratios usually correlated to low Chl *a* L^{-1} concentrations. The ratios of total carbon to chlorophyll *a* were highest at high irradiances in the cultures of the larger-celled species *Alexandrium catenella* and *Protoceratium reticulatum* ranging up to 346 with a similar ratio at high light levels in the smallest-celled diatom *Chaetoceros capense*. Those cultures which had a high total carbon to chlorophyll *a* ratio had low Chl *a* L^{-1} content. This influences the ratio calculations and resulted in an unusually high ratio particularly in the case of *Protoceratium reticulatum* (Expt. 7). In literature the particulate carbon to chlorophyll ratios reported for natural populations, ranging from 27 – 67 (Riemann *et al.* 1989), are much lower than those observed in this study. Species-specific increases and declines were noted for the C : Chl *a* ratios at the different irradiance levels which corresponded to similar fluctuations in P^*_m and α* values. Johnsen and Sakshaug (1993) state that the variations in Chl *a* : C^{-1} ratio are species-specific and varies inversely with irradiance, is highly dependent on day lengths in high light acclimated cells (Sakshaugh and Andresen 1986) (Chapters 3 and 4).

The carbon content per cell for the dinoflagellates ranged from 0.07 to 5.43 pg $cell^{-1}$. The larger cells i.e. *Protoceratium reticulatum, Alexandrium catenella* and *Prorocentrum micans* had the highest content (7.43,

5.69 and 5.10 pg cell^{-1} respectively). The smaller cells' carbon content ranged from 0.08 to 1.63 pg cell^{-1}. The carbon content per cell generally decreased from low to high light conditions in the diatoms although with a slight increase at medium light in *Chaetoceros capense* and *Chaetoceros pendulus* was noted. At higher irradiances the dinoflagellates showed an increase of total carbon per cell *i.e.* in *Protoceratium reticulatum, Alexandrium catenella* and *Gymnodinium zeta* by 15 to 25% respectively. This increase in carbon content during high irradiances may indicate that the cells are undergoing a period of unbalanced growth. A state of a high source-sink ratio is created whereby the capacity to harvest light is reduced and photosynthate (carbon) is stored. Source-sink regulation is an important process of photoacclimation (Cullen and MacIntyre 1998) and is of physiological significance. Thus both the diatoms and the majority of dinoflagellates, show an increase in carbon content with increasing light. Interestingly, unlike the other dinoflagellates, the species in the genus *Prorocentrum, Prorocentrum micans* and *Prorocentrum triestinum*, both responded in opposite fashion to the others with the total carbon per cell concentrations decreasing from low to high light environments by 13 and 55% respectively. On the other hand, this response in total carbon is overridden by the changed Chl *a* content so that the ratio of TC : Chl responded as expected (Chapter 3 and 4).

Generally the TC : Chl *a* ratio was higher in small cells than in larger ones which is in agreement with other findings (Le Bouteiller *et al.*2003). C : Chl *a* ratios were previously treated as being constant, however, variations in the C : Chl *a* ratio have been widely reported in literature (Wang *et al.* 2009). Values ranging from 40 to >200 have been documented. Wang *et al*. (2009) reported a ratio range at the surface of 80 – 140 g : g and a similar value near the bottom of the euphotic zone of 40 g : g. In phytoplankton cultures Taylor *et al*. (1997) found C : Chl *a* ratios from about 12 to > 200 g g. Sakshaugh and Andresen (1986) found that diatoms and dinoflagellates grown at high and continuous irradiances had a Chl *a* to C ratio ranging from 0.005 to 0.008 mg Chl *a* (mg C)$^{-1}$ in contrast to cells grown in 12 h which exhibited ratios ranging from 0.02 to 0.03 mg Chl *a* (mg C)$^{-1}$. Little is known about the relative roles of light, temperature and nutrients regulating the large scale variability in phytoplankton C : Chl *a* ratios. Models that simulate spatial and temporal variation in physical fields, ecosystem dynamics and biogeochemical fields (Wang *et al.* 2009) have been developed using *in situ* (Equatorial Pacific Ocean) and satellite derived chlorophyll *a.* Since laboratory studies have shown that phytoplankton photoacclimation respond to changes in light, nutrients, and temperature conditions by adjusting cellular pigment levels to match their new demands for photosynthesis and that this response is well quantified by changes in the ratio of chlorophyll to carbon biomass (Chl : C) (*e.g*. Geider 1987; Sakshaug *et al*. 1989; MacIntyre *et al*. 2002), it follows that a remote sensing index of Chl : C may provide a path for assessing global carbon cycling and phytoplankton physiology from space.

6.8 Statistical analyses - Ordination

A geometric approach similar to a principal component analysis (PCA) was used to summarise the multivariate data of the two groups (dinoflagellates and diatoms) in a graphical way. The result of

ordination is a two dimensional diagram in which the species are represented by points in two-dimensional space (ter Braak 1995). The aim of the ordination is such that the points that are close together correspond to species that are similar in their responses to variables (listed below) at different light levels. Points that are far apart correspond to species that are dissimilar in their responses to variables and irradiance levels. The results are based on 10 variables measured at different light levels for eight species of phytoplankton (five dinoflagellates and three diatom species). The parameters measured at low, medium and high irradiances were maximum photosynthetic rate (P^*_m), light utilisation coefficient (α^*), light saturation parameter (E_k) and the variables were Chl *a*–specific xanthophyll pool (diadinoxanthin + diatoxanthin), mean spectral absorption coefficient, Chl *a*-specific absorption (ratio - 440:675 nm), maximum quantum yield (ϕ_m), total chlorophyll *a* to total carbon ratio (TC : TChl *a*), % photosynthetic pigments to total pigment (%PSP : T. pig.), % photoprotective pigments to total pigments (%PPC : T.pig.).

The two-way data matrix contained the mean of each variable measured at three light levels. Because the variables were measured in different units they were first standardised ((value – mean) / standard deviation), then a resemblance matrix was constructed using Euclidean distance as a measure of dissimilarity or distance.

The ordination plot was produced using multidimensional scaling (MDS). The difference between dinoflagellates and diatoms was further investigated using a 1-way Analysis of Similarities (ANOSIM). The statistic for this non-parametric test is R. It compares the distance/resemblance within the group with that of distance/resemblance between the different groups, in an analogous way to 1-way ANOVA (Clarke and Gorley 2006; Clarke and Warwick 2001).

In Figure 6.1 the MDS Euclidean Distance bi-plot shows clearly the separation of the two groups. The two major axes correspond to two different factors. The horizontal axis separates the two groups (dinoflagellates and diatoms) whilst the second axis shows differences among the species within each group in terms of their photoacclimation responses to different light levels.

Figure 6.2 shows the distribution of R values calculated under the null hypothesis of no differences between diatom and dinoflagellate species for the sample (indicated by the vertical bars). The actual value for R is far removed from the values computed under the null hypothesis. The value of R for the global test is R=0.512 and this value is significantly different ($p<0.01$). Thus there is a statistically significant overall difference between the diatom and the dinoflagellate species, with respect to the 10 physiological variables calculated in this thesis.

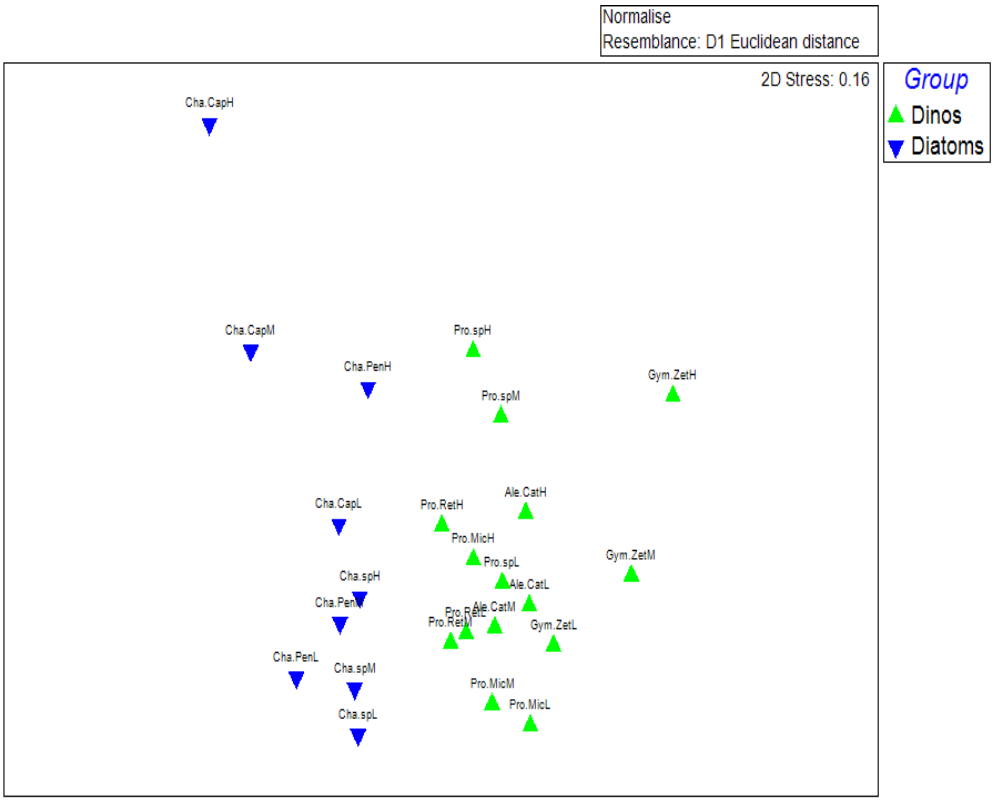

Figure 6.1: MDS plot of the data of the two main phytoplankton groups, based on their physiological responses at high, medium and low light intensities, showing that the dinoflagellate and diatom groups are clearly separated, using Euclidean Distance. Axes are relative values of Euclidean Distance and are therefore not given numerical values.

Legend: (Cha.spL,M,H - *Chaetoceros sp.*, Cha.PenL,M,H – *Chaetoceros pendulus*, Cha.Cap L,M,H – *Chaetoceros capense* Pro.spL,M,H – *Prorocentrum triestinum*, Pro.MicL,M,H - *Prorocentrum micans*, Ale.CatL,M,H – *Alexandrium catenella* Pro.Ret.L,M,H – *Protoceratium reticulatum*, Gym.ZetL,M,H – *Gymnodinium zeta*,

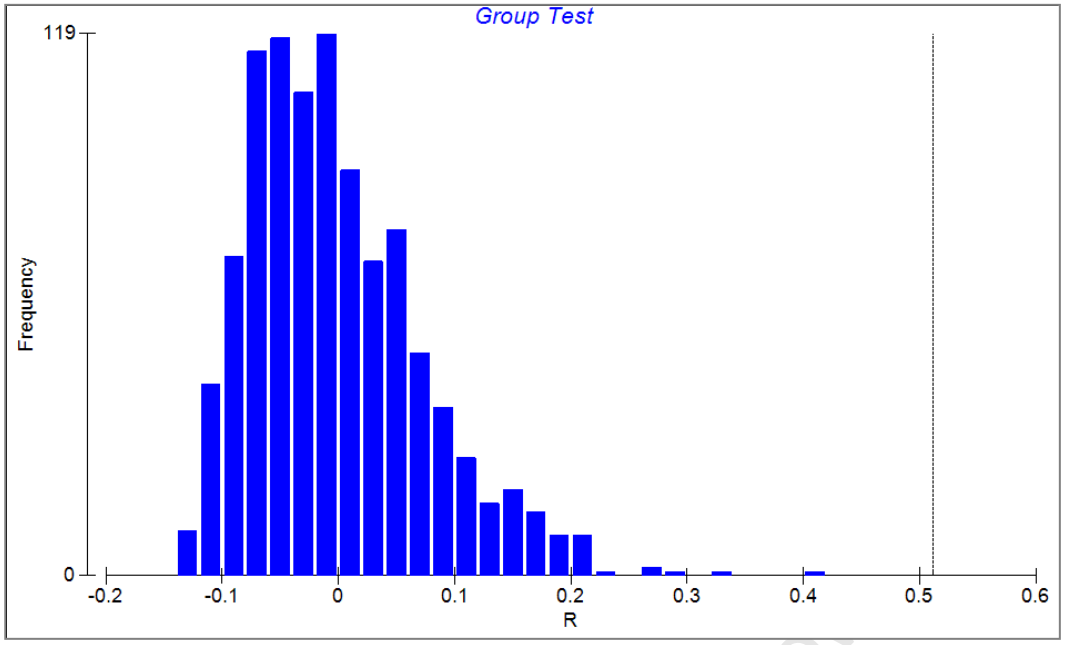

Figure 6.2: The distribution of R values (horizontal axis) calculated under a null hypothesis that the diatom and dinoflagellate species do not differ with respect to 10 physiological variables. (R = 0.512 , p < 0.01).

6.9 Synthesis: upwelling cycles and blooms

The most important theme investigated in this thesis was how the two groups, dinoflagellates and diatoms, differed in their responses to changing light environments and how the suite of adaptive strategies in each individual species compared in respect of photoacclimation.

Definite overall trends for each group were evident such as the diatoms' (mixers) capability to maximize light harvesting at rapidly varying irradiance levels by fast photoacclimation. The dinoflagellate (migrators) group adapted at a slower rate related to a need for diel vertical migration to remain at a low irradiance level in the water column where motile species can optimise the level of available light for growth.

An interesting schematic representation of how the phytoplankton life forms respond to the different regimes of turbulence and nutrient availability is given by Margalef (1978) (Figure 6.3). Turbulence is the principal factor whereby nutrients become available to phytoplankton cells. The adaptations, as observed in this study, have been shown to be characteristic of each group. Within each group the species demonstrate a set of species-specific adaptive strategies for survival within the realm of physiological, biochemical or morphological adaptations. Broad differences were noted between the two groups which were significant as

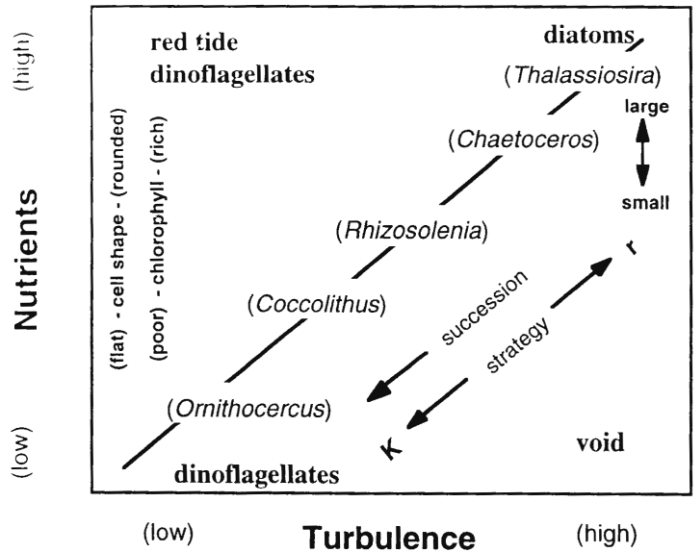

Figure 6.3. Schematic representation of how Margalef (1978) viewed responses of phytoplankton life forms to different turbulence regimes and available nutrients. The selective trends in cell size, pigment content, and ecological strategy are strongly influenced by nutrient availability. Depth regulation and vertical migration were not explicitly considered, although the dominance of red-tide dinoflagellates in low-turbulence, high-nutrient waters can be associated with swimming behaviour. (redrawn from Margalef, 1978; cited in Cullen and MacIntyre 1998).

supported by the ordination analysis (Figure 6.1 and 6.2) as the differences between the individual species were very variable, the results were not always easy to interpret. The reason for this variation could possibly be attributed to the size of the samples and the small volume of sample material available used for experimentation.

6.10 Sources of variation in data and results

The choice of the *P vs E* model used, *i.e.* that of Platt *et al.* (1980) fitted the data reasonably well and the function yielded a curvature between the Michaelis-Menten function (rate of reaction *versus* substrate concentration (Kirk 1996)) and that of the Blackman function (limitation in growth rate in cultures determined by availability of a limiting nutrient (MacIntyre and Cullen 2005)).

The parameter α* is reported to be particularly sensitive due to the regression being forced through a null intercept at the origin. If the function allowed the curve to have a non-null intercept at the origin, as stressed by Frenette *et al.* (1993), the α* values would have been more widely dispersed.

Several protocols are recommended in publications concerning the acquisition of accurate and precise photosynthetic parameters. The data, however, are usually estimated from several different measurements and lack a standard or absolute reference. This makes it complex when comparing the data resulting from my experiments with those in the literature.

Photosynthesis *versus* irradiance (*P vs E*) measurements for samples exposed to high light conditions in natural populations in actively mixing surface waters generally indicate a maximum P^*_m at midday when PAR is at its highest. This was also demonstrated in my data of the *P vs E*. However, when cultures in incubation bottles are exposed to high light, the carbon fixation is reduced. This illustrates that the duration of exposure to high light is an important factor possibly causing over excitation and subsequent inactivation of PSII reaction centres (Cullen *et al.* 1992; Behrenfeld *et al.* 1998).

Ambrust and Chisholm (1992) suggested that the cause of variation in experimental data of repeat experiments may be evolutionary. Clonal cultures do evolve over time even though they are established from a single cell by asexual propagation. However, mutation and sexual recombination within the culture are two mechanisms most likely to cause genotypic changes in the clonal cell culture. Also mitotic recombination may occur causing genetic variation.

6.11 Short-term and long-term photoacclimation

In literature photoacclimation has been characterised (Zonneveld 1997 and others) by the following changes:

1. Changes in the amount and ratios of photosynthetic and photoprotective pigments,
2. Changes in the photosynthetic parameters,
3. Changes in the enzymatic activities involved in photosynthesis and respiration,
4. Changes in cell volume, respiration rate and chemical composition.

In this study the first two changes, listed above, have been investigated in respect of the two groups of phytoplankton, the diatoms and dinoflagellates. As phytoplankton reacts in different ways with respect to light variations in order to achieve optimal conditions for growth. these adjustments can take the form of short-term processes involving time scales from a few seconds to less than 1 h and long-term photoacclimation processes that take 30–60 min and up to several days. The limit between short and long-term photoacclimation is imposed by a physiological constraint, *i.e*. the minimum time for synthesising new proteins or pigments, which is greater than 30–60 min (Garczarek 2000). In other words, short-term photoacclimation occurs without *de novo* synthesis of proteins/pigments, while long-term photoacclimation is a change in the stoichiometry of the phytoplankton that requires synthesis of protein, pigments or other cellular constituents.

6.11.1 *Short-term photoacclimation (without de novo synthesis of proteins)*

Phytoplankton maintain an equilibrium between photon absorption rate and electron transfer. Sakshaug *et al.* (1997) states that this balance is effective for irradiances around the light saturation parameter (E_k). At lower irradiances, the quantum yield is higher, but the photosynthetic rate is lower. The resulting data in this study are in agreement with these findings as it was noted, particularly during the light shift

experiments, that only minimal increases in the photosynthetic rate (P_m) took place at higher irradiances which suggested that hardly any *de novo* synthesis of photosynthetic pigments took place. Phytoplankton continuously adjusts its light saturation index by the interaction of two factors: the absorption cross-section of PSII ($\boldsymbol{\sigma_{PSII}}$) and minimum (or stationary) turnover time of electrons ($\boldsymbol{\tau}$) that determine E_k.

Under high-light conditions, the energy absorbed by light-harvesting pigments of phytoplankton may exceed their photosynthetic capacity and algae respond in short-term photo-protection through the deactivation of excitons formed by the absorption of photons. The excess excitons can be deactivated by thermal dissipation (Demmig-Adams and Adams 1992). This is thought to prevent over-reduction in the electron transfer chain and, therefore, provides protection from photo-damage.

It is thought that most of the energy-dependent quenching occurs directly within the light-harvesting antenna complexes of PSI and PSII and is an exothermic reaction involving the xanthophylls diadinoxanthin and diatoxanthin in diatoms (Anderson *et al*. 1998; Demers *et al*, 1991) and other eukaryotic algae (violaxanthin conversion, ultimately to zeaxanthin). This reaction is reversed under low light conditions and this reversible sequence of reactions is part of the xanthophyll cycle. Changes during the short-term photoacclimation experiments in the diadinoxanthin and diatoxanthin pigments was evident indicating that photoacclimation took place. This was mostly evident in the dinoflagellate *Prorocentrum triestinum*. Therefore experimental evidence of the role of the xanthophyll cycle in non-photochemical quenching, and therefore in excess energy dissipation, was confirmed in this study during the light-shift (short-term photoacclimation) experiments (Figure 5.7, Chapter 5).

6.11.2 *Long-term photoacclimation*

When exposed to frequently (and/or continuously) unfavourable light conditions, algae undertake structural and physiological changes to optimise light harvesting and minimise photo-inhibitory damage. These transformations occur over longer time scales than those previously considered (Baklouti *et al.* 2006) and two basic photoacclimation strategies have been identified in many algae: the first consists of an alteration of the amount of chlorophyll per PSU (i.e. the size of PSU), which changes their functional absorption cross-sections (e.g. σPSII), while the second affects the number of PSU (Falkowski and LaRoche, 1991). These strategies are both associated with changes in the cellular chlorophyll content, and therefore in the C:Chl intra-cellular ratio. Both the cellular chlorophyll content and the total carbon : chlorophyll ratios remained fairly constant over a period of 5 h during the short-term photoacclimation as expected in the light shift experiments but showed increases during the long-term experiments in high light and decreases in low light. The TC: Chl *a* ratios followed a similar pattern.

Leading on from the above and the points made by Zonneveld (1997) when attempting to develop a workable, mechanistic model of photoacclimation, I refer to the key questions tabulated in Chapter 1 which

formed the basis of this study. Of the tabulated key questions, items 1 to 3 are all related to long-term responses at the three irradiance levels, all of which were possible to measure adequately in the preconditioned, low light acclimated cultures. Definite photoacclimation responses, showing changes in the photosynthesis *versus* irradiance parameters, were clearly measurable. Pigment concentrations were all noticeable and discussed in each relevant chapter for dinoflagellates (Chapter 3) and diatoms (Chapter 4). The same applies to the Chl *a*-specific absorption coefficients which clearly showed the increases and decreases when exposed to the three light levels used and the relevancy of the light harvesting pigments present in the responses of the different species.

Answering the key questions related to the short-term photoacclimation responses during the light-shift experiment (Chapter 5) at 0.5 or 1 h intervals, the changes in the C : Chl *a* ratios were minimal without clear patterns emerging. This outcome is in agreement with the Behrenfeld *et al.* (1998) findings where they demonstrated that in light-shift experiments, Chl *a* turnover was firstly independent of light level and secondly in preconditioned, light acclimated cultures, physiologically poised to effectively dissipate excess energy, photo-oxidation and enzymatic degradation of Chl *a* takes place. This was illustrated in the photoprotective pigment concentrations where the pool of diadinoxanthin and diatoxanthin in dinoflagellates and diatoms showed a measureable and more realistic result which was an indication that photoacclimation had taken place within a short 0.5 and 1 h time span.

6.12 Importance of culture studies

Culture studies are important as they enable the description and analysis of natural processes that cannot be obtained easily from field studies. An insight into auto-ecology and systems ecology is thus provided by controlled comparison of environmental variables and effects. The extrapolation of culture studies to field studies aids in the management of natural environments and aquaculture sites. It is also useful in the prediction of harmful algal blooms i.e. temperature ranges which offer evidence of seasonal 'windows' for bloom initiation and decline as discussed by Kremp and Anderson (2000). However, it is not always easy to extrapolate culture data to field data. Wood (1989, cited in Wood *et al.* 2005) and others are of the opinion that certain measurements e.g. growth rates measured in cultures, cannot be extrapolated directly to field studies due to the clonal isolates and field species having a genetically determined difference in their acclimated growth rate. Knowledge of the processes and limitations of photoacclimation responses of specific phytoplankton species may aid algal culturing industries for economic purposes. The economics of such current ventures like the production of biodiesel from algal cultures necessitates an understanding of photoacclimation and its effect on limits imposed by quantum yields and profits per unit area (Dubinsky and Stambler 2009).

6.13 Future studies

This study has focussed on the differences of dinoflagellate and diatom photoacclimation responses grown

in the laboratory under controlled irradiance levels, 12 : 12 h light/dark regimes, set temperature (17º C) and nutrient replete conditions. Further investigation into the influence of different temperatures on *inter alia* cell volume of the individual dinoflagellate (thecate and athecate) and diatom species (chain-forming or single-celled) under LL, ML and HL would be useful for instance, in predicting phytoplankton blooms of individual toxic species under changing environmental conditions.

Cell volume is an important determinant of the optical properties of phytoplankton especially at 440 nm (Bouman *et al.* 2003) and an investigation into the relationship between a*(λ) and temperature under LL, ML and HL for the individual species of dinoflagellates and diatoms would be useful.

The effect of nutrient limitation (N) or nutrient loading (as in anthropogenic pollution of coastal areas) on growth rate under LL, ML and HL. Changes in community composition such as a shift from the diatom Skeletonema t*o Chaetoceros* dominance as a result of nutrient loading has been reported in the Seto Island Sea (Nishikawa *et al.* 2010).

To predict the future progress of any scientific discipline is difficult, particularly in the case of biological oceanography. There is, however, ample evidence to suggest that the field of biological oceanography will experience a rapid increase in technology and instrumentation over the next few decades. Researchers can anticipate increased automation in autonomous detection in phytoplankton organisms in respect of photoacclimation and their metabolic activities. Furthermore, advanced statistical treatments of laboratory and field data and the development of comprehensive models will provide a better understanding of the complex interactions between phytoplankton organisms and their environment. Exciting decades lie ahead.

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Appendix 1:

1.1: Motivation for the usage of a stable carbon isotope ^{13}C instead of the radioactive carbon isotope ^{14}C in marine research.

The isotopic technique based on the ^{14}C uptake developed by Steemann Nielsen (1952) to measure phytoplankton production has been applied to all autotrophic components of the marine ecosystem (phytoplankton, benthic microflora, macroalgae, epiphytes and seagrasses). In the Netherlands, the use of ^{14}C in the natural environment has been virtually abandoned in recent years because of the associated radioactive hazards (Mateo *et al.* 2001). Moreover, in South Africa, as elsewhere, the handling of radioactive materials requires a researcher to have special training and permits. In the field of phytoplankton research, such limitations have led to attempts to replace radioactive carbon isotope (^{14}C) with the stable carbon isotope (^{13}C). The ^{13}C technique has not been tried by other researchers in South Africa. However, Probyn (1985) has used a nonradioactive isotope of nitrogen (^{15}N) when investigating nitrogen uptake kinetics in phytoplankton in the southern Benguela upwelling system. An overall good statistical agreement between ^{14}C and ^{13}C techniques in experiments with marine phytoplankton was demonstrated as early as 1977 by Slawyk *et al.*, and later confirmed by other authors (Sakamoto 1984, Mousseau *et al.* 1995). It is generally accepted that the addition of ^{13}C constituting 10%, or even less, of the ambient DIC concentration of the incubation medium would allow detection of changes as small as 0.13% in ^{13}C concentrations in phytoplankton cells (Mateo *et al.* 2001). The response of carbon incorporation rates to increases of $NaH^{13}C_3$ additions in the range of 1.3 to 10.2% of the natural DIC concentrations in the incubation medium showed no significant effect on productivity. Comparing the production rates of ^{14}C with ^{13}C turned out to be remarkably similar and estimates achieved were very close to gross productivity after a 3 h incubation (Mateo *et al.* 2001).

A large amount and a great variety of radionuclides (or radioactive isotopes) have been introduced into the marine environment as a result of human activities. Fall out from testing atomic bombs, particularly between the years 1958 – 1965 and unintentional leaking from nuclear reactors are the major sources of artificial radionuclides*[1] (e.g. carbon fourteen (^{14}C)) now present in the oceans. Some radionuclides become strongly enriched in the tissues of marine organisms (e.g. polonium ^{120}Po), much like the naturally occurring trace metals. It may be argued that cosmic rays produce natural radionuclides*[2] continuously. The global inventory is approximately 340 MCi (7.5×10^4 kg) (cited in Libes SM 1992). However between 1958 and 1965 the anthropogenic input has increased the radio carbon inventory by 50% in the Northern Hemisphere.

*[1] Artificial radionuclides are isotopes produced by humans
*[2] Natural radionuclides are formed by spallation reactions that occur in the atmosphere.

Using ^{13}C instead of ^{14}C prevents additional pollution of the oceans with radioactive isotopes (^{14}C half life is 5680 years). Much dilution may well take place when invariably the waste of production experiments is dumped at sea. It is argued that dilution at sea is less problematic than waste disposal of radioactive materials on land, which could ultimately cause contamination of ground water. But why use radioactive isotopes at all when there are other alternatives? Japan has **banned** its use at sea.

In South Africa the Dumping at Sea (Control) Act (incorporating the MARPOL Convention) prohibits the dumping of low-level radioactive material in the sea, unless authorised by permit.

Appendix 2:

Appendix 2.1: Overview of mean number of diatom cells L^{-1} in samples collected during the St. Helena Bay Monitoring Line cruises between June 2000 and July 2001.

(n = number of samples collected during each cruise).

		Jun.2000 (n=18)	July.2000 (n=12)	Aug. 2000 (n=19)	Sept.2000 (n=15)	Dec.2000 (n=10)	Jan.2001 (n=7)	Feb. 2001 (n=12)	April.2001 (n=12)	May.2001 (n=8)	Jun.2001 (n=11)	Jul. 2001 (n=10)
Actinocyclus senarius			17									
Amphora spp.		2444	500	15384				179				780
	Flabellatus	22	33	0	37			0		50	173	65
Asterionellopsis	Glacialis	0	0	2763	2273	6000		296	67	0	0	12830
Bacteriastrum spp.		0	0	1316	0	0		0	0	0	0	0
	Hyalinum	0	0	0	0	0		0	0	0	0	580
Chaetoceros spp.		83	0	2279	353	1120	1771	1342	0	1050	636	5560
Chaetoceros	Curvisetus	0	0	789	0	270	0	0	0	0	0	0
	Compressus	0	0	0	0	590	0	0	0	0	0	600
	Convolutes	0	0	0	0	0	0	4629	0	0	0	0
	Debilis	0	25	0	0	0	0	0	0	0	0	0
	Decipiens	39	233	24516	0	3380	28650	32575	67	0	0	16560
	Diadema	0	0	0	0	0	0	1108	71	0	0	0
	Laciniosus	0	217	32105	53	0	0	0	0	0	0	0
	Peruvianus	0	0	0	0	0	0	0	71	0	18	0
	Radicans	0	0	2632	7050	39335	357143	83	67088	0	64	2000
	Simplex	0	0	0	0	0	0	0	0	0	18	0
Cocconeis spp.		0	0	0	0	0	0	0	8	0	0	0
Corethron criophilum		0	8	3	0	0	0	0	0	0	0	0
Coscinodiscus spp.		0	0	0	20	0	100	0	0	0	41	10
Coscinodiscus oculus iridis		0	17	0	20	0	0	0	0	0	0	0
Coscinodiscus	Granii	6	3708	3487	3	0	0	17	0	0	0	20
Cylindrotheca	Closterium	67	33	3	0	60	479	17	58	13	0	0
Dactyliosolen spp.		11	0	0	0	0	0	0	0	0	0	0

	Jun.2000 (n=18)	July.2000 (n=12)	Aug. 2000 (n=19)	Sept.2000 (n=15)	Dec.2000 (n=10)	Jan.2001 (n=7)	Feb. 2001 (n=12)	April.2001 (n=12)	May.2001 (n=8)	Jun.2001 (n=11)	Jul. 2001 (n=10)
Detonula pumila	0	0	8618	0	0	0	150	1358	0	0	0
Ditylum brightwelli	0	0	1579	210	85	0	0	0	0	0	770
Eucampia spp.	0	0	0	223	165	0	0	0	0	0	1080
Eucampia zodiacus.	0	0	0	0	0	0	33	0	0	0	70
Fragilariopsis spp.	6	4	5	0	15	0	0	0	13	9	5
Fragilariopsis ribbons (cells)	0	0	0	23	0	479	0	0	0	0	0
Fragilariopsis doliolus	78	229	9992	587	0	0	0	0	0	209	70
Fragilariopsis kerguelensis	0	0	0	0	0	0	17	25	0	0	0
Guinardia cylindrus	11	8	0	0	0	0	0	0	0	0	0
Guinardia delicatula	75	0	0	0	0	0	208	0	0	0	0
Guinardia flaccid	0	0	132	0	0	0	0	0	0	0	0
Hemiaulus hauckii	111	0	0	0	0	0	0	0	0	0	0
Leptocylindricus spp.	1325	175	0	600	0	236	0	0	0	0	780
Thalassionema Nitzschoides	22	0	0	0	0	0	0	0	0	0	0
Thalassiosira spp	114	0	0	7	0	0	17	0	0	0	0
Flaccida	0	0	1053	0	0	0	0	0	0	0	0
or Leptocylindricus	0	0	1447	0	0	0	0	0	25	0	0
Meuniera membranica	0	0	1053	0	0	0	0	0	0	0	0
Navicula spp.	6	29	0	0	0	0	25	4	38	50	15
Navicula Directa	0	0	0	0	35	0	0	0	0	0	0
Odontella mobiliensis	0	0	0	0	0	0	0	0	0	0	10
Longicruris	0	0	0	0	0	0	0	0	0	18	0
Nitzschia spp	78	25	2750	50	5	0	33	38	56	177	800
Nitzschia pelagila	6	8	0	0	0	0	0	0	0	9	10
Odontella aurita	0	0	1184	0	0	0	0	0	0	0	0
Odontella mobilensis	0	8	132	0	10	0	0	0	0	0	0
Planktoniella sol	0	0	53	0	0	0	0	0	0	0	0
Pleurosigma spp.	0	0	0	0	35	121	0	0	0	0	250
Pleurosigma Normanii	3	0	132	20	0	21	0	0	0	0	0
Directum	14	0	0	0	0	0	0	0	13	373	905
Pseudonitzschia spp.	464	771	35634	10613	1305	3229	533	74483	113	13409	12470

	Jun.2000 (n=18)	July.2000 (n=12)	Aug. 2000 (n=19)	Sept.2000 (n=15)	Dec.2000 (n=10)	Jan.2001 (n=7)	Feb. 2001 (n=12)	April.2001 (n=12)	May.2001 (n=8)	Jun.2001 (n=11)	Jul. 2001 (n=10)
Rhizosolenia spp.	0	0	0	0	0	0	8	0	0	0	5
Rhizosolenia accicularis	0	0	355	0	0	0	0	0	0	0	0
Rhizosolenia bergonii	3	0	42	0	0	0	29	0	0	0	40
Skeletonema costatum.	972	883	277763	27	12090	65329	0	81354	0	0	3910
Thalassionema spp.	0	0	0	0	0	1157	29	0	0	114	0
Thalassionema nitzschoides	778	975	38924	1067	930	3093	167	550	1700	2164	17040
Thalassiosira spp	606	2013	188132	7523	21415	14029	3408	43475	3344	3341	11930
Thalassiothrix spp.	0	8	42	0	0	0	29	4	0	27	155
Thalassiothrix Antarctica	0	17	0	10	15	0	0	0	0	5	90
Other	**19**	**100**		**0**	**0**	**9**	**0**	**0**	**0**	**0**	**0**
Diatoms total	**7361**	**10046**	**654297**	**30770**	**86860**	**475845**	**44933**	**268721**	**6413**	**20855**	**89410**

Appendix 2.2: Photosynthetron incubation procedure

(All handling of ^{14}C containing samples to be done in the fume cupboard)

Phytoplankton sample
(50 mL in beaker)
↓
Add ^{14}C while stirring
↓
Phytoplankton sample - → Remove 3 * 0.1 mL aliquots (Label **TA**1 – **TA**3)
↓ ↓
50 mL + ^{14}C Add 0.1 mL ethanolamine or similar
(Specific activity range Add scintillation cocktail
0.5 – 2.0 µCi/mL) (10 mL Optima Gold XR to which 0.01mL NaOH has been added)
↓
↓ Determine **T**otal **A**ctivity

Dispense 1 mL aliquots
into 30 scintillation vials + 3 more samples for **T**ime **Z**ero control
placed in Photosynthetron (Label **TZ** 1 – **TZ**3)
↓ ↓
Acidify immediately
(0.5 mL 6N HCL)
20 minutes incubation ↓
↓
Immediately remove ↙
Place on shaker tray under a
Fume hood and acidify
(0.5 mL 6N HCL)
↓
Shake for 1 hr ← ←
↓
Neutralize samples
(add 0.1 mL 6N NaOH)
↓

Labelling of lids

3 **T**otal **A**ctivity	-	**TA** 1 -**TA** 3
3 **T**ime **Z**ero	-	**TZ** 1 - **TZ** 3
30 Samples	-	**A**1 - **A**5
		B6 -**B**10
		C11–**C**15
		D16-**D**20
		E21-**E**25
		F26-**F**30

Add scintillation cocktail
(8 – 12 mL as required
to clear solution)

(Increase numbering for each subsequent experiment therefore Experiment 1 A&B = 1-60)
Experiment 1Experiment

Note:
Experiment 1 A&B = 1–60)

Once incubation started, complete **TA** samples

Examples:

50 µCi ampule + 50 mL sample → 1 µCi/mL

10 µCi ampule + 50 mL sample → 0.2 µCi/mL

Appendix 2.3: List of incubation dates for the dinoflagellate and diatom species experiments.

Experiment date	Experiment number	Species
16th July 2002	2	*Alexandrium catenella*
16th July 2002	2	*Chaetoceros* sp.
6th August 2002	3	*Gymnodinium zeta*
6th August 2002	3	*Prorocentrum micans*
8th August 2002	4	*Chaetoceros* sp.
8th August 2002	4	*Prorocentrum triestinum*
31st October 2002	5	*Chaetoceros capense*
31st October 2002	5	*Alexandrium catenella*
17th December 2002	6	*Chaetoceros capense*
17th December 2002	6	*Proroceratium reticulatum*
28th January 2003	7	*Chaetoceros* sp.
28th January 2003	7	*Protoceratium reticulatum*
14th February 2003	8	*Alexandrium catenella*
14th February 2003	8	*Prorocentrum micans*
22nd July 2003	9	*Gymnodinium zeta*
22nd July 2003	9	*Prorocentrum triestinum*
29th July 2003	10	*Chaetoceros pendulus*
29th July 2003	10	*Chaetoceros capense*
12th August 2003	11	*Prorocentrum micans*
12th August 2003	11	*Protoceratium reticulatum*
26th August 2003	12	*Chaetoceros pendulus*
26th August 2003	12	*Gymnodinium zeta*
9th September 2003	13	*Chaetoceros* sp.
9th September 2003	13	*Prorocentrum triestinum*

Appendix 3

Appendix 3.1: Comparison of chlorophyll *a* values measured using HPLC and fluorometry.

(% difference of fluorometer reading versus HPLC = (HPLC-Fluorometer reading/ HPLC) *100)

Experiment			(Chl *a*, Allo, Epi, Chlide) HPLC Tchl *a* n=1 µg L^{-1}	Fluorometer Chl *a* n=3 µg L^{-1}	Difference Chl *a* %
5	*Alexandrium catenella*	Low light	152.66	153.12	0.3
		Medium light	126.78	121.80	3.9
		High light	110.61	106.14	4
8	*Alexandrium catenella*	Low light	87.83	62.79	28
		Medium light	77.70	54.63	29
		High light	64.69	47.54	27
6	*Protoceratium reticulatum*	Low light	177.92	174.77	5
		Medium light	143.91	151.57	2
		High light	150.16	146.55	2
7	*Protoceratium reticulatum*	Low light	128.92	59.69	53
		Medium light	110.37	48.44	56
		High light	134.34	45.01	66
11	*Protoceratium reticulatum*	Low light	128.11	105.73	17
		Medium light	83.04	88.86	6.5
		High light	106.94	65.38	39
3	*Gymnodinium zeta*	Low light	3.26	4.06	20
		Medium light	2.43	2.28	6
		High light	1.77	1.7	4
9	*Gymnodinium zeta*	Low light	119.84	100.07	16
		Medium light	77.81	63.66	18
		High light	69.89	50.29	28
12	*Gymnodinium zeta*	Low light	107.89	79.99	25
		Medium light	94.02	78.95	16
		High light	69.33	72.08	3
4	*Prorocentrum triestinum*	Low light	31.27	47.33	12
		Medium light	14.34	35.50	70
		High light	10.83	31.09	65
9	*Prorocentrum triestinum*	Low light	70.82	118.53	40
		Medium light	55.19	108.04	49
		High light	47.75	87.64	46
13	*Prorocentrum triestinum.*	Low light	57.28	73.90	21
		Medium light	38.35	72.95	48
		High light	32.11	51.73	38
3	*Prorocentrum micans*	Low light	91.54	95.89	5
		Medium light	66.60	71.15	6
		High light	38.20	67.28	43
8	*Prorocentrum micans*	Low light	62.06	51.21	17
		Medium light	51.28	46.12	10
		High light	45.46	40	12
11	*Prorocentrum micans*	Low light	133.67	110.68	17
		Medium light	117.72	153.2	23
		High light	75.47	116.5	35

Appendix 3.2: A comparison of total chlorophyll *a* (TChl *a*) concentrations for the five different dinoflagellate species acclimated to three different light intensities. (Low light = ± 33 µmol quanta m^{-2} s^{-1}, Medium light = ± 178 µmol quanta m^{-2} s^{-1}, High light = ± 647 µmol quanta m^{-2} s^{-1}).

Experiment		Low light TChl *a* µg L^{-1}	Medium light TChl *a* µg L^{-1}	High light TChl *a* µg L^{-1}	Low light TChl *a* pg $cell^{-1}$	Medium light TChl *a* pg $cell^{-1}$	High light TChl *a* pg $cell^{-1}$
5	*Alexandrium catenella*	153.12	121.80	106.14	23.2	12.4	11.3
8	*Alexandrium catenella*	62.79	54.63	47.54	16.70	16.3	11.4
	Mean	**107.96**	**88.22**	**76.84**	**19.9**	**14.4**	**11.4**
	±SE	63.8	47.5	41.4	10.3	4.6	3.0
6	*Protoceratium reticulatum*	174.77	151.57	146.55	49.9	41.1	37.67
7	*Protoceratium reticulatum*	59.69	48.44	45.0	16.8	10.9	9.29
11	*Protoceratium reticulatum*	105.73	65.36	88.86	30.3	28.3	16.76
	Mean	**113.4**	**88.46**	**93.47**	**32.3**	**26.8**	**21.2**
	±SE	78.3	49.1	59.3	22.5	21.7	18.7
3	*Gymnodinium. zeta*	4.06	2.28	1.70	0.4	0.3	0.2
9	*Gymnodinium zeta*	100.07	63.66	50.29	1.2	0.8	0.8
12	*Gymnodinium. zeta*	79.99	78.95	72.08	1.4	0.8	1.1
	Mean	**61.37**	**48.30**	**41.36**	**1.0**	**0.6**	**0.7**
	±SE	47.4	18.2	10.9	0.3	0.1	0.1
4	*Prorocentrum triestinum*	47.33	35.5	31.09	8.7	5.8	4.0
9	*Prorocentrum triestinum*	118.53	108.04	87.64	5.0	2.7	1.6
13	*Prorocentrum triestinum*	73.9	72.95	51.73	2.9	1.6	1.0
	Mean	**72.92**	**72.16**	**56.82**	**5.6**	**3.4**	**2.2**
	±SE	47.3	43.9	37.8	0.7	0.7	0.8
3	*Prorocentrum micans*	95.89	71.15	67.28	31.4	23.6	23.0
8	*Prorocentrum micans*	51.21	46.12	40.0	22.4	17.5	17.6
11	*Prorocentrum micans*	110.68	153.2	156.50	24.0	21.1	15.6
	Mean	**85.93**	**90.16**	**87.93**	**25.9**	**20.7**	**18.5**
	±SE	42.5	54.0	58.7	4.3	3.7	1.0

Appendix 3.2 (a) Table for statistical outputs.

(a-1): Two-way ANOVA output for the effect of species and light level on "total chlorophyll *a* (TChl *a*) concentrations" in the five dinoflagellates.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Species2	4	82.74	20.6849	1.641	0.1898
Light level	2	5.56	2.7782	0.2204	0.8035
Species2:Light level	8	2.57	0.3208	0.0254	1
Residuals	30	378.14	12.6048		

(a-2): Two-way ANOVA output for the effect species and light level on "total chlorophyll *a* (TChl *a*) concentrations per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Species2	4	69.626	17.4064	94.1742	< 2e-16	***
Light level	2	1.783	0.8917	4.8245	0.01525	*
Species2:Light level	8	0.714	0.0893	0.4831	0.85839	
Residuals	30	5.545	0.1848			

Appendix 3.3: Cell densities (Cells . L^{-1}) in each subsample of the dinoflagellate species used for the incubation and other experiments.

Experiment	Species	Treatment	Cells L^{-1}
5	*A. catenella*	Low light	6.60
		Medium light	9.79
		High light	9-32
8	*A. catenella*	Low light	3.76
		Medium light	3.34
		High light	4.16
6	*P. reticulatum*	Low light	3.50
		Medium light	3.69
		High light	3.88
7	*P. reticulatum*	Low light	3.56
		Medium light	4.41
		High light	4.84
11	*P. reticulatum*	Low light	3.50
		Medium light	3.73
		High light	5.30
3	*G. zeta*	Low light	19.45
		Medium light	8.04
		High light	7.57
9	*G. zeta*	Low light	81.45
		Medium light	82.46
		High light	60.26
12	*G. zeta*	Low light	57.57
		Medium light	99.18
		High light	64.44
4	*P. triestinum*	Low light	5.41
		Medium light	6.16
		High light	7.69
9	*P. triestinum*	Low light	23.65
		Medium light	40.33
		High light	54.95
13	*P. triestinum*	Low light	25.23
		Medium light	44.47
		High light	52.40

Experiment	Species	Treatment	Cells L^{-1}
3	*P. micans*	Low light	3.06
		Medium light	3.02
		High light	3.03
8	*P. micans*	Low light	2.29
		Medium light	2.64
		High light	2.27
11	*P. micans*	Low light	4.63
		Medium light	7.26
		High light	10.04

Cell number = n x 10^6 L^{-1}

Appendix 3.4 (a): Comparison of the photosynthetic parameters of different species of dinoflagellates (*Alexandrium catenella, Protoceratium reticulatum, Gymnodinium zeta, Prorocentrum triestinum* and *Prorocentrum micans*). Sample statistics are mean ± standard error of three different experiments.

		Low light (33 µmol quanta m^{-2} s^{-1})			**Medium light** (178 µmol quanta m^{-2} s^{-1})			**High light** (647 µmol quanta m^{-2} s^{-1})		
	Experiment	**Maximum photosynthetic rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1}(mg Chl *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light Saturation Parameter** (µmol quanta $m^{-2}s^{-1}$)	**Maximum photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1} (mg Ch *a*) (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light Saturation Parameter** (µmol quanta m^{-2} s^{-1})	**Maximum photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization coefficient** (mg C m^{-3} h^{-1} (mg Ch *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1}) [1]	**Light saturation parameter** (µmol quanta m^{-2} s^{-1})$^{-}$
		P^*_m	α*	E_k	P^*_m	α*	E_k	P^*_m	α*	E_k
Alexandrium catenella	5	8.5	0.04	191	4.6	0.03	151	4.7	0.02	253
Alexandrium catenella	8	10.0	0.05	216	6.5	0.03	234	6.8	0.02	376
Mean		**9.3**	**0.05**	**204**	**5.5**	**0.03**	**193**	**5.8**	**0.02**	**314**
± SE		1.1	0.002	17	1.3	0.002	59	1.5	0.0003	87
Protoceratium reticulatum	6	4.5	0.03	173	2.7	0.02	154	2.8	0.02	153
Protoceratium reticulatum	7	11.4	0.06	195	8.7	0.04	194	8.8	0.03	284
Protoceratium reticulatum	11	6.2	0.03	219	5.3	0.03	207	5.2	0.02	323
Mean		**7.4**	**0.04**	**195**	**3.6**	**0.03**	**185**	**5.6**	**0.02**	**253**
± SE		3.6	0.02	23	3.0	0.01	27	3.0	0.01	89
Gymnodinium zeta	3	0.7	0.01	79	2.1	0.02	133	1.2	0.01	154
Gymnodinium zeta	9	3.0	0.02	153	5.0	0.02	268	2.4	0.01	481
Gymnodinium zeta	12	2.4	0.02	139	3.1	0.01	242	1.3	0.01	340
Mean		**2.0**	**0.02**	**124**	**3.4**	**0.02**	**214**	**1.7**	**0.01**	**325**
± SE		1.2	0.002	39	1.5	0.002	72	0.7	0.002	164
Prorocentrum triestinum	4	3.7	0.02	187	3.3	0.02	165	4.2	0.01	292
Prorocentrum triestinum	9	5.4	0.03	202	4.6	0.02	296	5.4	0.01	435
Prorocentrum triestinum	13	6.2	0.03	209	6.8	0.02	282	6.0	0.01	439

		Low light (33 µmol quanta m^{-2} s^{-1})			**Medium light** (178 µmol quanta m^{-2} s^{-1})			**High light** (647 µmol quanta m^{-2} s^{-1})		
	Experiment	**Maximum photosynthetic rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1}(mg Chl *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light Saturation Parameter** (µmol quanta $m^{-2}s^{-1}$)	**Maximum photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1} (mg Ch *a*) (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light Saturation Parameter** (µmol quanta m^{-2} s^{-1})	**Maximum photosynthetic Rate** (mg C (mg Chl *a*)$^{-1}$ h^{-1})	**Maximum light utilization coefficient** (mg C m^{-3} h^{-1} (mg Ch *a*)$^{-1}$ (µmol quanta m^{-2} s^{-1}) ^{1}	**Light saturation parameter** (µmol quanta m^{-2} s^{-1})$^{-}$
		P^*_m	α*	E_k	P^*_m	α*	E_k	P^*_m	α*	E_k
Mean		**7.061**	**0.03**	**199**	**4.9**	**0.02**	**248**	**5.2**	**0.01**	**388**
± SE		1.484	0.01	11	1.8	0.001	72	0.9	0.001	84
Prorocentrum micans	3	3.325	0.03	107	3.2	0.03	110	2.8	0.02	147
Prorocentrum micans	8	0.890	0.01	7.6	1.7	0.02	83	2.4	0.01	206
Prorocentrum micans	11	8.293	0.03	306	7.3	0.04	178	5.7	0.03	186
Mean		**4.169**	**0.03**	**163**	**4.0**	**0.03**	**124**	**3.6**	**0.02**	**180**
± SE		0..01	0.01	125	2.9	0.01	49	1.8	0.01	30

Appendix 3.4 a: Table of statistical outputs.
a-1 : Two-way ANOVA output for the effect of species and light level on "maximum light utilisation coefficient" (α*).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	0.00205	0.0005125	7.4797	0.0003433	***
Light level	2	0.001348	0.00067381	9.834	0.000619	***
species2:Light level	8	0.000486	0.00006071	0.8861	0.5408572	
Residuals	27	0.00185	0.00006852			
Signif. codes: 0 '*	**'	0.001	'**' 0.0	1 '*' 0.	05 '.' 0.	1 ' ' 1

a-2: Two-way ANOVA output for the effect of species and light level on "maximum photosynthetic rate" (P_m*).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	7.2192	1.80481	5.9369	0.001461	**
Light level	2	0.175	0.08751	0.2879	0.752134	
species2:Light level	8	1.2891	0.16114	0.5301	0.823385	
Residuals	27	8.208	0.304			
Signif. codes: 0 '*	**'	0.001	'**' 0.0	1 '*' 0.	05 '.' 0.	1 ' ' 1

a-3: Two-way ANOVA output for the effect of species and light level on "Light saturation parameter" (E_k).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	70739	17685	2.9975	0.036095	*
Light.level	2	108502	54251	9.1955	0.0009002	***
species2:Light.level	8	41851	5231	0.8867	5.40E-01	
Residuals	27	159293	5900			
Signif. codes: 0 '*	**'	0.001	'**' 0.0	1 '*' 0.	05 '.' 0.	1 ' ' 1

a-4 : Two-way ANOVA output for the effect of species and light level on "maximum light utilisation coefficient per cell" (α*).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	0.030939	0.0077347	29.8167	1.49E-09	***
Light.level	2	0.003267	0.0016337	6.2976	5.69E-03	**
species2:Light.level	8	0.001304	0.000163	0.6283	0.746818	
Residuals	27	0.007004	0.0002594			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

a-5: Two-way ANOVA output for the effect of species and light level on "maximum photosynthetic rate per cell" (P_m*).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	62.592	15.6479	30.6038	1.12E-09	***
Light.level	2	1.166	0.583	1.1402	3.35E-01	
species2:Light.level	8	1.114	0.1392	0.2723	0.9696	
Residuals	27	13.805	0.5113			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

a-6: Two-way ANOVA output for the effect of species and light level on "Light saturation parameter per cell" (E_k).

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	31.67	7.9175	17.1021	4.24E-07	***
Light.level	2	0.869	0.4343	0.9381	0.4038	
species2:Light.level	8	1.328	0.166	0.3586	0.9332	
Residuals	27	12.5	0.463			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 3.4 (b): Comparison of photosynthetic parameters of surface samples of natural populations measured during two cruises (Algoa 79 and Algoa 83) in August and September2000 along the west coast of South Africa during monitoring of the St Helena Bay Monitoring Line.

Sample statistics are mean ± standard error.

		Maximum Photosynthetic Rate (mg C (mg Chl a)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} h^{-1} (mg Chl a)$^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light Saturation Parameter** (µmol quanta m^{-2} s^{-1})
	Stations	P^*_m	**α***	E_k
Cruise Algoa 79	1	6.39	0.026	245.8
8th August 2000	2	5.05	0.026	194.2
	3	10.38	0.029	357.9
	4	10.13	0.019	533.2
	5	18.56	0.040	464.0
	6	11.23	0.059	190.3
	7	4.81	0.031	155.2
	8	4.49	0.023	195.2
	Mean	**8.88**	**0.032**	**292.0**
	±SE	1.69	0.004	50.39
Cruise Algoa 83				
11th September 2000	1	3.06	0.009	340.0
	2	3.64	0.025	145.6
	3	4.36	0.019	229.5
	4	9.70	0.066	147.0
	5	20.32	0.068	298.8
	6	25.05	0.115	217.8
	7	4.59	0.019	241.6
	8	0.55	0.007	78.6
	Mean	**8.91**	**0.041**	**212.4**
	±SE	3.17	0.01	30.35

Distance from the coast ranged between 1.70 to 221 km. Station 1 is near the coast. Station 8 is open ocean.

Appendix 3.4 (c) : Summary of some of the photosynthetic parameters from *P versus E* studies in different marine environments of the world.

Date	Location	Depth	Photosynthetic parameters			Reference
		m	P_m*	α*	E_k	
	Southern Ocean	n/d	0.44 - 1.12			Allanson *et al.* 1981.
	Southern Ocean	n/d	6.0 - 0.4			Laubscher (unpubl).
Early to late Arctic Summer	Lancaster Sound	5	0.81	0.045	18.66	Gallagos *et al.* 1983.
	Baffin Bay	5	1.96	0.074	30.84	
	JonesSound/Kane Basin	10	0.72	0.051	14.50	
4th December	McMurdock Sound	under snowfree ice	0.13	0.026	5.44	Palmisano *et al.* 1985
Antarctic summer	Southern Ocean	n/d	0.69	0.003	236.90	Tilzer *et al.* 1985
Antarctic winter	Southern Ocean	surface	1.19	0.021	56.67	Brightman and Smith 1989
Autumn	Agulhas Retroflection south of Africa	Surface - inside ring	1.44	0.056	26.21	Dower and Lucas. 1993
		Surface edge	1.51	0.055	30.22	
	42º 30' S, 22º 40' E	Surface - outside	1.75	0.039	44.69	
8 February 1992	Gulf of Coquimbo (coastal) - Chile	Surface	6.22	0.045	138.22	Montecino *et al.* 1996
26 - 27 April 1992		Surface	13.76	0.066	210.08	
24 June 1992		21-36	10.44	0.075	139.20	
27 October 1992		Surface	2.65	0.034	77.94	
25 September 1993		Surface	10.91	0.070	155.86	
28 January 1994		Surface	8.01	0.028	286.07	
29 January 1994		7	17.39	0.091	191.10	
30 January 1994		Surface	8.90	0.050	178.00	
31 January 1994		6	10.39	0.056	185.54	
1 February 1994		Surface	5.40	0.081	66.67	
27 September 1994		12	6.00	0.058	103.45	
6 February 1992	Gulf of Coquimbo - (open ocean)	n/d	8.59	0.050	171.80	
24 April 1992		40	8.33	0.056	149.21	
22 - 23 June 1992		48	6.77	0.066	103.25	
23 September 1993		32	2.98	0.025	119.00	
24 January 1994		27	7.81	0.024	325.42	
23 - 24 September 1994		15 - 32	13.08	0.078	168.71	
November	Gulf of California	n/d	3.17	0.230	13.78	Gaxiola-Castro *et al.* 1999
15 February 1996	Lambert's Bay, South Africa	Surface to 7m	3.995	0.019	210.26	Mitchell-Innes *et al.* 2000
20 February 1996		Surface	4.780	0.022	217.27	
22 February 1996		Surface to 7m	3.400	0.015	226.67	
28-29 February 1996		Surface to 7m	2.950	0.019	155.26	
6 March 1996		Surface to 7m	3.435	0.020	176.15	
08 August 2000	St Helena Bay, South Africa	Surface	8.880	0.032	277.50	Balarin 2000 (unpubl.)
11 September 2000		Surface	8.910	0.041	217.32	

Units used are P_m* mg C (mg Chl*a*)$^{-1}$ h^{-1}
α* (mg C m^{-3} h^{-1} (mgChl*a*)$^{-1}$ µmol quanta m^{-2} s^{-1}
E_k (µmol quanta m^{-2} s^{-1})

Appendix 3.5: Chlorophyll *a* concentrations of the five dinoflagellate species acclimated at low light, medium light and high light for four days generally showing a decrease from low light to high light conditions.

Experiment	**Species**		**T. Chl *a* µg L^{-1}**	**T. Chl *a* µg L^{-1}**	**T. Chl *a* µg L^{-1}**
			Low light 33 µmol quanta m^{-2} s^{-1}	**Medium light 178 µmol quanta m^{-2} s^{-1}**	**High light 647 µmol quanta m^{-2} s^{-1}**
Time series	*A. catenella*	Day 1			62.3
		Day 2	69.5		44.7
		Day 3	88.3		50.2
		Day 4	92.0		44.2
		Day 5	105.0		46.2
		Day 6	109.8		33.5
2	*A. catenella*		2.9	2.8	2.0
5	*A. catenella*		121.8	153.1	106.1
8	*A. catenella*		54.6	62.8	47.5
		mean	**59.8**	**72.9**	**51.2**
		±SD	49.0	61.8	32.8
3	*G. zeta*		4.1	2.3	1.8
9	*G. zeta*		101.7	63.71	50.3
12	*G. zeta*		80.0	79.0	72.1
		Mean	**61.6**	**48.3**	**41.4**
		±SD	41.5	33.1	29.4
3	*P. micans*		95.9	71.2	67.3
8	*P. micans*		51.2	46.1	40.0
11	*P. micans*		110.7	153.2	166.5
		Mean	**85.9**	**90.2**	**91.3**
		±SD	25.3	45.7	54.4
4	*P. triestinum*		35.5	47.3	31.1
9	*P. triestinum*		118.5	108.0	87.6
13	*P. triestinum*		73.0	73.9	51.7
		Mean	**75.7**	**76.4**	**56.8**
		±SD	34.0	25.0	23.4
6	*P. reticulatum*		151.6	174.8	146.5
7	*P. reticulatum*		48.4	59.7	45.0
11	*P. reticulatum*		65.4	105.7	88.9
		Mean	**88.5**	**113.4**	**93.5**
		±SD	45.2	47.3	41.6

Appendix 3.6: Comparison of the total photosynthetic pigments (PSCs) and photoprotective pigments (PPCs) of the five dinoflagellate species.

Sample				LL Total PSC µg L^{-1}	LL Total PPC µg L^{-1}	ML Total PSC µg L^{-1}	ML Total PPC µg L^{-1}	HL Total PSC µg L^{-1}	HL Total PPC µg L-1
Expt. 1	*Alexandrium catenella*		Day 1					116.73	10.48
			Day 2	126.7	11.9			87.7	11.6
			Day 3	161.9	14.8			98.2	18.6
			Day 4	168.5	15.0			87.1	15.1
			Day 5	193.5	17.4			90.2	16.9
			Day 6	199.5	17.2			64.6	12.9
Expt. 2	*Alexandrium catenella*		1	126.7	0.5	7.3	1.3	5.4	1.3
Expt 5	*Alexandrium catenella*		2	245.2	19.9	298.4	31.5	224.7	34.5
Expt. 8	*Alexandrium catenella*		3	173.0	13.9	153.1	16.2	129.6	21.2
		mean		**181.6**	**11.4**	**152.9**	**16.3**	**119.9**	**19.0**
		±SE		77.9	4.7	43.9	0.1	12.6	0.9
Expt. 3	*Gymnodinium* cf. *zeta*		1	6.6	1.1	5.0	1.1	3.6	0.9
Expt. 9	*Gymnodinium* cf. *zeta*		2	234.7	24.1	155.5	24.1	140.2	31.5
Expt 12	*Gymnodinium* cf. *zeta*		3	196.6	19.2	226.3	31.1	147.6	28.3
		mean		**146.0**	**14.8**	**128.9**	**18.8**	**97.1**	**20.2**
		±SE		108.7	0.8	80.1	2.1	20.2	3.4
Expt 3	*Prorocentrum micans*		1	174.1	15.8	113.8	15.8	67.0	11.3
Expt 8	*Prorocentrum micans*		2	119.5	10.6	94.9	10.7	82.6	10.7
Expt. 11	*Prorocentrum micans*		3	252.1	23.8	211.9	27.8	133.4	22.3
		mean		**181.9**	**16.7**	**140.2**	**18.1**	**94.3**	**14.7**
		±SE		76.5	0.2	22.3	1.8	24.5	2.5
Expt 4	*Prorocentrum triestinum*		1	60.0	5.3	27.8	3.5	20.7	3.6
Expt 9	*Prorocentrum triestinum*		2	143.7	13.7	110.7	15.3	94.4	17.8
Expt 13	*Prorocentrum triestinum*		3	111.7	10.1	76.3	10.2	64.4	10.9
		mean		**105.1**	**9.7**	**71.6**	**9.7**	**59.8**	**10.7**
		±SE		47.2	0.3	0.9	0.2	1.4	0.9
Expt 6	*Protoceratium reticulatum*		1	274.0	21.7	343.0	36.5	286.2	49.4
Expt 7	*Protoceratium reticulatum*		2	235.6	16.8	231.8	27.3	261.3	45.4
Expt 11	*Protoceratium reticulatum*		3	163.0	12.1	256.5	25.7	205.9	31.0
		mean		**224.2**	**16.9**	**277.1**	**29.8**	**251.1**	**41.9**
		±SE		14.0	1.2	55.2	2.8	33.1	1.4

Total PSC=Chl c_1 and c_2, Total peridin, Total Chl *a*
Total PPC = Diadinoxanthin, Diatoxanthin, Dinoxanthin and *B*-carotene.

Appendix 3.6 (a): Comparison of ratios, expressed as a percentage, of total photosynthetic pigments (PSPs) to total pigments, photoprotective pigments (PPCs) to total pigments and PSPs to PPCs of the five dinoflagellate species.

		Ratio PPC to T.Pig			Ratio PSC to T. Pig.			Ratio PPC:PSC		
	Species	LL	ML	HL	LL	ML	HL	LL	ML	HL
Expt. 2	*A. catenella*	9.4	16.4	22.2	90.6	83.6	77.8	10.3	19.6	28.5
Expt 5	*A. catenella*	7.6	9.5	13.5	92.4	90.5	86.5	8.3	10.5	15.6
Expt. 8	*A. catenella*	10.4	10.5	15.9	89.6	89.5	84.1	11.6	11.8	18.9
Expt. 3	*Gymnodinium zeta*	13.5	18.0	19.6	86.5	82.0	80.4	15.5	22.0	24.3
Expt. 9	*Gymnodinium zeta*	10.1	14.6	20.7	89.9	85.4	79.3	11.2	17.1	26.1
Expt 12	*Gymnodinium zeta*	9.5	13.6	15.4	90.5	86.4	84.6	10.5	15.7	18.2
Expt 3	*P. micans*	8.2	11.8	10.5	91.8	88.2	89.5	8.9	13.4	11.7
Expt 8	*P. micans*	8.9	10.7	12.2	91.1	89.3	87.8	9.8	11.9	13.8
Expt. 11	*P. micans*	9.4	10.1	9.4	90.6	89.9	90.6	10.4	11.2	10.4
Expt 4	*Prorocentrum triestinum*	7.6	5.5	8.0	92.4	94.5	92.0	8.3	5.8	8.7
Expt 9	*Prorocentrum triestinum*	6.7	8.5	11.7	93.3	91.5	88.3	7.2	9.3	13.2
Expt 13	*Prorocentrum triestinum*	7.4	8.4	11.5	92.6	91.6	88.5	7.9	9.1	12.9
Expt 6	*P. reticulatum*	7.1	9.7	14.9	92.9	90.3	85.1	7.7	10.7	17.5
Expt 7	*P. reticulatum*	8.8	14.4	20.9	91.2	85.6	79.1	9.7	16.8	26.4
Expt 11	*P. reticulatum*	7.7	9.9	14.2	92.3	90.1	85.8	8.3	11.0	16.5

a-1: Two-way ANOVA output for the effect of species and light level on "Ratio of photosynthetic pigments (PSC) to total pigments (T. Pig)".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	231.933	57.983	10.1338	2.59E-05	***
Light	2	260.942	130.471	22.8025	9.52E-07	***
species2:Light	8	68.883	8.61	1.5048	0.1972	
Residuals	30	171.653	5.722			

a-2: Two-way ANOVA output for the effect of species and light level on "Ratio of photoprotective pigments (PSC) to total pigments (T. Pig)".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	0.014629	0.003657	12.5139	4.19E-06	***
Light	2	0.013704	0.006852	23.4456	7.39E-07	***
species2:Light	8	0.003062	0.000383	1.3096	0.2765	
Residuals	30	0.008768	0.000292			

a-3: Two-way ANOVA output for the effect of species and light level on "Ratio of photosynthetic pigments (PSC) to photoprotective pigments (PPC)".

species2	4	0.01463	0.003658	12.5227	4.16E-06	***
Light	2	0.013712	0.006856	23.473	7.31E-07	***
species2:Light	8	0.003063	0.000383	1.3109	0.2759	
Residuals	30	0.008762	0.000292			

Appendix 3.7: Comparison of xanthophyll pools (DD + DT) normalised to Chl *a* in the five dinoflagellate species, *A. catenella*, *G. zeta*, *P. micans*, *P. triestinum* and *P. reticulatum*, expressed as a ratio when grown under low (LL), medium (ML) and high light (HL) conditions.

Experiment	**Species**		**Chl *a*-specific DD+DT LL**	**Chl *a*-specific DD+DT ML**	**Chl *a*-specific DD+DT HL**
Expt. 1	*A. catenella*	Day 1			0.10
		Day 2	0.11		0.20
		Day 3	0.1		0.31
		Day 4	0.1		0.28
		Day 5	0.1		0.32
		Day 6	0.1		0.33
2	*A. catenella*		0.09	0.2	0.25
5	*A. catenella*		0.1	0.13	0.24
8	*A. catenella*		0.1	0.15	0.26
		Mean	**0.1**	**0.16**	**0.25**
		±SE	0.002	0.02	0.004
3	*G. zeta*		0.25	0.32	0.32
9	*G. zeta*		0.17	0.28	0.41
12	*G. zeta*		0.14	0.23	0.36
		Mean	**0.19**	**0.27**	**0.36**
		±SE	0.03	0.03	0.03
3	*P. micans*		0.12	0.20	0.23
8	*P. micans*		0.12	0.16	0.19
11	*P. micans*		0.13	0.18	0.23
		Mean	**0.13**	**0.18**	**0.22**
		±SE	0.004	0.01	0.01
4	*P. triestinum*		0.1	0.18	0.22
9	*P. triestinum*		0.15	0.22	0.31
13	*P. triestinum*		0.12	0.18	0.25
		Mean	**0.13**	**0.2**	**0.26**
		±SE	0.02	0.02	0.03
6	*P. reticulatum*		0.1	0.17	0.28
7	*P. reticulatum*		0.1	0.15	0.27
11	*P. reticulatum*		0.1	0.15	0.24
		Mean	**0.1**	**0.18**	**0.26**
		±SE	0.001	0.007	0.01

Appendix 3.7 (a):Two-way ANOVA output for the effect of species and light level on "xanthophyll pools (DD + DT) normalised to Chl *a* ".

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	8.529719	1	8.529719	8249.417	0.000000
Species	0.079703	4	0.019926	19.271	0.000000
Light level	0.202146	2	0.101073	97.751	0.000000
Species*Light level	0.007909	8	0.000989	0.956	0.487478
Error	0.031019	30	0.001034		

Appendix 3.8: Diadinoxanthin percentage of total carotenoids in the five dinoflagellate species grown at three different levels of irradiance (low light (LL), medium light (ML) and high light (HL)

	Species		% Diad. LL	% Diad. ML	% Diad. HL
Time series	*A. catenella*	Day 1			11.5
		Day 2	13.8		8.3
		Day 3	13.3		9.6
		Day 4	12.8		12.2
		Day 5	12.8		22.7
		Day 6	14.0		20.5
	Mean		**13.4**		**14.1**
	±SE		0.3		2.4
Experiment					
2	*A. catenella*		4.1	10.9	7.5
5	*A. catenella*		11.4	13.9	20.9
8	*A. catenella*		11.7	14.9	20.1
	Mean		**9.1**	**13.3**	**16.2**
	±SE		2.5	1.2	4.3
3	*G. zeta*		17.4	17.5	14.0
9	*G. zeta*		18.0	26.2	25.8
12	*G. zeta*		14.0	19.4	24.2
	Mean		**16.4**	**21.1**	**21.3**
	±SE		1.3	2.6	3.7
3	*P. micans*		14.0	22.2	15.9
8	*P. micans*		15.0	16.7	10.4
11	*P. micans*		15.5	20.2	24.1
	Mean		**14.9**	**19.7**	**16.8**
	±SE		0.4	1.6	4.0
4	*P. triestinum*		13.7	20.2	17.6
9	*P. triestinum*		18.1	23.1	23.8
13	*P. triestinum*		16.2	19.4	21.5
	Mean		**16.0**	**20.9**	**21.0**
	±SE		1.3	1.1	1.8
6	*P. reticulatum*		13.9	21.6	24.5
7	*P. reticulatum*		13.3	18.9	22.3
11	*P. reticulatum*		13.7	17.6	24.6
	Mean		**13.6**	**19.4**	**23.8**
	±SE		0.2	1.2	0.8

Appendix 3.8 (a): Two-way ANOVA output for the effect of species and light level on "Diadinoxanthin percentage".

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	13877.59	1	13877.59	917.6974	0.000000
Species	285.35	4	71.34	4.7175	0.004500
Light level	291.44	2	145.72	9.6362	0.000586
Species*Light level	69.81	8	8.73	0.5770	0.788370
Error	453.67	30	15.12		

Appendix 3.9: Chl *a*-specific mean spectral absorption coefficients for the five dinoflagellate species grown at low, medium and high light conditions.

Experiment		**Mean coefficients (m^{-2} (mg Chl *a*)$^{-1}$)**		
		Low light	Medium light	High light
2	*Alexandrium catenella*	n/d	n/d	n/d
5	*Alexandrium catenella*	0.026	0.029	0.08
8	*Alexandrium catenella*	0.029	0.028	0.034
	Mean	**0.027**	**0.028**	**0.057**
	±SE	0.001	0.001	0.023
3	*Gymnodinium zeta*	0.062	n/d	n/d
9	*Gymnodinium zeta*	0.036	0.053	0.056
12	*Gymnodinium zeta*	0.035	0.042	0.027
	Mean	**0.044**	**0.031**	**0.042**
	±SE	0.009	0.005	0.014
4	*Prorocentrum triestinum*	0.027	0.025	0.028
9	*Prorocentrum triestinum*	0.025	0.028	0.027
13	*Prorocentrum triestinum*	0.027	0.031	0.035
	Mean	**0.026**	**0.028**	**0.03**
	±SE	0.001	0.002	0.003
3	*Prorocentrum micans*	0.021	0.027	0.029
8	*Prorocentrum micans*	0.04	0.038	0.036
11	*Prorocentrum micans*	0.034	0.030	0.026
	Mean	**0.032**	**0.032**	**0.03**
	±SE	0.005	0.003	0.003
6	*Protoceratium reticulatum*	0.016	0.019	0.02
7	*Protoceratium reticulatum*	0.048	0.049	n/d
11	*Protoceratium reticulatum*	0.029	0.069	0.026
	Mean	**0.031**	**0.046**	**0.023**
	±SE	0.009	0.015	0.003

Appendix 3.9 (a): Table of outputs.

a-1: Two-way ANOVA output for the effect of species and light level on "Chl *a*- specific mean spectral absorption coefficients" of five species of dinoflagellates.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
species2	4	0.83913	0.209783	1.7236	0.1777
Light.level	2	0.08457	0.042284	0.3474	0.71
species2:Light.level	8	0.71065	0.088831	0.7298	0.6643
Residuals	24	2.92113	0.121714		

Appendix 3.10: The ratio of Chl *a*-specific absorption at 440 nm to that at 675 nm of the five dinoflagellate species measured at low light, medium light and high light conditions.

Experiment			Low light	Medium light	High light
2	*A. catenella*		n/d	n/d	n/d
5	*A. catenella*		1.53	1.42	1.73
8	*A. catenella*		1.67	1.92	2.23
		Mean	**1.62**	**1.70**	**2.03**
		±SE	0.09	0.2	0.3
6	*P. reticulatum*		1.77	1.62	0.98
7	*P. reticulatum*		1.65	1.59	n/d
11	*P. reticulatum*		1.94	1.81	2.78
		Mean	**1.76**	**1.70**	**1.88**
		±SE	0.2	0.1	0.2
3	*G. zeta*		2.73	2.75	2.92
9	*G. zeta*		1.93	2.04	2.14
12	*G. zeta*		1.81	1.94	1.92
		Mean	**2.19**	**2.30**	**2.36**
		±SE	0.2	0.2	0.2
4	*P. triestinum*		1.82	1.65	1.82
9	*P. triestinum*		1.41	1.53	1.57
13	*P. triestinum*		1.38	1.49	1.74
		Mean	**1.53**	**1.55**	**1.71**
		±SE	0.2	0.1	0.2
3	*P. micans*		1.51	1.52	1.47
8	*P. micans*		1.89	1.83	1.78
11	*P. micans*		1.78	1.55	1.57
		Mean	**1.76**	**1.64**	**1.61**
		±SE	0.2	0.2	0.2

Appendix 3.10 (a): Two-way ANOVA output for the effect of species and light level on "ratio of Chl *a*-specific absorption at 440 nm to that at 675 nm".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	0.453	0.11325	1.8192	0.1567	
Light.level	2	2.19872	1.09936	17.6597	1.65E-05	***
species2:Light.level	8	0.24371	0.03046	0.4894	8.52E-01	
Residuals	25	1.55631	0.06225			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 3.11: Comparison of the estimated maximum quantum yields of five dinoflagellate species acclimated to different irradiances as shown.

Species	Low light **mol C (mol quanta m^{-2} s^{-1})**	Medium light **mol C (mol quanta m^{-2} s^{-1})**	High light **mol C (mol quanta m^{-2} s^{-1})**
Alexandrium catenella			
Alexandrium catenella	0.40	0.24	0.05
Alexandrium catenella	0.38	0.23	0.12
Mean	**0.39**	**0.24**	**0.09**
±SE	0.01	0.01	0.01
Protoceratium reticulatum	0.37	0.21	0.21
Protoceratium reticulatum	0.28	0.21	n/d
Protoceratium reticulatum\\\\\\\\\	0.23	0.09	0.14
Mean	**0.29**	**0.17**	**0.18**
±SE	0.03	0.05	0.15
Gymnodinium zeta	0.03	n/d	n/d
Gymnodinium zeta	0.13	0.08	0.02
Gymnodinium zeta	0.11	0.07	0.03
Mean	**0.09**	**0.08**	**0.03**
±SE	0.04	0.01	0.01
Prorocentrum triestinum	0.17	019	012
Prorocentrum triestinum	0.25	0.13	0.11
Prorocentrum triestinum	0.26	0.18	0.09
Mean	**0.23**	**0.17**	**0.10**
±SE	0.3	0.05	0.00
Prorocentrum micans	0.32	0.24	0.15
Prorocentrum micans	0.08	0.12	0.07
Prorocentrum micans	0.22	0.32	0.28
Mean	**0.21**	**0.23**	**0.17**
±SE	0.15	0.13	0.11
Overall mean for all species	**0.24**	**0.17**	**0.11**
±SE	0.13	0.05	0.06

Appendix 3.11 (a-1): Two-way ANOVA output for the effect of species and light level on "Maximum quantum yields".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	8.4144	2.10361	9.7477	7.89E-05	***
Light.level	2	4.1229	2.06143	9.5523	0.000888	***
species2:Light.level	8	2.0714	0.25893	1.1998	0.340265	
Residuals	24	5.1793	0.2158			

Appendix 3.11 (a-2): Two-way ANOVA output for the effect of species and light level on "Maximum quantum yields per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	54.426	13.6064	2.5262	0.06718	.
Light.level	2	3.215	1.6076	0.2985	0.74467	
species2:Light.level	8	20.599	2.5748	0.4781	0.85955	
Residuals	24	129.265	5.386			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 3.12: Total Carbon concentrations of the five dinoflagellates grown under low, medium and high irradiances and cellular carbon (TC) to chlorophyll *a* (T Chl *a*) ratios.

Date	Expt.		Total carbon mg L^{-1} Low light	Total carbon mg L^{-1} Medium light	Total carbon mg L^{-1} High light	TC : T Chl *a* Low light	TC : T Chl *a* Medium light	TC : T Chl *a* High light
Alexandrium catenella								
2002/07/16	2		5.43	5.12	5.84	187	180	296
2002/10/31	5		20.1	16.81	n/d	165	109	n/d
2003/02/14	8		15.48	19.0	18.85	283	302	396
		Mean	**13.67**	**13.64**	**12.35**	**211**	**197**	**346**
		±SE	7.9	3.9	10.0	57	107	48
Protoceratium reticulatum								
2002/12/17	6		15.58	22.42	21.48	102	128	146
2003/01/28	7		25.77	21.84	32.38	532	365	719
2002/08/12	11		6.93	10.47	11.52	106	99	129
		Mean	**16.09**	**18.24**	**21.80**	**246**	**197**	**331**
		±SE	11.9	4.4	13.0	349	172	276
Gymnodinium zeta								
2002/08/06	3		12.91	12.36	14.36	n/d	n/d	n/d
2003/07/22	9		8.81	12.73	10.55	62	142	149
2003/08/26	12		4.5	7.71	6.55	56	97	90
		Mean	**8.74**	**10.93**	**10.49**	**39**	**120**	**80**
		±SE	0.1	2.2	0.1	28	22	45
Prorocentrum triestinum								
2002/10/08	4		8.46	10.02	n/d	238	211	n/d
2003/09/09	9		17.72	25.28	25.38	106	166	206
2003/07/22	13		6.93	11.69	16.13	95	158	311
		Mean	**11.04**	**15.66**	**13.84**	**146**	**178**	**172**
		±SE	8.2	11.8	14.0	49	18	40
Prorocentrum micans								
2002/08/06	3		2.23	2.61	2.28	23	36	33
2003/02/14	8		11.68	12.15	10.39	228	236	259
2003/08/12	11		10.1	14.59	15.09	91	95	90
		Mean	**8.0**	**9.79**	**9.25**	**114**	**131**	**128**
		±SE	4.5	2.9.	1.4	139	98	28

Appendix 3.12(a): Two-way ANOVA output for the effect of groups, species and light level on "total Carbon (TC) concentration".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	597.11	149.278	2.8902	0.0428	*
Light.level	2	82.75	41.375	0.8011	0.46	
species2:Light.level	8	101.65	12.706	0.246	0.9773	
Residuals	25	1291.26	51.65			

Appendix 3.12(b): Two-way ANOVA output for the effect of species and light level on "carbon (TC) to chlorophyll *a* (T Chl *a*) ratios".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	5.9647	1.49116	2.9381	0.04045	*
Light.level	2	1.1268	0.56339	1.1101	0.34523	
species2:Light.level	8	0.7714	0.09643	0.19	0.99	
Residuals	25	12.688	0.50752			

Appendix 3.12(c): Two-way ANOVA output for the effect of species and light level on "carbon (TC) to chlorophyll *a* (T Chl *a*) ratios per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	4	60.424	15.1059	34.3347	8.08E-10	***
Light.level	2	0.018	0.0089	0.0201	9.80E-01	
species2:Light.level	8	0.856	0.107	0.2432	0.9781	
Residuals	25	10.999	0.44			

Signifiance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 4

Appendix 4.1: Comparison of chlorophyll *a* values measured using HPLC and fluorometry (% estimation of fluorometer reading versus HPLC = (HPLC-fluorometer reading/ HPLC) *100)

Experiment			HPLC Tchl *a* *n*=1 µg L^{-1}	HPLC Chl *a* *n*=1 µg L^{-1}	Fluorometer Chl *a* *n*=3 mg m^{-3}	Difference %
2	*Chaetoceros* sp.	Low light	36.75	20.22	73.66	50
		Medium light	63.37	30.14	82.36	23
		High light	37.33	13.81	64.38	42
4	*Chaetoceros* sp.	Low light	134.63	89.33	135.26	0.5
		Medium light	96.82	13.67	162.69	40
		High light	94.01	13.75	124.12	24
7	*Chaetoceros* sp.	Low light	211.88	57.20	253.43	16
		Medium light	138.78	12.89	212.33	35
		High light	103.94	7.03	178.09	42
13	*Chaetoceros* sp.	Low light	91.57	37.79	107.21	15
		Medium light	93.24	28.49	115.9	20
		High light	82.39	21.20	88.77	7
5	*Chaetoceros capense*	Low light	18.58	4.65	49.42	62
		Medium light	16.64	2.27	50.46	67
		High light	9.66	3.35	22.45	56
6	*Chaetoceros capense*	Low light	47.69	40.19	54.09	13
		Medium light	36.67	22.92	48.72	25
		High light	30.71	11.29	43.3	29
10	*Chaetoceros capense*	Low light	13.36	3.60	27.26	51
		Medium light	11.39	1.18	29.44	61
		High light	7.04	0.90	21.06	67
10	*Chaetoceros pendulus*	Low light	47.40	37.51	51.28	8
		Medium light	70.01	32.03	82.97	17
		High light	42.76	10.69	65.01	34
12	*Chaetoceros pendulus*	Low light	54.96	42.59	81.56	33
		Medium light	69.37	22.03	56.95	17
		High light	50.61	16.71	59.47	15

Appendix 4.1 (a) Table for statistical outputs.
(Chl *a* was measured fluorometrically)
(a-1): Two-way ANOVA output for the effect of species and light level on "total chlorophyll *a* (TChl *a*) concentrations" in the three diatom species.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Species2	2	7.5988	3.7994	24.8782	6.59E-06	***
Light.level	2	0.5521	0.2761	1.8077	0.1926	
Species2:Light.level	4	0.0962	0.024	0.1575	0.9571	
Residuals	18	2.7489	0.1527			

(a-2): Two-way ANOVA output for the effect species and light level on "total chlorophyll *a* (TChl *a*) concentrations per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Species2	2	30.5908	15.2954	47.7851	6.31E-08	***
Light.level	2	3.0522	1.5261	4.7677	0.0218	*
Species2:Light.level	4	0.26	0.065	0.2031	0.9334	
Residuals	18	5.7616	0.3201			

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 4.2: Cell densities (Cells L^{-1}) in each replica sample used for the incubation experiments.

Experiment	Species	Treatment	Cell counts L^{-1}
2	*Chaetoceros* sp.	Low light	2.80
		Medium light	6.18
		High light	6.10
4	*Chaetoceros* sp.	Low light	4.66
		Medium light	9.85
		High light	10.30
7	*Chaetoceros* sp.	Low light	7.14
		Medium light	6.84
		High light	13.67
13	*Chaetoceros* sp.	Low light	9.76
		Medium light	11.92
		High light	15.53
5	C. *capense*	Low light	18.09
		Medium light	19.38
		High light	20.74
6	C. *capense*	Low light	20.47
		Medium light	22.33
		High light	29.60
10	C. *capense*	Low light	9.88
		Medium light	10.86
		High light	11.27
10	*C. pendulus*	Low light	32.05
		Medium light	50.52
		High light	53.55
12	*C. pendulus*	Low light	39.90
		Medium light	53.26
		High light	66.03

Cell number = n x 10^6 L^{-1}

Appendix 4.3: Comparison of photosynthetic parameters of different species of diatoms. (*Chaetoceros* sp., *Chaetoceros capense* and *Chaetoceros* cf. *pendulus.*

Sample statistics are mean ± standard error.

		Low light (33 µmol quanta m^{-2} s^{-1})			**Medium light** (178 µmol quanta m^{-2} s^{-1})			**High light** (647 µmol quanta m^{-2} s^{-1})		
Species	**Experiment**	**Maximum photosynthetic Rate** (mg C (mg Chl*a*)$^{-1}$ h^{-1})	**Maximum light utilization Coefficient** (mg C m^{-3} (mg Chl*a*)$^{-1}$ (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light Saturation Parameter** (µmol quanta m^{-2} s^{-1})	**Maximum photosynthetic Rate** (mg C m^{-3} (mg Chla^{-1}) h^{-1})	**Maximum light utilization coefficient** (mg C m^{-3} (mg Chla^{-1}) (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light saturation parameter** (µmol quanta m^{-2} s^{-1})	**Maximum photosynthetic Rate** (mg C m^{-3} (mg Chla^{-1}) h^{-1})	**Maximum light utilization coefficient** (mg C m^{-3} (mg Chla^{-1}) (µmol quanta m^{-2} s^{-1})$^{-1}$	**Light saturation parameter** (µmol quanta m^{-2} s^{-1})
		P^*_m	α^*	E_k	P^*_m	α^*	E_k	P^*_m	α^*	E_k
Chaetoceros sp.	2	2.37	0.02	105	5.26	0.04	131	5.40	0.04	138
Chaetoceros sp.	4	1.28	0.01	120	2.01	0.01	146	2.25	0.01	191
Chaetoceros sp.	7	1.79	0.02	87	2.73	0.03	100	2.59	0.2	131
Chaetoceros sp.	13	0.78	0.006	157	2.59	0.01	259	3.18	0.01	268
Mean		**1.56**	**0.01**	**117**	**3.15**	**0.02**	**159**	**3.35**	**0.02**	**182**
± SE		0.9	1.2	1.3	1.2	1.1	1.2	1.3	1.1	1.2
C. capense	5	3.71	0.02	153	5.93	0.02	255	17.09	0.05	356
C. capense	6	0.54	0.01	94	1.31	0.01	130	2.28	0.01	204
C. capense	10	7.90	0.03	234	17.97	0.05	330	20.05	0.05	403
Mean		**4.05**	**0.02**	**160**	**8.40**	**0.03**	**238**	**13.14**	**0.04**	**321**
± SE		1.2	1.3	1.2	1.0	1.0	1.3	1.4	1.4	1.2
C. cf. *pendulus*	10	3.43	0.02	155	5.68	0.02	232	5.17	0.02	314
C. cf. *pendulus*	12	3.76	0.03	147	3.96	0.02	200	3.87	0.01	324
Mean		**3.59**	**0.02**	**151**	**4.82**	**0.02**	**216**	**4.52**	**0.01**	**319**
± SE		0.2	0.002	4.0	0.9	0.002	16.2	0.7	0.002	5.3

Appendix 4.3 (a – 1): Two-way ANOVA output for the effect of species and light level on "Maximum photosynthetic rate".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
species2	2	3.0889	1.54446	2.3228	0.1266
Light.level	2	3.3635	1.68175	2.5293	0.1076
species2:Light.level	4	0.7903	0.19757	0.2971	0.876
Residuals	18	11.9682	0.6649		

Appendix 4.3 (a – 2): Two-way ANOVA output for the effect of species and light level on "Maximum light utilisation coefficient (α*)".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
species2	2	0.000485	0.000243	1.1567	0.3368
Light.level	2	0.000278	0.000139	0.6623	0.5278
species2:Light.level	4	0.000469	0.000117	0.5596	0.6949
Residuals	18	0.003775	0.00021		

Appendix 4.3 (a – 3): Two-way ANOVA output for the effect of species and light level on "Light saturation parameter (E_k)".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	45994	22996.8	5.0932	0.01766	*
Light.level	2	55878	27939.2	6.1878	0.00901	**
species2:Light.level	4	20534	5133.6	1.137	0.3707	
Residuals	18	81274	4515.2			

Appendix 4.3 (a – 4): Two-way ANOVA output for the effect of species and light level on "Maximum photosynthetic rate per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	7.3593	3.6796	6.5633	0.007232	**
Light.level	2	1.1102	0.5551	0.9901	0.390882	
species2:Light.level	4	1.4255	0.3564	0.6357	0.643631	
Residuals	18	10.0915	0.5606			

Appendix 4.3 (a – 5): Two-way ANOVA output for the effect of species and light level on "Maximum light utilisation coefficient (α*) per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	3473.9	1736.93	20.5289	2.27E-05	***
Light.level	2	56.5	28.23	0.3336	0.7206	
species2:Light.level	4	487.1	121.78	1.4393	0.2617	
Residuals	18	1523	84.61			

Appendix 4.3 (a – 6): Two-way ANOVA output for the effect of species and light level on "Light saturation parameter (E_k) per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	0.22951	0.114755	71.9524	2.60E-09	***
Light.level	2	0.004462	0.002231	1.3987	0.2725	
species2:Light.level	4	0.01284	0.00321	2.0128	0.1357	
Residuals	18	0.028708	0.001595			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 4.4: Pigment ratios for the diatom species. Chlorophyll *a* (Chl *a*), chlorophyll $c_{1,2}$ (Chlc), fucoxanthin (Fuc), diadinoxanthin (Dia) diatoxanthin (Diato), β-carotene (Bcar).

	Low light						**Medium light**						**High light**					
	TChl *a* :Tpig	**Chl *c* :Tpig**	**Fuc:Tpig**	**Diad:Tpig**	**Diato:Tpig**	**Bcar:Tpig**	**TChl *a* :Tpig**	**Chl *c*:Tpig**	**Fuc:Tpig**	**Diad:Tpig**	**Diato:Tpig**	**Bcar:Tpig**	**TChl *a* : Tpig**	**Chlc:Tpig**	**Fuc:Tpig**	**Diad:Tpig**	**Diato:Tpig**	**Bcar:Tpig**
Chaetoceros sp.	0.52	0.17	0.22	0.06	0.004	0.01	0.53	0.20	0.18	0.06	0.006	0.010	0.50	0.22	0.15	0.09	0.020	0.012
Chaetoceros sp.	0.55	0.15	0.22	0.06	0.001	0.01	0.54	0.24	0.14	0.07	0.005	0.009	0.51	0.24	0.08	0.13	0.020	0.014
Chaetoceros sp.	0.52	0.17	0.23	0.04	0.001	0.01	0.56	0.23	0.13	0.08	0.008	0.009	0.51	0.27	0.08	0.11	0.020	0.008
Chaetoceros sp.	0.56	0.12	0.23	0.07	0.004	0.01	0.52	0.16	0.24	0.05	0.006	0.012	0.53	0.16	0.21	0.06	0.018	0.013
Mean	**0.54**	**0.16**	**0.23**	**0.06**	**0.003**	**0.01**	**0.53**	**0.20**	**0.18**	**0.07**	**0.006**	**0.010**	**0.51**	**0.22**	**0.13**	**0.1**	**0.020**	**0.012**
±SE	0.01	0.01	0.003	0.006	0.001	0.0001	0.003	0.02	0.03	0.008	0.001	0.001	0.007	0.02	0.03	0.02	0.001	0.001
C. capense	0.56	0.26	0.08	0.04	0.009	0.01	0.51	0.30	0.09	0.04	0.011	0.018	0.48	0.38	0.03	0.03	0.012	0.012
C. capense	0.54	0.12	0.25	0.06	0.013	0.01	0.52	0.12	0.24	0.08	0.021	0.017	0.52	0.09	0.19	0.11	0.070	0.022
C. capense	0.56	0.27	0.08	0.03	0.019	0.02	0.43	0.45	0.05	0.03	0.015	0.020	0.49	0.38	0.04	0.03	0.012	0.023
Mean	**0.55**	**0.22**	**0.14**	**0.04**	**0.014**	**0.02**	**0.49**	**0.29**	**0.13**	**0.05**	**0.016**	**0.018**	**0.50**	**0.28**	**0.09**	**0.06**	**0.031**	**0.019**
±SE	0.009	0.05	0.06	0.009	0.003	0.001	0.03	0.01	0.06	0.01	0.003	0.001	0.01	0.01	0.05	0.03	0.019	0.004
C. cf. *pendulus*	0.51	0.14	0.25	0.04	0.008	0.02	0.50	0.16	0.18	0.06	0.011	0.018	0.48	0.13	0.14	0.14	0.031	0.019
C. cf. *pendulus*	0.49	0.02	0.34	0.04	0.004	0.02	0.42	0.18	0.17	0.07	0.012	0.012	0.46	0.11	0.18	0.13	0.037	0.014
Mean	**0.50**	**0.08**	**0.29**	**0.04**	**0.006**	**0.02**	**0.46**	**0.17**	**0.18**	**0.06**	**0.012**	**0.015**	**0.47**	**0.12**	**0.16**	**0.13**	**0.034**	**0.017**
±SE	0.007	0.06	0.07	0.0002	0.002	0.002	0.04	0.008	0.009	0.004	0.001	0.003	0.01	0.009	0.02	0.005	0.003	0.003

Appendix 4.4 (a – 1): Two-way ANOVA output for the effect of species and light level on "Photosynthetic pigments (PSC) : total pigment (T.pig.) ratio".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	27.78	13.892	0.6243	0.54683	
Light	2	176.8	88.398	3.9728	0.03723	*
species2:Light	4	40.12	10.03	0.4508	0.77055	
Residuals	18	400.52	22.251			

Appendix 4.4 (a – 2): Two-way ANOVA output for the effect of species and light level on "Photoprotective pigments (PPC) : total pigment (T.pig.) ratio".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	41.65	20.823	0.8426	0.44686	
Light	2	156.38	78.189	3.1641	0.06644	.
species2:Light	4	43.68	10.92	0.4419	0.77675	
Residuals	18	444.8	24.711			

Appendix 4.5: Photosynthetic and photoprotective pigment ratios of the three diatom species acclimated to low (LL), medium (ML) and high (HL) irradiances calculated as a percentage of total pigment content.

	Low light		**Medium light**		**High light**	
	PSC : T. pig.	**PPC : T. pig.**	**PSC : T. pig.**	**PPC : T. pig.**	**PSC : T. pig.**	**PPC : T. pig.**
Chaetoceros sp.	22.87	5.12	16.05	7.48	12.69	11.51
C. capense	18.45	6.23	16.30	7.83	13.32	13.16
C. pendulus	29.76	4.69	17.47	7.25	16.47	16.36

% difference between the mean PSC and PPC concentrations at different light levels in three diatom species

	LL	ML	HL
	%	%	%
Chaetoceros sp.	17.75	8.57	1.17
C. capense	12.23	8.48	0.16
C. pendulus	25.06	10.22	0.11

Photosynthetic pigments (PSC) = Fucoxanthin

Photoprotective pigments (PPC) = Diadinoxanthin and diatoxanthin

Appendix 4.5 (a – 1): Two-way ANOVA output for the effect of species and light level on "PSP : PPC ratio".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
species2	2	1.3631	0.68155	1.9678	0.1687
Light	2	1.4988	0.74942	2.1637	0.1438
species2:Light	4	0.558	0.13949	0.4027	0.8042
Residuals	18	6.2345	0.34636		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 4.6: Diadinoxanthin percentage of the total carotenoids in the three diatom species grown at three different levels of irradiance (low light (LL), medium light (ML) and high light (HL)).

Experiment	Species	% Diad. LL	% Diad. ML	% Diad. HL
2	*Chaetoceros* sp.	20.9	20.6	32.6
4	*Chaetoceros* sp.	19.9	32.5	52.1
7	*Chaetoceros* sp.	n/d	35.5	n/d
13	*Chaetoceros* sp.	13.6	36.5	50.7
13	*Chaetoceros* sp.	18.8	16.4	18.6
	Mean	**18.3**	**35.4**	**38.5**
	±SE	1.6	4.1	8.0
5	*C. capense*	26.3	24.6	37.9
6	*C. capense*	20.3	29.1	29.0
10	*C. capense*	17.5	22.6	28.5
	Mean	**21.3**	**25.4**	**31.8**
	±SE	2.6	1.9	3.0
10	*C. pendulus*	13.5	20.9	41.3
12	*C. pendulus*	10.3	25.9	34.7
	Mean	**11.9**	**23.4**	**38.0**
	±SE	1.6	2.5	3.3

Appendix 4.6 (a): Two-way ANOVA output for the effect of species and light level on "Diadinoxanthin percentage of total carotenoids".

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	17563.76	1	17563.76	258.2153	0.000000
Species	67.13	2	33.57	0.4935	0.618110
Light level	1492.40	2	746.20	10.9703	0.000680
Species*Light level	161.80	4	40.45	0.5947	0.670781
Error	1292.38	19	68.02		

Appendix 4.7: Comparison of xanthophyll pools (DD+DT) normalised to Chl *a* in the three diatom species, *Chaetoceros* sp., *Chaetoceros capense* and *Chaetoceros* cf. *pendulus*, expressed as a ratio, when grown under low (LL), medium (ML) and high (HL) conditions.

Experiment	**Species**	**Chl *a*-specific DD+DT** LL	**Chl *a*-specific DD+DT** ML	**Chl *a*-specific DD+DT** HL
2	*Chaetoceros* sp.	0.55	0.48	0.49
4	*Chaetoceros* sp.	0.51	0.36	0.40
7	*Chaetoceros* sp.	0.52	0.40	0.37
13	*Chaetoceros* sp.	0.50	0.56	0.50
	Mean	**0.52**	**0.45**	**0.44**
	±SE	0.01	0.04	0.03
5	*C. capense*	0.21	0.25	0.14
6	*C. capense*	0.20	0.18	0.15
10	*C. capense*	0.57	0.62	0.58
	Mean	**0.33**	**0.35**	**0.29**
	±SE	0.12	0.14	0.15
10	*C. pendulus*	0.57	0.48	0.57
12	*C. pendulus.*	0.77	0.48	0.67
	Mean	**0.67**	**0.48**	**0.62**
	±SE	0.10	0.003	0.05

Appendix 4.8: Changes in the concentrations of diadinoxanthin (DD) and diatoxanthin (DT) as a result of interconversion between DD and DT during xanthophyll cycling in diatoms. A comparison between cultures grown at different light levels.

		Low light			**Medium light**			**High light**		
Experiment	**Species**	**Chl *a*-specific** DD+DT µg L^{-1}	**Chl *a*-specific** DD µg L^{-1}	**Chl *a*-specific** DT µg L^{-1}	**Chl *a*-specific** DD+DT µg L^{-1}	**Chl *a*-specific** DD µg L^{-1}	**Chl *a*-specific** DT µg L^{-1}	**Chl *a*-specific** DD+DT µg L^{-1}	**Chl *a*-specific** DD µg L^{-1}	**Chl *a*-specific** DT µg L^{-1}
2	*Chaetoceros* sp.	0.55	0.43	0.12	0.48	0.37	0.10	0.49	0.31	0.18
4	*Chaetoceros* sp.	0.51	0.40	0.11	0.36	0.22	0.14	0.40	0.16	0.24
7	*Chaetoceros* sp.	0.52	0.45	0.07	0.40	0.25	0.16	0.37	0.16	0.22
13	*Chaetoceros* sp.	0.50	0.40	0.10	0.56	0.46	0.10	0.50	0.40	0.10
	Mean	**0.52**	**0.42**	**0.10**	**0.45**	**0.33**	**0.12**	**0.44**	**0.26**	**0.19**
	±SE	0.01	0.01	0.01	0.04	0.06	0.01	0.03	0.06	0.03
5	*Chaetoceros capense*	0.21	0.14	0.07	0.25	0.17	0.07	0.14	0.07	0.07
6	*Chaetoceros capense*	0.20	0.14	0.05	0.18	0.11	0.08	0.15	0.09	0.06
10	*Chaetoceros capense*	0.57	0.46	0.11	0.62	0.47	0.16	0.58	0.36	0.22
	Mean	**0.33**	**0.25**	**0.08**	**0.35**	**0.25**	**0.10**	**0.29**	**0.17**	**0.12**
	±SE	0.12	0.11	0.02	0.14	0.11	0.03	0.15	0.10	0.05
10	*Chaetoceros pendulus*	0.57	0.48	0.08	0.48	0.37	0.11	0.57	0.29	0.28
12	*Chaetoceros pendulus*	0.77	0.68	0.08	0.48	0.35	0.14	0.67	0.40	0.27
	Mean	**0.67**	**0.58**	**0.08**	**0.48**	**0.36**	**0.12**	**0.62**	**0.35**	**0.28**
	±SE	0.10	0.10	0.001	0.00	0.01	0.01	0.05	0.05	0.00

Appendix 4.8 (a - 1): Two-way ANOVA output for the effect of species and light level on "Diadinoxanthin concentration".

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	2.692545	1	2.692545	159.0795	0.000000
species	0.157350	2	0.078675	4.6482	0.023577
Light level	0.109341	2	0.054670	3.2300	0.063291
species*Light level	0.033320	4	0.008330	0.4921	0.741582
Error	0.304664	18	0.016926		

Appendix 4.8 (a - 2): Two-way ANOVA output for the effect of species and light level on "Diatoxanthin concentration".

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	0.374234	1	0.374234	243.7914	0.000000
species	0.011624	2	0.005812	3.7863	0.042410
Light level	0.037009	2	0.018504	12.0545	0.000477
species*Light level	0.012253	4	0.003063	1.9955	0.138402
Error	0.027631	18	0.001535		

Appendix 4.9: The Chl *a*-specific absorption coefficients at excitation wavelengths 440 and 675 nm of the three diatom species acclimated to low (LL), medium (ML) and high light (HL) conditions.

Species		Wavelengths (λ)					
		440 nm	**675 nm**	**440 nm**	**675 nm**	**440 nm**	**675 nm**
		LL	**LL**	**ML**	**ML**	**HL**	**HL**
Chaetoceros sp.		0.023	0.020	0.030	0.026	0.038	0.026
Chaetoceros sp.		0.022	0.017	0.034	0.022	0.026	0.018
Chaetoceros sp.		0.025	0.018	0.002	0.002	0.032	0.021
Chaetoceros sp.		0.033	0.020	0.032	0.020	0.031	0.017
	Mean	**0.026**	**0.019**	**0.024**	**0.018**	**0.032**	**0.020**
	±SE	0.007	0.006	0.005	0.003	0.007	0.005
Chaetoceros capense		0.034	0.021	0.055	0.028	0.106	0.059
Chaetoceros capense		0.036	0.023	0.041	0.024	0.046	0.024
Chaetoceros capense		0.067	0.042	0.075	0.042	0.096	0.049
	Mean	**0.046**	**0.029**	**0.057**	**0.032**	**0.083**	**0.044**
	±SE	0.027	0.017	0.044	0.023	0.070	0.036
Chaetoceros pendulus		0.047	0.030	0.044	0.027	0.049	0.027
Chaetoceros. Pendulus		0.061	0.040	n/d	n/d	0.072	0.038
	Mean	**0.044**	**0.036**	**0.044**	**0.027**	**0.055**	**0.029**
	±SE	0.005	0.004	0.000	0.000	0.002	0.002

Appendix 4.10: The ratio of absorption at 440 nm to that of 675 nm of the three diatom species measured at low light (LL), medium light (ML) and high light conditions (HL).

Experiment		440:675 nm LL	440:675 nm ML	440:675 nm HL
2	*Chaetoceros* sp.	1.2	1.1	1.5
4	*Chaetoceros* sp.	1.3	1.5	1.5
7	*Chaetoceros* sp.	1.3	1.5	1.6
13	*Chaetoceros* sp.	1.6	1.6	1.8
	Mean	**1.4**	**1.4**	**1.6**
	±SE	0.09	0.1	0.07
5	*Chaetoceros capense*	1.6	1.9	1.8
6	*Chaetoceros capense*	1.5	1.7	1.9
10	*Chaetoceros capense*	1.6	1.8	2.0
	Mean	**1.6**	**1.8**	**1.9**
	±SE	0.01	0.08	0.04
10	*Chaetoceros pendulus*	1.6	1.6	1.8
12	*Chaetoceros pendulus*	1.5	n/d	1.9
	Mean	**1.5**	**1.6**	**1.9**
	±SE	0.03	n/d	0.06

Appendix 4.10 (a): Two-way ANOVA output for the effect of species and light level on "Ratio of absorption at wavelengths 440 and 675 nm".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	0.202948	0.101474	11.092	0.000827	***
Light.level	2	0.130499	0.065249	7.1323	0.005635	**
species2:Light.level	4	0.008371	0.002093	0.2288	0.91846	
Residuals	17	0.155524	0.009148			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 4.11: Chl *a*-specific mean spectral absorption coefficients for the three diatom species grown at low, medium and high light conditions.

Experiment	Species		Mean spectral coefficients (m^{-2} (mg Chl *a*)$^{-1}$) Low light	Medium light	High light
2	*Chaetoceros* sp.		0.013	0.017	0.019
4	*Chaetoceros* sp.		0.013	0.018	0.014
7	*Chaetoceros* sp.		0.015	0.001	0.016
13	*Chaetoceros* sp.		0.017	0.017	0.015
		Mean	**0.015**	**0.013**	**0.016**
		±SE	0.001	0.004	0.001
5	*Chaetoceros capense*		0.026	0.029	0.08
6	*Chaetoceros capense*		0.019	0.021	0.021
10	*Chaetoceros capense*		0.036	0.039	0.049
		Mean	**0.027**	**0.030**	**0.050**
		±SE	0.005	0.005	0.017
10	*Chaetoceros* cf. *pendulus*		0.024	0.022	0.023
12	*Chaetoceros* cf. *pendulus*		0.021	n/d	0.028
		Mean	**0.022**	**0.022**	**0.025**
		±SE	0.001	0.0	0.003

Appendix 4.11 a: Table of outputs.
a-1: Two-way ANOVA output for the effect of species and light level on "Chl *a*- specific mean spectral absorption coefficients" of three diatom species.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	4.7569	2.37843	5.3588	0.01568	*
Light.level	2	0.9386	0.46932	1.0574	0.36912	
species2:Light.level	4	0.4329	0.10822	0.2438	0.90944	
Residuals	17	7.5452	0.44383			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 4.12: Comparison of the estimated maximum quantum yields of the three diatom species acclimated to different irradiances as indicated

Species	Low light		Medium light		High light	
	mol C (Chl *a*$^{-1}$)	mol quanta m^{-2} s^{-1}	mol C (Chl *a*$^{-1}$)	mol quanta m^{-2} s^{-1}	mol C (Chl *a*$^{-1}$)	mol quanta m^{-2} s^{-1}
Chaetoceros sp.	0.39		0.49		0.43	
Chaetoceros sp.	0.09		0.18		0.19	
Chaetoceros sp.	0.32		0.52		0.28	
Chaetoceros sp.	0.07		0.14		0.19	
Mean	**0.24**		**0.33**		**0.27**	
±SE	0.1		0.2		0.1	
Chaetoceros capense	0.22		0.18		0.14	
Chaetoceros capense	0.07		0.11		0.14	
Chaetoceros capense	0.41		0.65		0.45	
Mean	**0.23**		**0.31**		**0.24**	
±SE	0.2		0.2		0.15	
Chaetoceros cf. *pendulus*	0.43		0.33		0.34	
Chaetoceros cf. *pendulus*	0.27		n/d		0.10	
Mean	**0.35**		**0.33**		**0.22**	
±SE						
Overall **mean** for all species	**0.21**		**0.24**		**0.17**	
±SE	0.02		0.09		0.08	

Appendix 4.12 (a-1): Two-way ANOVA output for the effect of species and light level on "Maximum quantum yields".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
species2	2	0.192	0.09599	0.1781	0.8384
Light.level	2	0.4024	0.20122	0.3733	0.6939
species2:Light.level	4	0.7436	0.1859	0.3449	0.8439
Residuals	17	9.1629	0.539		

Appendix 4.12 (a-2): Two-way ANOVA output for the effect of species and light level on "Maximum quantum yields per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	53.292	26.6458	4.6484	0.02453	*
Light.level	2	4.367	2.1837	0.381	0.6889	
species2:Light.level	4	5.94	1.485	0.2591	0.90008	
Residuals	17	97.449	5.7323			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 4.13: Total carbon concentrations of the three diatom species grown under low, medium and high irradiances and total carbon (TC) to chlorophyll *a* (Chl *a*) ratios.

.			Total carbon mg L^{-1}	Total carbon mg L^{-1}	Total carbon mg L^{-1}	TC : TChl *a* ratio	TC : TChl *a* ratio Medium	TC : TChl *a* Ratio
Date	**Experiment**		**Low light**	**Medium light**	**High light**	**Low light**	**light**	**High light**
Chaetoceros sp								
2002/07/16	2		4.49	6.09	5.47	60	74	85
2002/10/08	4		9.10	13.08	13.82	67	80	111
2003/01/28	7		16.91	16.46	18.62	67	78	104
		Mean	**10.17**	**11.87**	**12.64**	**65**	**77**	**100**
		±SE	1.3	1.5	1.5	2	4	16
Chaetoceros capense								
2002/10/31	5		13.32	10.82	11.58	269	214	516
2003/07/29	6		7.19	10.20	6.79	130	211	156
2003/08/26	10		5.12	6.23	6.39	252	260	369
		Mean	**8.54**	**9.08**	**8.25**	**217**	**229**	**347**
		±SE	1.7	1.4	1.8	106	23	230
Chaetoceros cf**. *pendulus***								
2002/12/17	10		6.91	11.03	9.67	85	87	162
2003/07/29	12		4.46	7.28	9.95	87	193	152
		Mean	**5.69**	**9.15**	**9.81**	**86**	**140**	**157**
		±SE	0.0	0.0	0.0	0.0	0.0	0.0

Appendix 4.13 (a-1): Two-way ANOVA output for the effect of species and light level on "Total carbon".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
species2	2	0.3331	0.166573	0.7597	0.485
Light.level	2	0.3028	0.151388	0.6905	0.5166
species2:Light.level	4	0.1977	0.049416	0.2254	0.92
Residuals	15	3.2888	0.219252		

Appendix 4.13 (a-2): Two-way ANOVA output for the effect of species and light level on "Total carbon to Chlorophyll *a* ratio".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	1.37E-05	6.85E-06	55.3434	1.19E-07	***
Light.level	2	2.83E-06	1.42E-06	11.4416	0.0009602	***
species2:Light.level	4	9.18E-07	2.30E-07	1.8542	0.1710505	
Residuals	15	1.86E-06	1.24E-07			

Appendix 4.13 (a-3): Two-way ANOVA output for the effect of species and light level on "Total carbon to Chlorophyll *a* ratio per cell".

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
species2	2	18.6733	9.3366	35.5296	2.04E-06	***
Light.level	2	0.2163	0.1081	0.4115	0.6699	
species2:Light.level	4	0.6174	0.1543	0.5874	0.6768	
Residuals	15	3.9418	0.2628			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Appendix 5

Appendix 5.1: Comparison of chlorophyll *a* values measured using HPLC and fluorometry

			Time	**HPLC Tchl *a*** (n=1) µg.L^{-1}	**HPLC Chl *a*** (n=1) µg.L^{-1}	**Fluorometer Chl *a*** (n=3) mg m^{-3}	**Difference** %
Expt. 14	*Chaetoceros capense*	Low light	0	23.52	16.47	19.86	16
		High light	0.5	24.44	18.41	18.28	25
		High light	1	18.18	8.97	20.12	10
			1.5				
		High light	2	9.69	1.39	18.62	48
		Low light	2.5	15.06	7.00	18.61	19
		Low light	3	16.52	8.48	20.17	18
			3.5				
		Low light	4	12.56	1.46	20.24	38
		High light	4.5	11.13	1.74	18.96	41
		High light	5	10.24	1.35	19.27	47
Expt. 15							
	Prorocentrum triestinum	Low light	0	86.36	85.02	37.8	56
		High light	0.5	58.63	57.82	42.36	27
		High light	1	59.28	58.24	40.08	32
			1.5				
		High light	2	44.68	43.69	43.91	2
		Low light	2.5	53.01	51.67	51.58	3
		Low light	3	50.08	48.64	46.76	7
			3.5				
		Low light	4	49.63	46.93	46.84	6
		High light	4.5	45.90	44.76	49.94	8
		High light	5	63.13	61.51	46.84	26

Appendix 5.2: Cell density in the diatom and dinoflagellate samples used in the light shift experiment

Sample #	Time (h)		Diatom *Chaetoceros capense* Cells L^{-1} (Cell number x 10^{-6})	Dinoflagellate *Prorocentrum triestinum* Cells L^{-1} (Cell number x 10^{-6})
1	0	Low light	9	8
2	0.5	High light	14	11
3	1	High light	19	14
4	2	High light	15	15
5	2.5	Low light	12	9
6	3	Low light	10	12
7	4	Low light	11	11
8	4.5	High light	14	13
9	5	High light	19	16

Appendix 5.3: Comparison of photosynthetic parameters between the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* after transfer from low to high irradiance levels and *vice versa* at half hourly and hourly intervals. (LL = 33, ML = 178, HL = 647 µmol quanta m^{-2} s^{-1}).

	Sample		Time (h)	Maximum photosynthetic rate (mg C (mg Chl *a*$^{-1}$) m^{-3} hr^{-1}) P^*_m	Maximum light utilization Coefficient (mg C m^{-3} (mg Chl *a*$^{-1}$) µmol quanta m^{-2} s^{-1} α*	Light Saturation Parameter µmol quanta m^{-2} s^{-1} E_k
Chaetoceros capense	1	Low light	0	9.02	0.046	223
	2	High light	0.5	9.29	0.031	297
	3	High light	1	9.31	0.031	297
	4	High light	2	10.51	0.039	272
	5	Low light	2.5	11.18	0.041	271
	6	Low light	3	9.35	0.036	259
	7	Low light	4	11.65	0.045	260
	8	High light	4.5	11.98	0.038	313
	9	High light	5	13.45	0.042	320
Prorocentrum triestinum	1	Low light	0	7.71	0.033	236
	2	High light	0.5	7.25	0.029	249
	3	High light	1	8.41	0.031	274
	4	High light	2	8.45	0.024	347
	5	Low light	2.5	6.73	0.021	324
	6	Low light	3	8.07	0.026	309
	7	Low light	4	7.21	0.025	285
	8	High light	4.5	6.72	0.024	279
	9	High light	5	8.30	0.032	259

Appendix 5.3 (a-1): Two-way ANOVA output for the effect of species and light level on "Maximum photosynthetic rate (P^*_m)".

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	81.89132	1	81.89132	577.0752	0.000000
Species	5.03579	1	5.03579	35.4864	0.000035
Light level	0.08679	1	0.08679	0.6116	0.447201
Species*Light level	0.05420	1	0.05420	0.3819	0.546487
Error	1.98671	14	0.14191		

Appendix 5.3 (a-2): Two-way ANOVA output for the effect of species and light level on "Maximum light utilisation coefficient (α*)".

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	625.1548	1	625.1548	744.2597	0.000000
Species	41.4612	1	41.4612	49.3604	0.000006
Light level	0.4368	1	0.4368	0.5200	0.482720
Species*Light level	0.0453	1	0.0453	0.0539	0.819810
Error	11.7596	14	0.8400		

Appendix 5.4: A comparison of the changes of total chlorophyll *a* concentrations per cell of the diatom *Chaetoceros capense* and the dinoflagellate *Prorocentrum triestinum* when subjected to three light shifts over a period of 5 hours.

Time (h.)	Treatment	***Prorocentrum triestinum*** **Chl *a* per cell (pg)** **($x10^{-6}$)**	***Chaetoceros capense*** **Chl *a* per cell (pg)** **($x10^{-6}$)**
0	Low light	4.5	2.1
0.5	High light	4.1	1.2
1	High light	3.0	1.1
2	High light	3.1	1.2
2.5	Low light	5.7	1.5
3	Low light	3.8	1.9
4	Low light	4.1	1.8
4.5	High light	4.1	1.5
5	High light	1.8	1.0

Appendix 5.5: A comparison of the changes in the diadinoxanthin and diatoxanthin pool, including normalisation to chlorophyll *a*, in the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* during transfers from low light to high light environments and *vice versa*.

Chaetoceros capense	**Cells L^{-1}**	**Time**	**Diad**	**Diato**	**Diad :Diato**	**Diad+Diato**	**T. Chl a**	**Chl *a*-specific DD+DT**	**Diad :Diato**	**Chl *a*-specific DD-DT per cell**
Treatment	$N = 10^6$	(h)	µg L^{-1}	µg L^{-1}	µg:µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	(pg)
Low light	9.5	0	1.1	0.04	28.7	1.1	19.9	0.06	1.44	0.006
High light	14.9	0.5	1.3	0.2	7.8	1.5	18.3	0.08	0.43	0.005
High light	20.8	1	1.4	0.09	15.8	1.6	20.1	0.08	0.79	0.004
		1.5								
High light	15.0	2	1.1	0.2	5.3	1.3	18.6	0.07	0.29	0.005
Low light	12.1	2.5	1.3	0.1	9.9	1.5	18.6	0.08	0.53	0.007
Low light	10.5	3	1.4	0.1	11.7	1.5	20.2	0.08	0.58	0.007
		3.5								
Low light	11.2	4	1.7	0.09	18.2	1.7	20.2	0.09	0.90	0.008
High light	13.5	4.5	1.2	0.2	6.1	1.4	19.0	0.08	0.32	0.006
High light	18.7	5	1.2	0.3	4.2	1.5	19.3	0.08	0.22	0.004

Prorocentrum triestinum	**Cells L^{-1}**	**Time**	**Diad**	**Diato**	**Diad : Diato**	**Diad + Diato**	**T. Chl a**	**Chl *a*-specific DD+DT**	**Diad :Diato**	**Chl *a*-specific DD+DT per cell**
Treatment	$N = 10^6$	(h)	µg L^{-1}	µg L^{-1}	µg:µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	(pg)
Low light	8.3	0	11.7	0.5	23.9	12.2	37.8	0.32	0.63	0.039
High light	10.7	0.5	8.6	0.8	11.1	9.4	42.4	0.22	0.26	0.021
High light	13.7	1	9.5	1.2	8.2	10.6	40.2	0.27	0.20	0.019
		1.5								
High light	14.9	2	8.1	1.1	7.5	9.0	43.9	0.21	0.17	0.014
Low light	9.8	2.5	10.1	1.0	10.1	11.0	51.6	0.21	0.20	0.024
Low light	12.2	3	9.1	0.8	11.6	9.9	46.8	0.21	0.25	0.017
		3.5								
Low light	11.2	4	8.1	0.6	13.0	8.6	46.8	0.18	0.26	0.016
High light	13.2	4.5	7.9	0.7	10.6	8.6	49.9	0.17	0.21	0.013
High light	25.8	5	11.0	1.0	10.2	11.5	46.8	0.25	0.22	0.015

Appendix 5.6: A comparison of the changes in photosynthetic and photoprotective pigments and ratios in the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* during transfers from low light to high light environments and *vice versa*.

PSP = Photosynthetic pigments (chlorophyll *a*, chlorophyll c_1,c_2, peridinin (dinoflagellates and fucoxanthin (diatoms); PPC = Photoprotective carotenoids (diadinoxanthin, diatoxanthin, β-carotene), T. Pig. = total pigments.

		Total pigments µg L^{-1}	Total PSP µg L^{-1}	Total PPC µg L^{-1}	T. Pig:PSP ratio %	PSP:PPC ratio %	PSP:Chl *a* ratio %	Chl *a*:PPC ratio %
C. capense	Low light	49.1	46.9	1.9	95.5	4.0	42.4	9.3
	High light	47.7	45.0	2.4	94.3	5.2	40.6	12.9
	High light	54.1	51.2	2.5	94.7	4.8	39.3	12.2
	High light	49.7	47.3	2.0	95.2	4.2	39.3	10.7
	Low light	50.1	47.5	2.2	94.9	4.6	39.1	11.8
	Low light	53.5	50.7	2.3	94.8	4.6	39.8	11.5
	Low light	58.9	55.8	2.6	94.6	4.7	36.2	13.0
	High light	53.1	50.4	2.2	94.9	4.3	37.7	11.5
	High light	51.7	49.1	2.2	95.0	4.4	39.3	11.2
P.triestinum	Low light	151.2	134.4	16.5	88.9	12.3	28.1	43.6
	High light	121.6	108.8	12.6	89.4	11.6	39.0	29.7
	High light	117.6	103.3	14.1	87.8	13.6	38.7	35.2
	High light	103.8	91.6	12.0	88.2	13.1	47.9	27.2
	Low light	123.2	108.8	14.2	88.3	13.1	47.4	27.5
	Low light	112.7	100.0	12.4	88.7	12.4	46.8	26.5
	Low light	108.2	96.6	11.3	89.3	11.7	48.4	24.1
	High light	110.2	98.1	11.8	89.1	12.0	50.9	23.5
	High light	129.5	113.8	15.4	87.9	13.6	41.1	33.0

Appendix 5.7 (a): Comparison of pigments concentrations during transient light conditions in the diato*m* *Chaetoceros capense* and *Prorocentrum triestinum* during the time course of 5 h. (T. Chl *a* - total chlorophyll *a*, T. Fuc. - total fucoxanthin, D T. Per. - total peridinin, Diad. - diadinoxanthin, Dino – dinoxanthin, Diat. - diatoxanthin, Zea – zeaxanthin, *B*-car.- β carotene, T. pig. - total pigment).

	Time (h)	Chl c_1 c_2 µg L^{-1}	Fuc µg L^{-1}	Diad µg L^{-1}	Dino µg L^{-1}	Diato µg L^{-1}	Zea µg L^{-1}	*B*-Car µg L^{-1}	T. Chl *a* µg L^{-1}	T. Pig. µg L^{-1}
C. capense										
LL	0	22.96	4.03	1.10	0	0.04	0.34	0.71	19.3	48.5
HL	0.5	22.30	4.43	1.33	0	0.17	0.35	0.85	21.5	51.0
HL	1	27.15	3.94	1.48	0	0.09	0.42	0.89	10.5	44.5
	1.5									
HL	2	26.18	2.57	1.07	0	0.20	0.40	0.72	1.6	32.8
LL	2.5	25.87	3.06	1.33	0	0.13	0.35	0.73	8.2	39.7
LL	3	27.18	3.31	1.42	0	0.12	0.47	0.78	9.9	43.2
	3.5									
LL	4	31.29	4.27	1.65	0	0.09	0.55	0.89	1.7	40.5
HL	4.5	28.46	2.90	1.23	0	0.20	0.53	0.75	2.0	36.1
HL	5	27.18	2.64	1.17	0	0.28	0.41	0.70	1.6	34.0
P. triestinum			**T.Per**							
LL	0	45.08	51.56	11.74	2.12	0.49	0.28	2.12	37.8	151.2
HL	0.5	29.78	36.58	8.64	1.51	0.78	0.25	1.66	42.4	121.6
HL	1	26.60	36.72	9.45	1.65	1.16	0.24	1.82	40.2	117.8
	1.5									
HL	2	18.63	29.03	7.97	1.37	1.06	0.26	1.56	43.9	103.8
LL	2.5	24.05	33.17	9.99	1.41	0.99	0.21	1.83	51.6	123.2
LL	3	21.13	32.08	9.12	1.05	0.79	0.29	1.46	46.8	112.7
	3.5									
LL	4	19.57	30.27	7.98	1.41	0.61	0.25	1.28	46.8	108.2
HL	4.5	18.61	29.61	7.87	1.40	0.74	0.28	1.74	49.9	110.2
HL	5	26.33	40.71	10.50	1.87	1.03	0.26	2.04	46.8	129.5

Appendix 5.7 (b): Comparison of pigments per cell during transient light conditions in the diatom C. *capense* and *Prorocentrum triestinum* during the time course of 5 hours. (T. Chl *a* - total chlorophyll *a*, T. Fuc. - total fucoxanthin, D T. Per. - total peridinin, Diad. - diadinoxanthin, Diat. - diatoxanthin, B-car.- β carotene, T. pig. - total pigment).

		Time	Cells	T. Chl *a* cell^{-1}	T. Fuc cell^{-1}	Diad cell^{-1}	Diato cell^{-1}	B-car cell^{-1}	T.pig cell^{-1}
		(h)	L^{-1}	pg	pg	pg	pg	pg	Pg
Chaetoceros capense	Low light	0	9451079	2.1	0.43	0.12	0.004	0.08	5.2
	High light	0.5	14945893	1.2	0.30	0.09	0.01	0.06	3.2
	High light	1	19815167	1.0	0.20	0.07	0.004	0.04	2.6
	High light	2	14978454	1.2	0.17	0.07	0.01	0.05	3.3
	Low light	2.5	12145573	1.5	0.26	0.11	0.01	0.06	4.1
	Low light	3	10476778	1.9	0.32	0.14	0.01	0.07	5.1
	Low light	4	11225700	1.8	0.38	0.15	0.008	0.08	5.3
	High light	4.5	13545733	1.5	0.21	0.09	0.02	0.06	4.0
	High light	5	18698647	1.0	0.14	0.06	0.02	0.04	2.8
					T.Per cell^{-1}				
		(h)	L^{-1}	pg	pg	pg	pg	pg	P
Prorocentrum triestinum	Low light	1	8343976	4.5	6.18	1.41	0.08	0.3	12.3
	High light	2	10664008	4.1	3.43	0.81	0.07	0.2	11.4
	High light	3	13651559	2.9	2.69	0.69	0.08	0.1	8.6
	High light	4	14872628	2.6	1.95	0.54	0.07	0.1	7.1
	Low light	5	9127775	3.6	3.67	1.11	0.11	0.2	8.5
	Low light	6	12235118	3.8	2.62	0.75	0.08	0.1	9.2
	Low light	7	11176858	4.2	2.71	0.71	0.05	0.1	9.7
	High light	8	13171271	3.8	2.25	0.60	0.06	0.1	8.4
	High light	9	18845974	1.8	2.17	0.41	0.04	0.08	5.0

Appendix 5.8: Mean Chl *a*-specific absorption coefficients (m^2 (mg Chl *a*)$^{-1}$) for the diatom *Chaetoceros capense* and the dinoflagellate *Prorocentrum triestinum* at different wavelengths (nm).

Chaetoceros capense Light condition	Time (h)	Mean absorption coefficient	440 nm	460 nm	490 nm	550 nm	660 nm	675 nm	Ratio 440/675
LL	0.0	0.093	0.181	0.150	0.110	0.053	0.081	0.128	1.42
HL	0.5	0.094	0.172	0.13	0.108	0.058	0.077	0.124	1.39
HL	1.0	0.107	0.199	0.168	0.126	0.068	0.086	0.132	1.50
	1.5								
HL	2.0	0.107	0.205	0.167	0.124	0.061	0.088	0.143	1.43
LL	2.5	0.093	0.168	0.132	0.102	0.054	0.080	0.127	1.33
LL	3.0	0.100	0.187	0.150	0.112	0.057	0.085	0.129	1.44
	3.5								
LL	4.0	0.851	0.157	0.127	0.097	0.050	0.066	0.106	1.48
HL	4.5	0.123	0.242	0.189	0.135	0.066	0.106	0.165	1.46
HL	5.0	0.096	0.186	0.142	0.108	0.051	0.082	0.132	1.41

Prorocenttrum triestinum Light condition	Time	Absorption coefficient	440 nm	460 nm	490 nm	550 nm	660 nm	675 nm	440/675 nm
LL	0.0	0.084	0.139	0.140	0.111	0.067	0.075	0.123	1.13
HL	0.5	0.080	0.140	0.141	0.108	0.059	0.067	0.111	1.26
HL	1.0	0.083	0.150	0.148	0.112	0.061	0.067	0.115	1.30
	1.5								
HL	2.0	0.086	0.148	0.149	0.121	0.065	0.070	0.114	1.30
LL	2.5	0.067	0.117	0.117	0.093	0.050	0.054	0.088	1.32
LL	3.0	0.078	0.138	0.138	0.106	0.058	0.064	0.106	1.30
	3.5								
LL	4.0	0.079	0.138	0.138	0.108	0.060	0.065	0.107	1.29
HL	4.5	0.076	0.133	0.133	0.103	0.057	0.062	0.102	1.30
HL	5.0	0.085	0.151	0.152	0.115	0.063	0.070	0.115	1.32

Appendix 5.9: Estimated maximum quantum yield changes during transient light conditions of a diatom (*Chaetoceros capense*) and a dinoflagellate (*Prorocentrum triestinum*)

Diatom	Treatment	Time	Mean Chl *a*-specific Spectral absorption Coefficient ($a(\lambda)$ m^{-1}) mg C (Chl a^{-1}) µmol quanta m^{-2} s^{-1}	Maximum light utilization Coefficient (α^*) (mg C m^{-3} (mg Chl a^{-1}) µmol quanta m^{-2} s^{-1})		Estimated quantum yield ($ø_m$) mol C (mol quanta m^{-2})
Chaetoceros capense	Low light	0	0.09	0.04		0.10
	High light	0.5	0.09	0.03		0.08
	High light	1	0.11	0.04		0.07
		1.5				
	High light	2	0.10	0.04		0.08
	Low light	2.5	0.10	0.04		0.09
	Low light	3	0.10	0.04		0.08
		3.5				
	Low light	4	0.09	0.04		0.10
	High light	4.5	0.10	0.04		0.09
	High light	5	0.10	0.04		0.10
					Mean	**0.09**
					±SE	0.01
Dinoflagellate						
Prorocentrum triestinum	Low light	0	0.08	0.03		0.09
	High light	0.5	0.08	0.03		0.08
	High light	1	0.08	0.03		0.08
		1.5				
	High light	2	0.09	0.02		0.06
	Low light	2.5	0.07	0.02		0.07
	Low light	3	0.08	0.03		0.08
		3.5				
	Low light	4	0.08	0.03		0.07
	High light	4.5	0.08	0.02		0.07
	High light	5	0.09	0.03		0.09
					Mean	**0.08**
					±SE	0.01

Appendix 5.10: Comparison of changes taking place in total carbon, nitrogen and total chlorophyll *a* concentrations in the diatom and dinoflagellate species during transient light conditions.

Chaetoceros capense		**Total carbon**	**Total nitrogen**	**T.Chl a**	**Ratio**	**Ratio**
Hours		mg L^{-1}	mg L^{-1}	mg L^{-1}	C : Chl *a*	C : N
0	Low light	3.25	0.72	0.020	164	4.5
0.5	High light	3.73	0.76	0.018	204	4.9
1	High light	3.58	0.71	0.020	178	5.1
2	High light	3.32	0.65	0.019	178	5.1
2.5	Low light	3.52	0.74	0.019	189	4.8
3	Low light	3.35	0.70	0.020	166	4.8
4	Low light	3.86	0.79	0.020	191	4.9
4.5	High light	3.45	0.71	0.019	181	4.8
5	High light	3.62	0.74	0.019	188	4.9
Prorocentrum triestinum						
Hours						
0	Low light	3.97	0.75	0.038	105	5.3
0.5	High light	3.81	0.66	0.042	90	5.8
1	High light	3.50	0.61	0.040	87	5.8
2	High light	4.11	0.71	0.044	94	5.8
2.5	Low light	4.87	0.80	0.052	94	6.1
3	Low light	4.25	0.73	0.047	91	5.8
4	Low light	4.47	0.76	0.047	95	5.9
4.5	High light	4.80	0.79	0.050	96	6.1
5	High light	4.69	0.74	0.047	100	6.3

Appendix 5.11: Comparison of the ratio of total carbon (TC) to total chlorophyll *a* (T Chl *a*) and total chlorophyll *a* to nitrogen in the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum* sampled at 0.5 and 1 h interval during the light shift experiment.

Chaetoceros capense		**T C:T Chl *a***	**T Chl *a*:N**
Time (h)			
0	Low light	0.6	2.9
0.5	High light	0.3	1.6
1	High light	0.3	1.4
2	High light	0.4	1.9
2.5	Low light	0.4	2.1
3	Low light	0.6	2.7
4	Low light	0.5	2.3
4.5	High light	0.4	2.0
5	High light	0.3	1.4
Prorocentrum triestinum			
Time (h)			
0	Low light	1.1	6.1
0.5	High light	1.0	6.0
1	High light	1.8	4.8
2	High light	0.7	4.1
2.5	Low light	1.2	7.1
3	Low light	0.9	5.2
4	Low light	0.9	5.5
4.5	High light	0.8	4.8
5	High light	0.4	2.4

Appendix 5.12: Comparison of the ratio of total chlorophyll *a* (T Chl *a*) to diadinoxanthin (Diad) and chlorophyll c1, c_2 (Chl $c_{1,2}$) to diadinoxanthin in the diatom *Chaetoceros capense* and dinoflagellate *Prorocentrum triestinum*.

C. capense		Time (h)	Chl c_1 c_2	T Chl *a*	Fuc	Chl a: Diad	Chl c_1c_2:Diad
	Low light	0.0	23.0	19.9	4.0	18.05	1.15
	High light	0.5	22.3	18.3	4.4	13.77	1.22
	High light	1.0	27.2	20.1	3.9	13.63	1.35
		1.5					
	High light	2.0	26.2	18.6	2.6	17.39	1.41
	Low light	2.5	25.9	18.6	3.1	13.94	1.39
	Low light	3.0	27.2	20.2	3.3	14.22	1.35
		3.5					
	Low light	4.0	31.3	20.2	4.3	12.23	1.55
	High light	4.5	28.5	19	2.9	15.48	1.50
	High light	5.0	27.2	19.3	2.6	16.43	1.41
					T. Per		
P. triestinum	Low light	0.0	45.1	37.8	51.6	3.22	1.19
	High light	0.5	29.8	42.4	36.6	4.91	0.70
	High light	1.0	26.6	40	36.7	4.23	0.66
		1.5					
	High light	2.0	18.6	43.9	29.0	5.51	0.42
	Low light	2.5	24.1	51.6	33.2	5.17	0.47
	Low light	3.0	21.1	46.8	32.1	5.13	0.45
		3.5					
	Low light	4.0	19.6	46.8	30.3	5.86	0.42
	High light	4.5	18.6	49.9	29.6	6.34	0.37
	High light	5.0	26.3	46.8	40.7	4.46	0.56

There are only two ways to live your life. One is as though nothing is a miracle. The other is as though everything is a miracle.
Albert Einstein