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Factors Affecting the Kinetics of Light Intensity Adaptation in Marine Phytoplankton

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FACTORS AFFECTING THE KINETICS OF LIGHT INTENSITY ADAPTATION IN MARINE PHYTOPLANKTON

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ABSTRACT

FACTORS AFFECTING THE KINETICS OF
LIGHT INTENSITY ADAPTATION IN MARINE PHYTOPLANKTON

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It has been suggested that the recent light history of phytoplankton and the kinetics of photoadaptation can be used to provide information about the vertical mixing processes in the upper mixed layer. To be useful as a parameter in a model of photoadaptation and vertical mixing, the response of a photoadaptive variable to changes in growth irradiance must be monotonic, significant, and comparable in time rate scale to mixing processes. Previous studies of photoadaptation kinetics have focused on the response of phytoplankton to changes in light intensity under continuous illumination. This dissertation attempts to elucidate the effects of light:dark cycle, nutrient concentration, growth rate and species difference on the photoadaptation kinetics of marine phytoplankton in a series of light transition experiments. The study found that: (1) Hysteresis of photoadaptive response exists between the two reciprocal (low-to-high vs high-to-low) light transitions. The increase in photoadaptive cellular properties following a light shift is better described by a logistic model, whereas the decrease in the same properties

following the reverse light shift is better described by first order kinetics model. Shift-up and shift-down are not simply mechanistically reverse processes. (2) Phytoplankton do not shade-adapt at night; however, cellular photoadaptive variables, including pigment components and chemical composition (C, N), undergo diurnal variations, causing the light response curve of these variables to be non-monotonic under natural light:dark cycles. (3) Different algal species are different in their responsiveness to changes in growth irradiance. The difference exists both between and within taxonomic groups, and may be related to the growth characteristics or selective strategies of individual species. (4) Phytoplankton growth, as controlled by nutrient-limitation, has a positive effect on photoadaptation. As growth rate increases, so does the rate of photoadaptation. However, the increase is not homogeneous among different photoadaptive variables and is not proportional to the increase in growth rate, suggesting that biosynthesis and cell division are uncoupled and that growth is unbalanced during photoadaptation. These results revealed that factors other than light intensity can cause large variabilities in the rate and pattern of photoadaptation in marine phytoplankton. Hence, previous photoadaptation-vertical mixing model based on simple first order kinetic light response of photoadaptive variables should be re-evaluated and refined.

To my parents, Qingju Zhang and Wenjiao Guo,
who always believe that I can do it;

To my wife, Beirong Zhang,
without whom I couldn't have done it.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Marine phytoplankton grow in a rapidly changing environment. Hydrodynamic processes expose phytoplankton cells to large changes in light intensity. Phytoplankton are able to respond to such changes by adjusting a number of biochemical and physiological characteristics in the process collectively known as photoadaptation. The photoadaptation status of phytoplankton affect their ability to optimize light utilization under prevailing light conditions, thereby affecting primary production. In addition, it has been suggested that the recent light history of phytoplankton and the kinetics of photoadaptation can be used to provide information about the vertical mixing processes in the mixed layer of the ocean (e.g., Falkowski 1980, 1983, Lewis et al 1984, Cullen and Lewis 1988, Therriault et al 1990). In principle, if the rate of photoadaptation of some photoadaptive parameter exceeds the rate of mixing, then the parameter will not be homogeneously distributed with depth. By observing the variation with depth of a number of photoadaptive variables, each with different rates of photoadaptation, the upper and lower bound of vertical mixing rate can be estimated (Lewis et al 1984). So far, a number of photoadaptive properties has been described which may be useful for estimating, either

qualitatively or quantitatively, the degree of vertical mixing. They include pigment composition, chemical composition, photosynthetic parameters, *in vivo* fluorescence properties and photosynthetic unit (PSU) size (e.g., Steemann Nielsen and Hansen 1959, Ryther and Menzel 1959, Falkowski 1980, 1983, Harris 1980, Putt et al 1987, Cullen and Lewis 1988, Therriault et al 1990). However, previous studies failed to consider the effect of light/dark cycle on the photoadaptation kinetics. In addition, the effects of growth rate, nutrient limitation and species difference have not been studied. These effects may have important consequences in estimating vertical mixing rate. The purpose of this study is to elucidate such effects as well as studying the feasibility of using selected pigment ratios to examine photoadaptation of marine phytoplankton. This approach has the potential of distinguishing specific groups of phytoplankton. Newly evolving methodology of HPLC (high performance liquid chromatography) allows us to make simultaneous measurements of a wide variety of pigments quickly and with relative ease.

While initially examining a number of possible pigments to be used, the most suitable for photoadaptation studies seemed to be the following marker pigment : chl *a* ratios (will be called pigment ratios for simplicity throughout the rest of the text):

(1) For diatoms and chrysophytes, fucoxanthin : chl *a;*

(2) For dinoflagellates, peridinin : chl *a;*

(3) For green algae, chl *b* : chl *a;*

(4) For cyanobacteria, zeaxanthin : chl *a.*

These marker pigments (fucoxanthin, peridinin, chl *b* and zeaxanthin) are chosen because they are group specific, sensitive to light changes (except zeaxanthin), and not involved in any light/dark xanthophyll cycles where certain pairs of pigments convert to each other due to epoxidation or de-epoxidation depending on the photoperiod.

1.2 Literature Review

Photoadaptation In Marine Phytoplankton

Light is one of the major factors controlling algal growth, competition and succession. Because of turbulence and other vertical mixing processes, phytoplankton are potentially subject to large fluctuations in light intensity during the photoperiod. Phytoplankton can respond to changes in the natural light field by a number of phenotypic adaptations. As early as 1934, Steeman Nielsen (1934) characterized the dinoflagellate *Ceratium* as sun-adapted when living in the well-lit surface waters and shade-adapted when living in the dim light in deep waters. Later, Ryther and Menzel (1959) noted that sun-shade adaptation was modified by seasonal variations and mixing processes. From photosynthetic characteristics, they found that all phytoplankters are sun-adapted during winter when the water was well mixed throughout the euphotic zone; in contrast, only surface populations were sun-adapted during the period of summer stratification. Steemann Nielsen and Hansen (1959) pointed out that shade-adapted deep water planktonic algae were able to utilize low-intensity light more efficiently than sun-adapted forms. On the other hand, sun-adapted species showed a large increase in dark enzyme reaction rates over shade-adapted cells. On the basis of unialgal experiments, Steemann Nielsen and co-workers (1962, 1968) and Jorgensen (1964, 1969) summarized two basic types of responses: the "*Chlorella* type" which responds to new

light conditions by changing the light limited cellular photosynthetic rate, and the "*Cyclotella* type" which responds by changing photosynthetic capacity. Subsequent studies have indicated that many phytoplankton species can photoadapt to changes in light intensity by:

(1) changing the content and ratio of photosynthetic pigments so as to optimize the level of light harvesting in the new light regime (Myers and Graham 1971, Mandelli 1972, Beardall and Morris 1976, Marra 1978, Prezelin and Sweeney 1978, Falkowski 1980, Falkowski and Owens 1980, Prezelin and Matlick 1980, and Post et al 1984). Available data are, however, largely restricted to changes in various forms of chlorophylls.

(2) Changing chemical composition, including carbon, nitrogen, lipid, carbohydrate and protein (Falkowski 1980, Perry et al 1981, Post et al 1985, Sukenik et al 1989).

(3) changing the size or number of photosynthetic units (Schmid and Graffron 1968, Myers and Graham 1971, Prezelin and Alberte 1978, Prezelin and Sweeney 1978, 1979, Falkowski 1980, Falkowski and Owens 1980, Perry et al 1981, and Post et al 1985). Changes in the size or number of photosynthetic units will, in turn, change the shape of the photosynthesis vs. irradiance profile [i.e. the light limiting slope (α), and the photosynthetic capacity (P_{max})].

(4) changing cell volume (Myers and Graham 1971, Prezelin and Sweeney 1978

and Falkowski and Owens 1980).

(5) changing the cellular enzymatic activitiy (Beardall and Morris 1976, Senger and Fleischhacker 1978, Rivkin et al 1982, and Rivkin 1990).

(6) changing both *in vivo* and DCMU-induced fluorescence characteristics (Harris 1980, Lewis et al 1984, Putt et al 1987, Cullen and Lewis 1988, Therriault et al 1990).

Light-Intensity Adaptation in Algal Pigment Synthesis

Marine phytoplankton respond to changes in light intensity by changing the concentration and ratios of photosynthetic pigments. Because different pigments are synthesized and degraded at different rates, changes in pigment ratios occur. Except at very low light intensities (<20 uE m^{-2} s^{-1}), where light stress could cause a decrease in pigment concentration due to bleaching, as observed in the dinoflagellate *Gonyaulax,* (Prezelin and Sweeney 1978), a decrease in light intensity usually results in an increase in the photosynthetic pigment contents. For example, in the green alga *Dunaliella tertiolecta,* the cellular chlorophyll content can vary by a factor of five (Falkowski and Owens 1980). Many other marine and freshwater chlorophytes adapt in the same way (e.g., Steemann Nielsen and Jorgensen 1968). Their response to irradiance resembles the green macroalgae and the higher plants: shade-adapted forms of a species contain higher pigment concentrations as well as higher chl *b* : chl *a* ratios than the light-adapted forms. Myers and

Graham (1971) and Falkowski and Owens (1980) found that in some green algae, as cells became adapted to low light intensities, the chl *b* : chl *a* ratios increased dramatically (by as much as 50%).

Similar responses in chl *c* : chl *a* ratios have been found in diatoms and dinoflagellates as in green algae (Falkowski and Owens 1980, Prezelin and Matlick 1980). Specific light harvesting components, that is, the antenna pigments (e.g., chl *b* -chl *a* -protein complex in green algae and chl *c* -chl *a* -protein complex in diatoms and dinoflagellates) contain higher accessory chlorophylls (*b, c*) to chl *a* ratio than the whole photosynthetic apparatus that includes the reaction center chl *a* (for review, see Prezelin, 1981). Therefore, chl *b* (or chl *c*) to chl *a* ratio will increase as phytoplankton respond to decreases in irradiance by increasing the cellular content of specific light harvesting components.

The structural information on the organization of carotenoid-chl *a* -protein complexes may provide some clues regarding changes in specific carotenoid to chl *a* ratios in response to light. One of the successfully isolated and well characterized complexes is the peridinin-chl *a* -protein complex (PCP), which has been shown to contain higher peridinin : chl *a* ratios than in the whole cell (e.g., Haxo et al 1976, Prezelin and Haxo 1976, Siegelman et al 1977). Prezelin (1976) showed that PCP increased at low growth irradiance while other components of the photosynthetic apparatus remained constant. Thus, it is not surprising that a decrease in light intensity will cause an increase in peridinin : chl *a* ratio in dinoflagellates,

as has already been demonstrated in *Amphidinium* (Mandelli 1972) and *Glenodinium* (Prezelin and Matlick 1980). In diatoms, the major light harvesting component is the chl *a* -chl *c* -fucoxanthin-protein complex (Larkum and Barret 1983, Anderson 1986), which contains higher fucoxanthin : chl *a* ratios than in the entire photosynthetic apparatus (Friedman and Alberte 1984, Owens and Wold 1986). Thus, we can expect that a decrease in irradiance will lead to an increase in fucoxanthin : chl *a* ratios in diatoms and chrysophytes. Zeaxanthin is a carotenoid marker for picoplanktonic cyanobacteria which are widespread in the world oceans (Guillard et al 1985). Its function differs from chl *b* , fucoxanthin and peridinin in that it primarily serves photoprotective purposes (Kana et al 1988). Kana et al (1988) showed that zeaxanthin content in cells of *Synechococcus* is relatively constant to light changes. However, zeaxanthin : chl *a* ratio will increase at high light due to a decrease in cellular chl *a* content.

The adaptation of marine phytoplankton to new light conditions is usually swift. Prezelin and Matlick (1980) showed that cellular chl *a* content in *Glenodinium* reached a new steady state after three days following a high-to-low light transition. In *Pyrocystis noctiluca,* cellular chl *a* content reaches new equilibrium after about four days of transition from high to low light (Rivkin et al 1982). Similarly, Post et al (1984) showed that in four days the light-acclimated diatoms *Thalassiosira weisflogii* became fully shade-adapted with respect to cellular chl *a* content. The time course of changes in cellular chl *a* content usually can be approximated by first order kinetics, with rate constants normally in the order of 10^{-2} hr^{-1} (Rivkin

et al 1982, Falkowski 1984, Post et al 1984). It has been shown, for both cultured species and natural populations of phytoplankton, that changes in chl *a* : P700 ratios (P700 is the Photosystem I reaction center chl *a* having maximum absorption at 700 nm wavelength) in response to changes in light intensity can also be described by first order kinetics with similar rate constant as that for cellular chl *a* (Falkowski 1980, 1983, Falkowski and Owens 1980). Thus, there is reason to expect that the selected marker pigment to chl *a* ratios will follow a similar pattern of change.

Vertical Mixing And Photoadaptation

In natural waters, light-shade adaptation occurs when phytoplankton experience variations in light intensity. At any given depth in the euphotic zone, low frequency (months) temporal fluctuations of light intensity are primarily related to the seasonal cycles of solar radiation. Such temporal variabilities may be manifested in the seasonal periodicities of cellular and photosynthetic properties. Higher frequency (hours to days) fluctuations are primarily related to light/dark cycles or turbulent mixing. Post et al (1984) found that phytoplankton cells can distinguish between light/dark cycles and variations in light intensity. As a result, they do not shade-adapt at night. Given the exponential attenuation of light intensity with depth, Marra (1980) pointed out, perhaps the most important variations in light intensity that phytoplankton experience, are those caused by vertical mixing

processes. Thanks to light-shade adaptation, these variations are "recorded" in the biochemical and physiological features of the cells that have been vertically displaced. Thus, it has been suggested that information contained in phytoplankton cells about their recent light history can be used to estimate the vertical displacement rates of the cells *in situ* (e.g., Falkowski 1980, 1983, Lewis et al 1984, Cullen and Lewis 1988, Therriault et al 1990), which should also provide boundary values about the vertical mixing rates. As Falkowski (1983) put it: "If the time scale of change in the adaptive variable is known, it is possible to estimate the maximum rate of vertical displacement of the cell in the water column. If mixing processes occur on a time scale shorter than it takes for the cells to adapt to the variations in the light regime, the vertical distribution of the light-dependent physiological characteristic would be expected to be more uniformly distributed. From knowledge of the time scale of change of the adaptive variable, a minimum vertical mixing rate can be estimated".

In field studies, one of the most frequently measured physiological characteristics of phytoplankton is the photosynthesis-irradiance relationship. The essential photosynthetic parameters include light limited slope (α), light saturated maximum (P_{max}) and the light intensity at the onset of light saturation (I_k) (Jassby and Platt 1976). It has been suggested that vertical displacement rates of phytoplankton cells can be estimated by generating P vs I curves from samples taken at various light depths (e.g. Steemann Nielsen and Hansen 1961), assuming that differences in photosynthetic parameters with depth reflect recent light history of phytoplankton.

Steemann Nielsen and Hansen (1959), Ryther and Menzel (1959) and Falkowski (1980) have qualitatively related the photosynthetic characteristics to the degree of vertical mixing. Generally, when the water column is well mixed, photosynthetic parameters are similar for samples taken from different light depths. However, when the water column is highly stratified, light adapted cells (i.e. from surface populations) have characteristically higher P_{max} than shade adapted cells (i.e. from deep populations). Aside from being cumbersome, one major problem of this approach has been the necessity of incubating samples for a period of time. The physiological parameters determined will be dependent upon the length of incubation. If incubations are long relative to the time scales of photoadaptation, the photosynthetic parameters may reflect the physiological state of phytoplankton under the incubating conditions (Marra 1980), and the information about recent light history contained in the physiological features *in situ* may be "missed".

Another approach for estimating the light history of phytoplankton is to look for changes in the size of photosynthetic units of phytoplankton samples taken from different light depths (Falkowski 1980, 1983). This is based on the hypothesis that PSU size increases as cells experience a decrease in irradiance and become shade adapted, and that the change in PSU size follows first order kinetics. The PSU size can be estimated from chl *a* : P700 or chl *a* : O_2 ratios. By measuring changes in chl *a* : P700 ratios at different depths, Falkowski (1983) estimated vertical displacement rates (i.e. piston velocities) for phytoplankton cells in several marine environments at different times of the year, and found net displacement

rates ranging from the order of 10^{-3} cm/sec to 10^{-1} cm/sec. This method does not require incubation and can quantitatively relate the light history of phytoplankton to their vertical displacement rates. However, as the author also pointed out, the technique is hardly routine, relatively insensitive and has high coefficients of variations. In addition, chl *a* : P700 ratios only reflect changes in PSU size, whereas many species of phytoplankton have been shown to photoadapt by changing PSU number. Moreover, implicit in this method, as is in the P. vs I. method, is the assumption that phytoplankton species composition is homogeneous throughout the euphotic zone, which may not be true unless there is intensive vertical mixing. The inability to distinguish among various phytoplankton groups dominating at different depths may introduce some uncertainties in the estimation of vertical displacement rates, because the physiological characteristics are different for different phytolankton groups.

Vertical profiles of certain fluorescence properties of phytoplankton can also provide information about vertical mixing. Both lab and field studies indicate that DCMU-induced fluorescence response is a sensitive indicator of previous exposure of phytoplankton to bright light (Lewis et el 1984, Putt et al 1987). The vertical distribution of DCMU-induced fluorescence response in natural phytoplankton populations has been qualitatively related to the degree of wind mixing (Harris 1980). Recently, Therriault et al (1990) measured the photoadaptation status of phytoplankton by an in vivo fluorescence ratio (fluorescence after and before exposure to bright light). They further used the ratio to estimate vertical excursion of

phytoplankton cells. The results were consistent with wind strength data. Because of the rapid response of fluorescence to changes in light intensity, fluorescence parameters prove most useful in estimating the vertical displacement of cells in systems where mixing rate is rapid (hours). On the other hand, due to their sensitive nature to first order variations in light (e.g., light/dark cycle, passing clouds), they may not be appropriate for systems where mixing rate is slow (days).

Pigment Ratios As Photoadaptation Parameters

In view of the recent deveploment of rapid and sensitive HPLC methods for separating and quantifying chlorophylls and carotenoids from seawater samples (e.g. Mantoura and Llewellyn 1983), it may be feasible to use changes in phytoplankton pigment composition (i.e., pigment ratios as outlined in the Introduction section) to estimate vertical displacement of the cells, since changes in pigment composition are related to recent light history of phytoplankton. This approach requires no incubation. In addition, both types of change in PSU organization (number or size) in response to light are reflected by pigment ratio changes. Moreover, different phytoplankton groups can be distinguished by using the class specific pigment markers. Finally, using pigment ratios is more effective than using pigment contents because it minimizes variations due to cell size changes as well as variations associated with the determination of cell concentration.

To use pigment ratios as photoadaptive parameters to infer vertical mixing processes, the following theoretical questions relating to the effects of mixed species groups, selective grazing and physical processes, are discussed:

(1) Effect of Mixed Species Groups

The information about the pattern and rate of change in pigment ratios obtained in the laboratory can be applied to the field to determine how far apart in time cells (of the same group) from two depths are separated. The separation in time can be determined by using curves generated in the lab. With known distance between the two depths, the displacement rate ("piston velocity") can be calculated. Since marker pigments are specific only at the taxon level, there is an implicit assumption that all species within the same taxonomic group behave similarly.

This approach works best when one group of phytoplankton predominates, e.g., a diatom or dinoflagellate bloom in coastal regions, or *Synechococcus sp.* in the open ocean. When phytoplankton assemblages of diverse species groups occur, the analysis becomes more complicated though it can still be effective. Since different species groups tend to have different size distributions, we can use size fractionation to separate them. As an example, in pure cultures of *Synechococcus sp* and *Isochrysis galbana,* chl *a* : zeaxanthin and chl *a* : fucoxanthin ratio are 2.683 and 2.167 respectively. The two pure cultures are mixed and filtered sequentially through 2.0 and 0.2 um nuclepore filters to obtain two size fractions. The two fractions are extracted separately in 90% acetone and then combined in different

proportions. For each combination, total chl *a,* zeaxanthin and fucoxanthin are determined. From the multiple regression: total chl *a* = c1*zeaxanthin + c2*fucoxanthin, the coefficients c1 (2.688) and c2 (2.117) are obtained. These coefficients represent estimates of chl *a* : zeaxanthin ratio and chl *a* : fucoxanthin ratios in pure cultures of *Synechococcus sp.* and *Isochrysis galbana* respectively. They are very close to the ratios obtained from the pure cultures.

(2) Effect of Selective Grazing

Pigment ratios for each population of phytoplankton are inherent parameters. Grazing should not affect them. Within each group of phytoplankton, some cells will be grazed, some not. The pigment information contained in the living cells reflects cellular responses to changes in light fields as they are vertically displaced due to sinking and/or mixing. The fraction of a group of phytoplankton that has already been eaten will not affect the pigment composition of the remaining fraction that is left uneaten.

(3) Physical Processes

The described approach works only with a one layer system, i.e., in the upper layer of the ocean within the euphotic zone. If a research site is vertically separated into distinctive layers, each moving in a different direction or at a different horizontal speed, (e.g., the stratefied layers of an estuary), phytoplankton cells in the deeper layer may not result from immediate sinking of the cells in the overlying layer of water. False information about the vertical displacement rate would result from comparison of the pigment ratios between samples from the two

different layers. However, we can still say something about the photoadaptation status of phytoplankton in each layer.

1.3 Objectives

Although previous studies have established that photoadaptive variables change significantly, according to first order kinetics and thus can be used for estimating vertical mixing rates, several critical questions remain. First, since almost all of the previous studies used continuous illumination in their experiments, it is not entirely clear if and how light/dark cycles (which all phytoplankton experience in reality) would affect the kinetics of photoadaptation. Second, it is not known under similar conditions how much difference exists between different species or species groups in their rates of photoadaptation. In natural systems, phytoplankton often occur in mixed assemblages. Species difference, if significant, could result in erroneous estimate of vertical mixing rates. Third, previous studies focused solely on the effects of light intensity on photoadaptive parameters, although these parameters may also be affected by other physiological or environmental factors, such as growth rate and nutrient limitation. If these factors are important, it would be necessary under field conditions to determine whether changes in certain photoadaptive parameters in the water column are the sole result of changes in light intensity.

To study the effects of environmental and physiological factors on the kinetics of photoadaptation, and to examine the feasibility of using pigment ratios as photoadaptive parameters to infer vertical mixing processes, this research has the following objectives:

(1) To determine experimentally whether significant pigment ratio changes occur in association with changes in light history of phytoplankton.

(2) To quantify temporal changes in pigment ratios and other photoadaptive parameters. Knowledge of the kinetics may be useful for infering vertical displacement of cells in nature.

(3) To understand the effect of light/dark cycles on the kinetics of photoadaptation.

(4) To understand the effects of growth rate and nutrient limitation on the kinetics of photoadaptation.

(5) To compare algal species within and between taxonomic groups and examine whether they behave similarly.

CHAPTER 2

MATERIALS AND METHODS

2.1 Experimental Organisms

The kinetics of photoadaptation with respect to changes in pigment composition will be examined for seven species of marine phytoplankton. They include *Skeletonema costatum* (Bacillariophyceae), *Thalassiosira oceanica* (Bacillariophyceae), *Isochrysis galbana* (Prymnesiophyceae), *Dunaliella tertiolecta* (Chlorophyceae), *Tetraselmis levis* (Prasinophyceae), *Prorocentrum micans* (Dinophyceae), and *Synechococcus sp* (Cyanophyceae). These species are selected to represent major groups of marine phytoplankton with distinct types of pigment composition. Three fucoxanthin-containing species are selected to determine whether all species within the same taxonomic group will behave similarly in the pattern and rate of their photoadpative response to light transitions.

Batch cultures of the selected species have been maintained in f/2 medium (Guillard and Ryther 1962) at 20 °C on 12/12h, light/dark (L/D) cycles. Illumination for the algal culture is provided by cool-white fluorescence bulbs (Philips) at ca. 50 uE m^{-2} s^{-1}. Cells from exponential growth phase are transferred into fresh media (f/10), and allowed to adapt to the new growth irradiance [low light (LL) or high light (HL), see below for exact intensity) before photoadaptation experiment

begins.

2.2 Continuous Culture Experiments

Initial experiments were conducted for *Skeletonema costatum, Dunaliella tertiolecta* and *Tetraselmis levis,* using continuous culture. The phytoplankton cells were grown in f/10 medium at 20 °C in 4-L culture vessels with a 25% dilution rate. Illumination is provided by banks of high output cool-white fluorescence tubes (Philips) on 12:12 L:D cycles. The irradiance is controlled by varying the number of tubes used to give low light (LL, 50 uE m^{-2} s^{-1}) and high light (HL, 650 uE m^{-2} s^{-1}) intensities. Scalar irradiance is measured by a LI-185 photometer equipped with a LI-193SB spherical quantum sensor.

After at least one week of acclimation to the growth irradiance (LL or HL) and as cell density stablizes, the light condition is changed in one single step at the begining of the light period to start a light transition experiment (either LL->HL, or vice versa). The time course of changes in cellular pigments, carbon and nitrogen are followed by sampling daily at the beginning of the light period to avoid possible diurnal variations. These experiments primarily examine the difference between reciprocal light changes. The diurnal variation will be examined in further experiments described later. The ideal experimental conditions to be achieved should have the following features:

(a) exponentially growing cells;

(b) relatively constant cell density throughout the sampling period;

(c) constant temperature.

2.3 Sample Analysis

Aliquotes of samples are used for cell counting, and for analysis of pigments, as well as particulate carbon and nitrogen. Cells are counted using a haemocytometer. Growth rate of phytoplankton can be calculated from changes in cell number and the dilution rate. Pigments are analyzed by reverse phase high performance liquid chromatography (HPLC, see below).

For particulate carbon and nitrogen analysis, water samples are filtered through pre-cleaned 13 mm Gelman type A/E glass fiber filters and stored frozen until analysis. Prior to analysis, the sample filters are dried at 35 ^{o}C. After drying, the samples are run directly in a Carlo Erba ANA 1500 NCS analyzer according to Cutter (1991).

For pigment analysis, samples withdrawn from phytoplankton cultures are filtered onto Gelman A/E filters and kept at -20 ^{0}C in dark until analysis. Pigments are extracted by grinding in 90% acetone with a tissue grinder. The homogenates are transfered to centrifuge tubes and centrifuged to remove cell residue. Before injection into the HPLC, a 1000 uL aliquot of clear pigment extract is mixed with

300 uL of an ion pairing agent (IPA, prepared from 15 g of tetrabutylammonium acetate and 77 g of ammonium acetate in 1 liter of D.I. water, Mantoura and Llewellyn 1983).

Pigments are separated with a Spectra-physics model SP8800 liquid chromatograph, an autosampler (model SP8780) with 100 uL sample loop and a Radial-pak C18 column (0.8x10 cm, particle size 10 um, Waters Assoc.) at a flow rate of 1.5 mL/min. The mobile phase consists of three elution solvents. Solvent A is methanol. Solvent B is 25% IPA in D.I. water (v/v) and solvent C is isopropanol. The elution solvents were degassed before use.

To optimize the separation, we designed a 2-step linear gradient elution scheme. The first step involves use of solvent A and B, from 80% A and 20% B at t=0 min to 100% A at t=10 min. The second step involves use of solvent A and C, from 100% A at t=10 min to 50% A and 50% C at t=25 min. After passage through the column, pigments are detected with an absorbance detector (Waters Assoc. model 440) at 436 nm wavelength. Peak areas are integated with a computing integrator (Spectra-physics model SP4270).

Pigment standards are obtained from Sigma Chem. Co. (chl *a*, *b* and β-carotene) or purified by thin layer chromatography (Jeffrey 1981) or preparative HPLC from algal cultures whose pigment composition has been well documented. Pigments are quantified using Beer's law. Extinction coefficients are given by Davis (1976) and Mantoura and Llewellyn (1983). Examples of HPLC chromatograms are shown in Fig. 1 (mixtures of pigments from phytoplankton cultures and

natural samples) and in Fig. 2 (pigments from pure phytoplankton cultures).

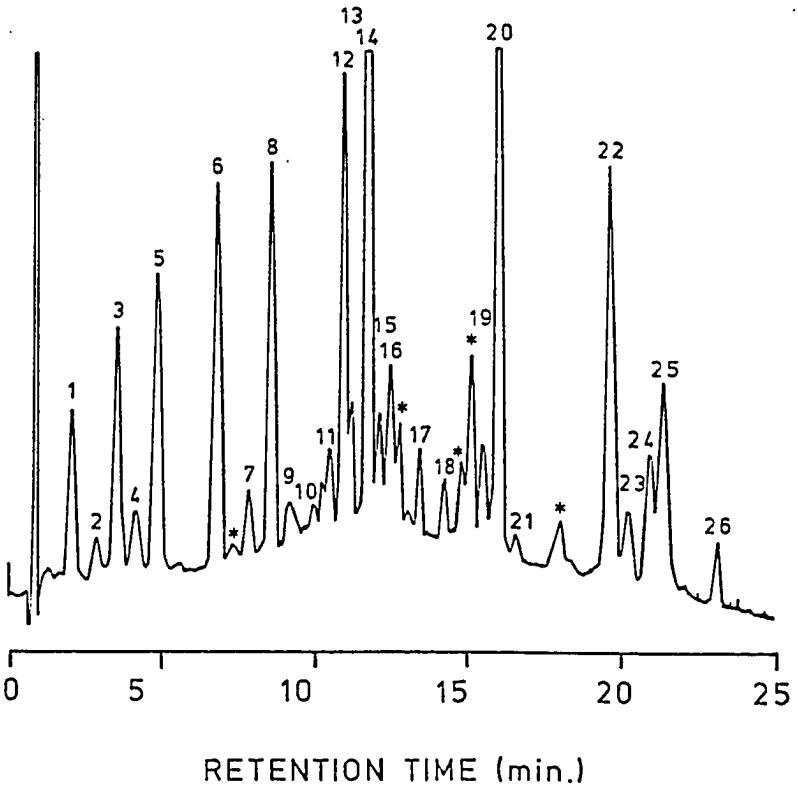


Figure 1. HPLC chromatogram of a mixture of pigments from partially acidified pigment extracts of phytoplankton cultures and natural phytoplankton samples. Peak identities: 1 chlorophyllide b; 2 chlorophyllide a derivative; 3 chlorophyllide a; 4 chlorophyllide a'; 5 chlorophyll c1+c2; 6 peridinin; 7 phaeophorbide a; 8 fucoxanthin; 9 neoxanthin; 10 violaxanthin; 11 cis-fucoxanthin (?); 12 diadinoxanthin; 13 antheraxanthin; 14 alloxanthin; 15 diatoxanthin; 16 lutein/zeaxanthin; 17 chlorophyll b derivative; 18 chlorophyll b; 19 chlorophyll a derivative; 20 chlorophyll a; 21 chlorophyll a'; 22 phaeophytin b; 23 phaeophytin b'; 24 α-carotene; 25 β-carotene; 26 phaeophytin a; * unidentified.

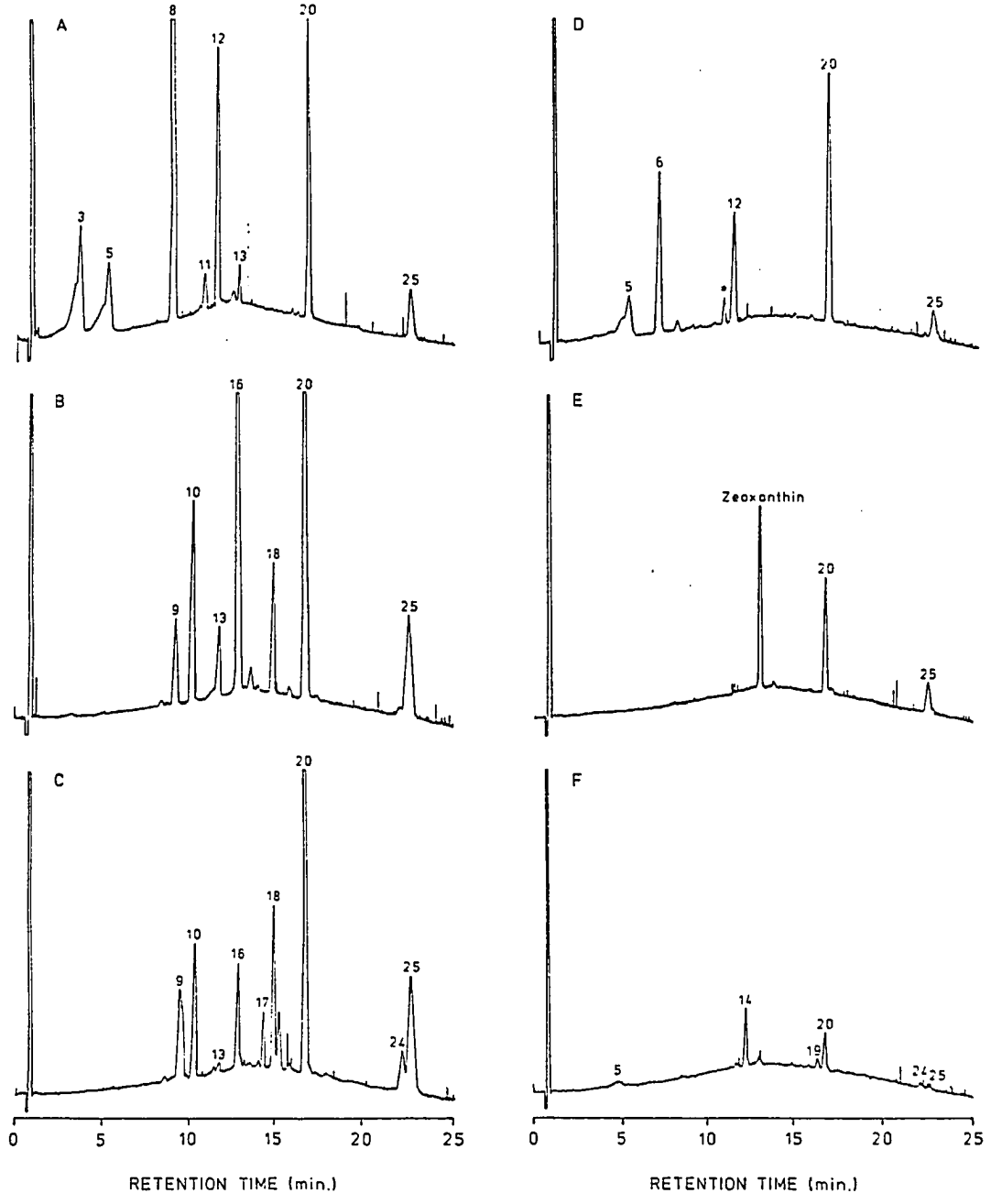


Figure 2. HPLC chromatograms of algal pigment extracts from phytoplankton cultures. (A) *Skeletonema costatum;* (B) *Dunaliella tertiolecta;* (C) *Tetraselmis levis;* (D) *Prorocentrum micans;* (E) *Synechococcus sp.* and (F) *Cryptomonas sp.* Peak identities as in Figure 1.

2.4 Kinetic Analysis

As discussed earlier, changes in photoadaptive variables following transitions of growth irradiance probably can be approximated by first order kinetics (e.g., Falkowski and Owens 1980, Falkowski 1984, Post et al 1984, Hoffmann and Senger 1988, Sukenik et al 1990). For each photoadaptive variable R (i.e., pigment contents, pigment ratios, C : pigment ratios or C : N ratios etc), measurements made during the time course of experiments can be fitted by:

$$R_t=(R_o-R_\infty)e^{-kt}+R_\infty$$

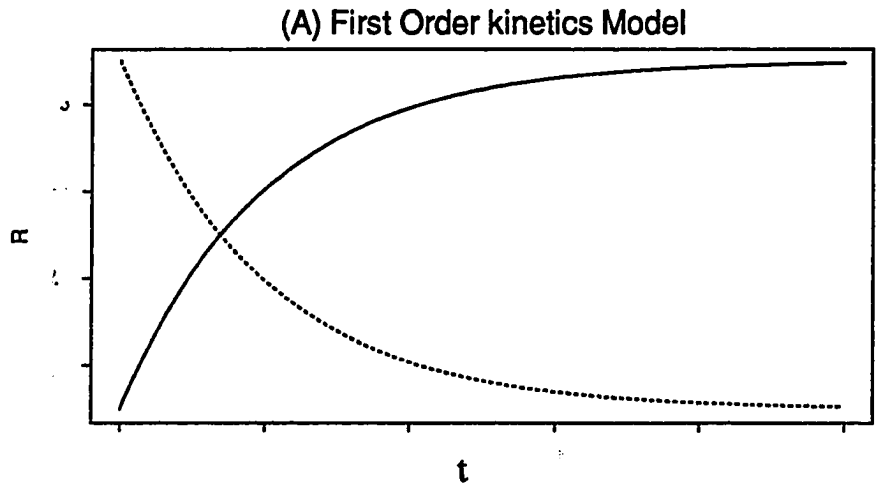
Where R_0 is initial value, R_t is the value at time t, R_∞ is the asymptotic value, and k is the first order rate constant. The first order rate constant, together with the initial and asymptotic values, can be estimated by regression analysis of the first order kinetic model (Fig. 3A). In this study, the Marquardt method of the NLIN procedure of SAS (SAS Institute Inc. 1988) is used in the nonlinear regression analysis. Each time course data point presented is the average of duplicate experiments, with relative errors normally within 10%. The data points in the time course are treated statistically as if they are from independent sampling, although they are from repeated sampling within the same culture vessel during the time course measurement. In this study, the difference between independent sampling and repeated sampling is believed to be small hence statistical procedures are considered robust, because sampling was carried out in such a way that would not alter any growth conditions.

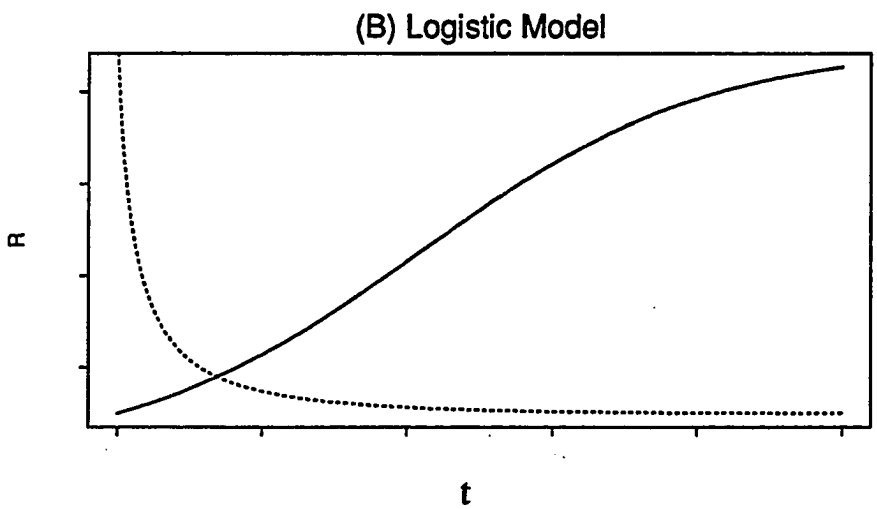
An alternative model to describe the time course of photoadaptation is the logistic model (Fig. 3B), which has the form of:

$$R_t = \frac{R_\infty}{1+[(R_\infty - R_o)/R_o]e^{-pt}}$$

Where p is the rate constant of photoadaption (p is used in the logistic model to distinguish it from k in the first order kinetic model). Other parameters are as above. The logistic model will be mentioned later in the text; however, it is not used in the calculation of the photoadaptation rate constants. To facilitate comparison, only first order kinetics model is used in the estimation of the rate constants of photoadaptation.

Fig. 3. Kinetic models of photoadaptation. (A) first order kinetics model, (B) logistic model. R (y–axis) is a photoadaptive variable, t (x–axis) is time following changes in light intensity.





CHAPTER 3

RESULTS FROM CONTINUOUS CULTURE STUDIES

3.1 Cellular Response To Changes In Growth Irradiance

Pigment Composition

Three species of marine phytoplankton were used in the preliminary continuous culture experiments. They are *Skeletonema costatum, Dunaliella tertiolecta* and *Tetraselmis levis*. Growth rates (ca. 0.25 d^{-1} for all experiments) were controlled by the media dilution rates. Following a one-step change in growth irradiance, all three species responded with changes in cellular pigmentation. Results are shown in Fig. 4.

In *Skeletonema costatum,* a marine diatom, both fucoxanthin and chl *a* $cell^{-1}$ decreased following transfer from low light to high light (LL->HL, Fig. 4). Fucoxanthin changed by a factor of five from 0.26 to 0.05 pg $cell^{-1}$, while chl *a* content changed from 0.7 to 0.2 pg $cell^{-1}$, a factor of 3.5. Because of the differential rates of change, fucoxanthin : chl *a* ratio decreased from 0.40 to 0.25 g g^{-1}, a change of about 60%. Inversely, during the HL->LL transition, *Skeletonema costatum* increased both the fucoxanthin and chl *a* contents. Fucoxanthin content increased by a factor of three, from 0.10 to 0.30 pg $cell^{-1}$, while chl *a* changed by a factor of

about two, increasing from 0.4 to 0.7 pg $cell^{-1}$, resulting in a change of fucoxanthin : chl *a* ratio from 0.27 to 0.40 g g^{-1}.

In *Dunaliella tertiolecta,* a marine chlorophyte, chl *b* is the major accessary and marker pigment. After transfer from LL to HL, the cellular contents of both chl *b* and chl *a* decreased (Fig. 5). Chl *b* decreased by a factor of three from 0.90 to 0.30 pg $cell^{-1}$, while chl *a* decreased from 2.5 to 1.0 pg $cell^{-1}$. As a result, chl *b* and chl *a* ratio decreased from 0.34 to 0.24 g g^{-1}. In contrast, during the transition from HL to LL, both chl *b* and chl *a* contents of the cell increased rapidly. Chl *b* increased from 0.2 to 1.0 pg $cell^{-1}$, a five fold change, whereas chl *a* content increased from 1.0 to 2.7 pg $cell^{-1}$, a change of less than three fold. This differential change resulted in a chl *b* : chl *a* ratio increasing from 0.24 to 0.34 g g^{-1}.

Tetraselmis levis is a prasinophycean, It contains similar pigment matrix as the chlorophycean *Dunaliella tertiolecta.* The general trends of change in *T. levis* were similar to those in *D. tertiolecta,* i.e., both chl *b* and chl *a* increased following a HL->LL transition, and vice versa. The magnitude of change were smaller however, usually within a factor of two (Fig. 6). In the LL->HL transition, chl *b* : chl *a* ratio did not show monotonic changes over time. In the HL->LL transition, the ratio had a lag time of one day during which no significant changes occur, but increased from the second day on.

Fig. 4. Time course changes in cellular pigment contents and pigment ratios in *Skeletonema costatum,* following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in fucoxanthin and chl *a* contents (pg $cell^{-1}$) during LL–>HL (Fig. 4A), during HL–>LL (Fig. 4B), and changes in fucoxanthin : chl *a* ratio (g g^{-1}) following each light transition (Fig. 4C). Light transitions occurred at time zero, which coincided with the beginning of the light period. All sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

Fig. 4A

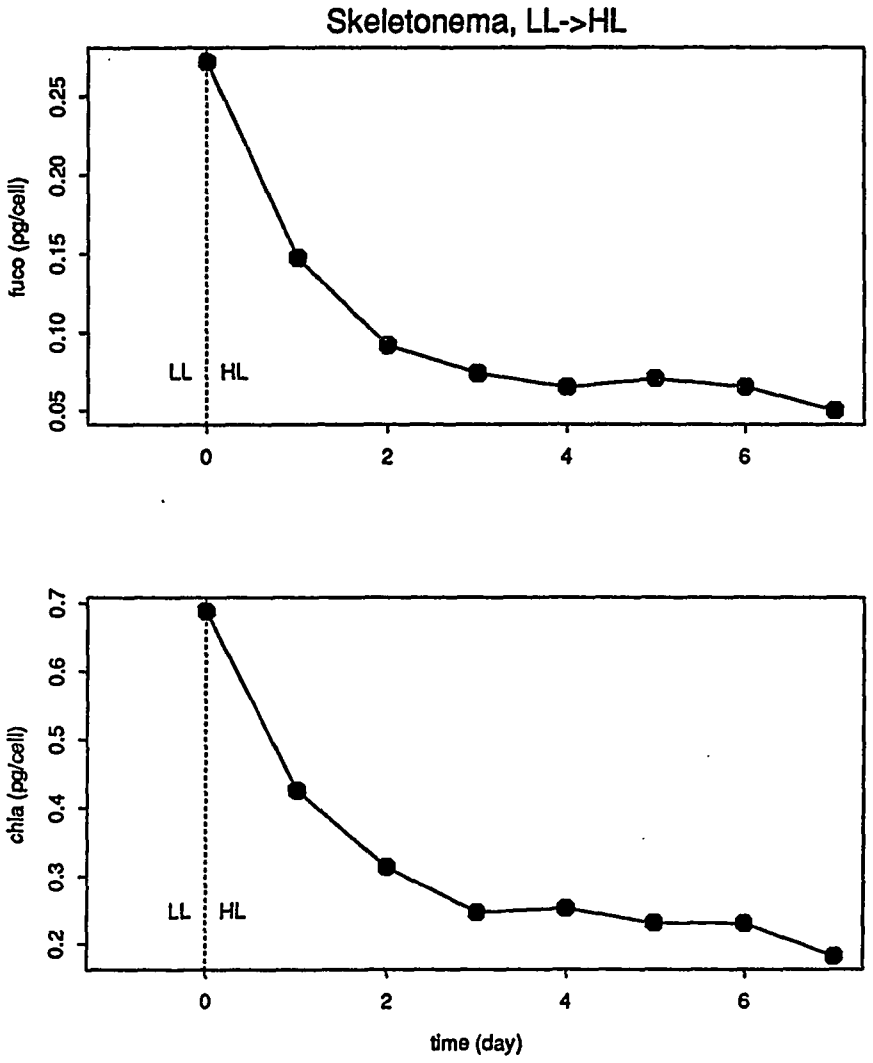


Fig. 4B

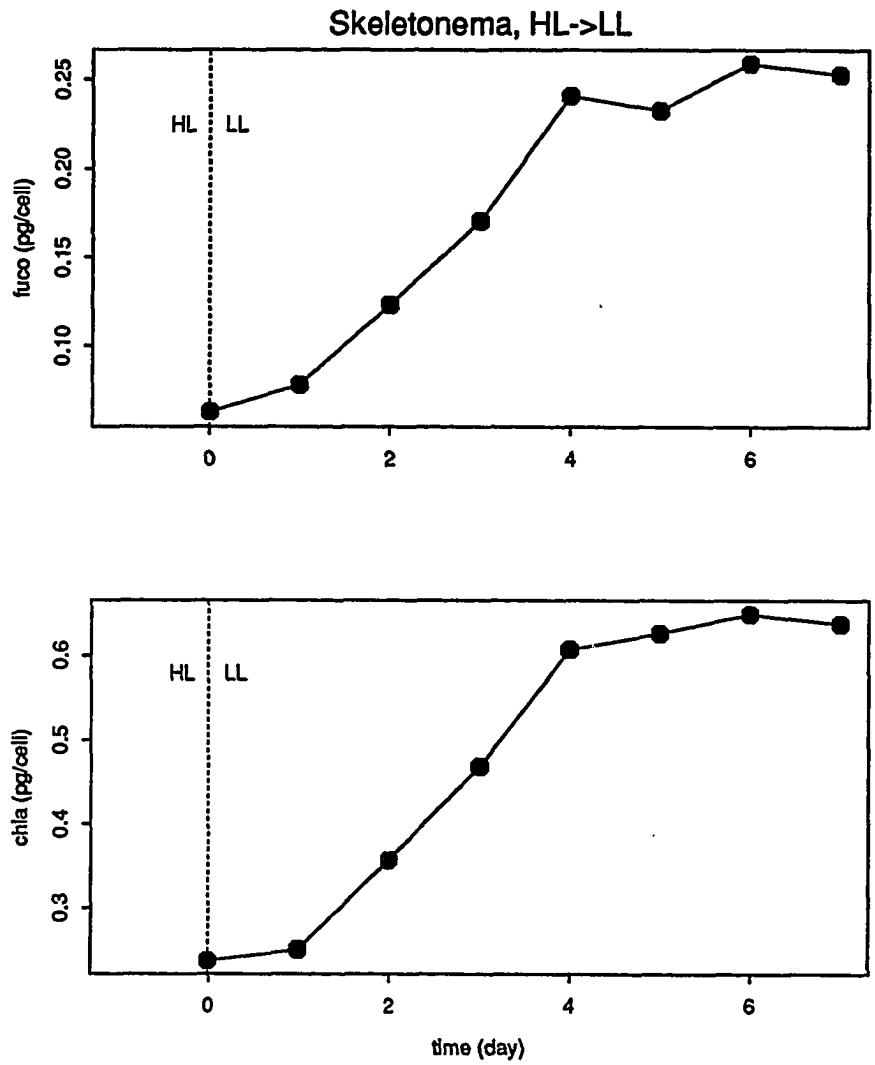


Fig. 4C

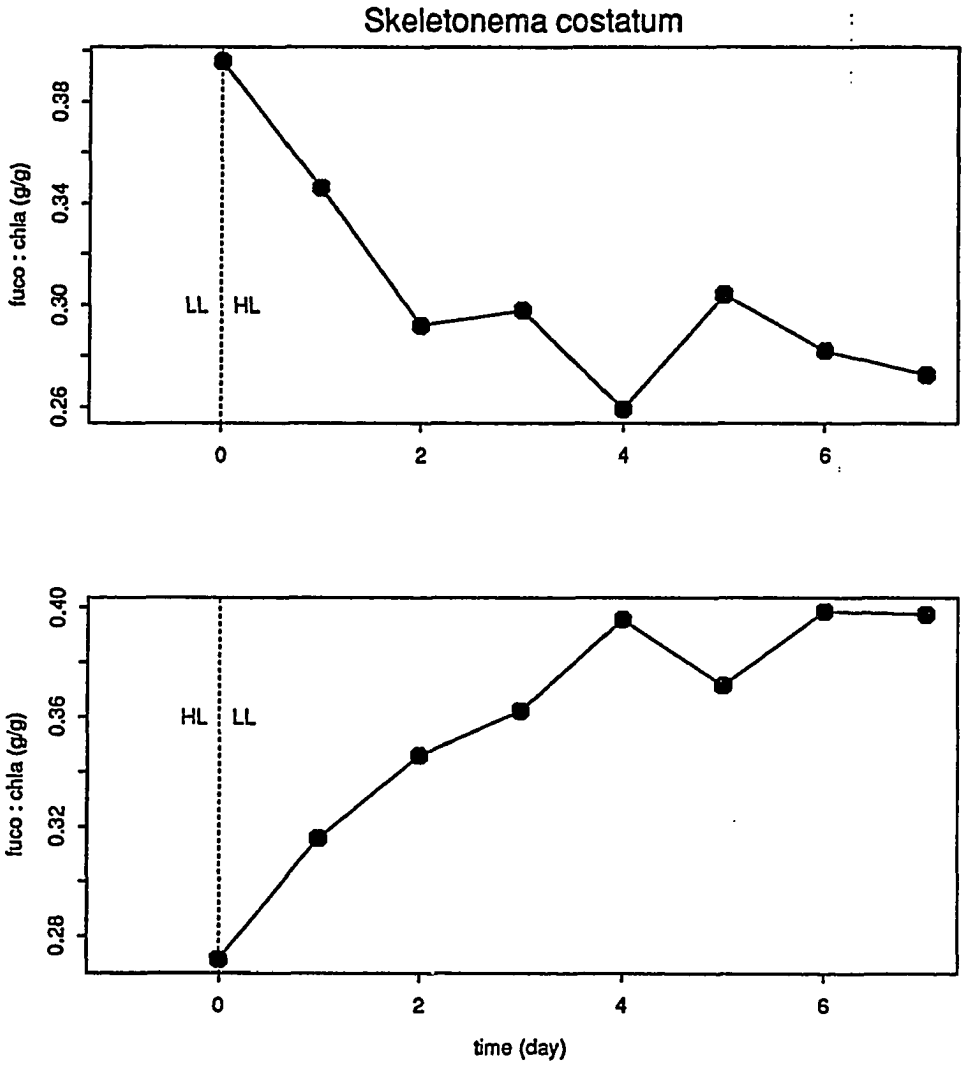


Fig. 5. Time course changes in cellular pigment contents and pigment ratios in *Dunaliella tertiolecta*, following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in chl *b* and chl *a* contents (pg cell^{-1}) during LL–>HL (Fig. 5A) and during HL–>LL (Fig. 5B), and changes in chl *b* : chl *a* ratios (g g^{-1}) following each light transition (Fig. 5C). Light transitions occurred at time zero, which coincided with the beginning of the light period. All sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

Fig. 5A

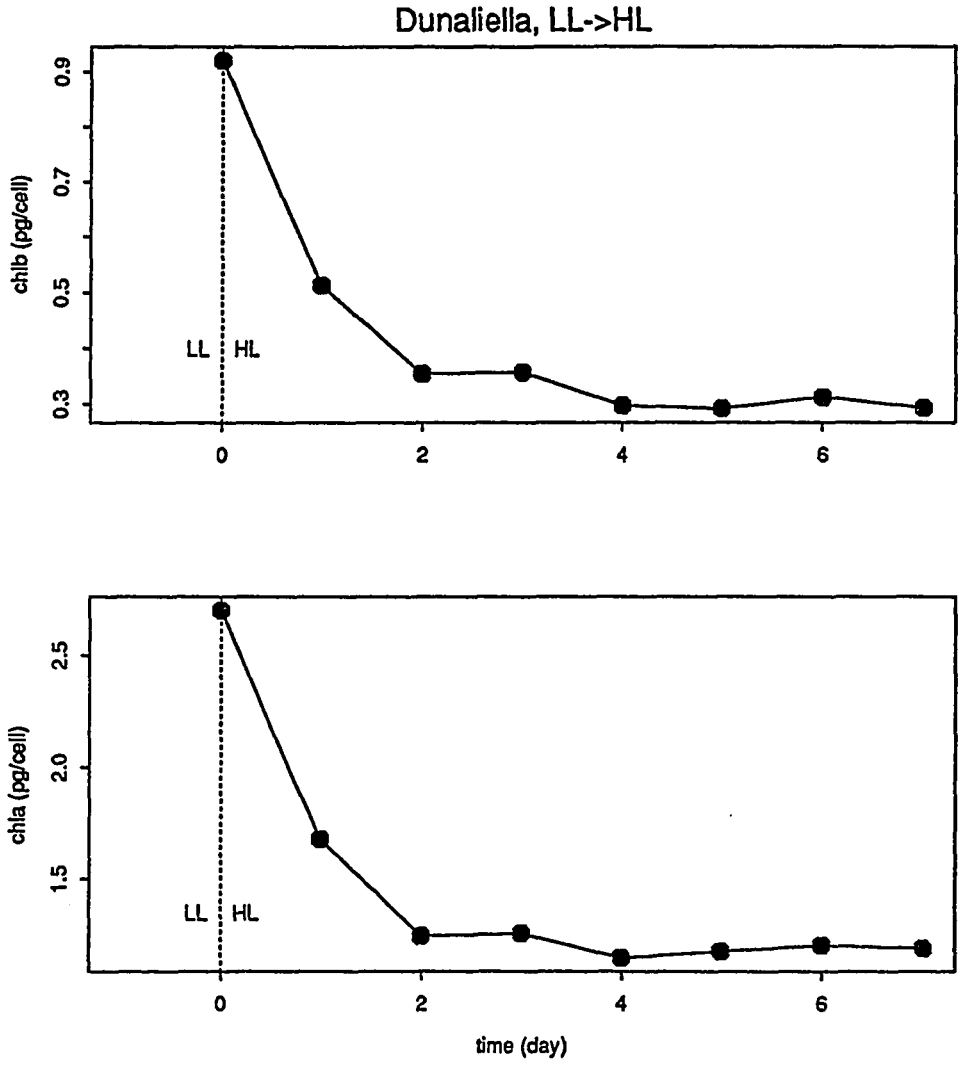


Fig. 5B

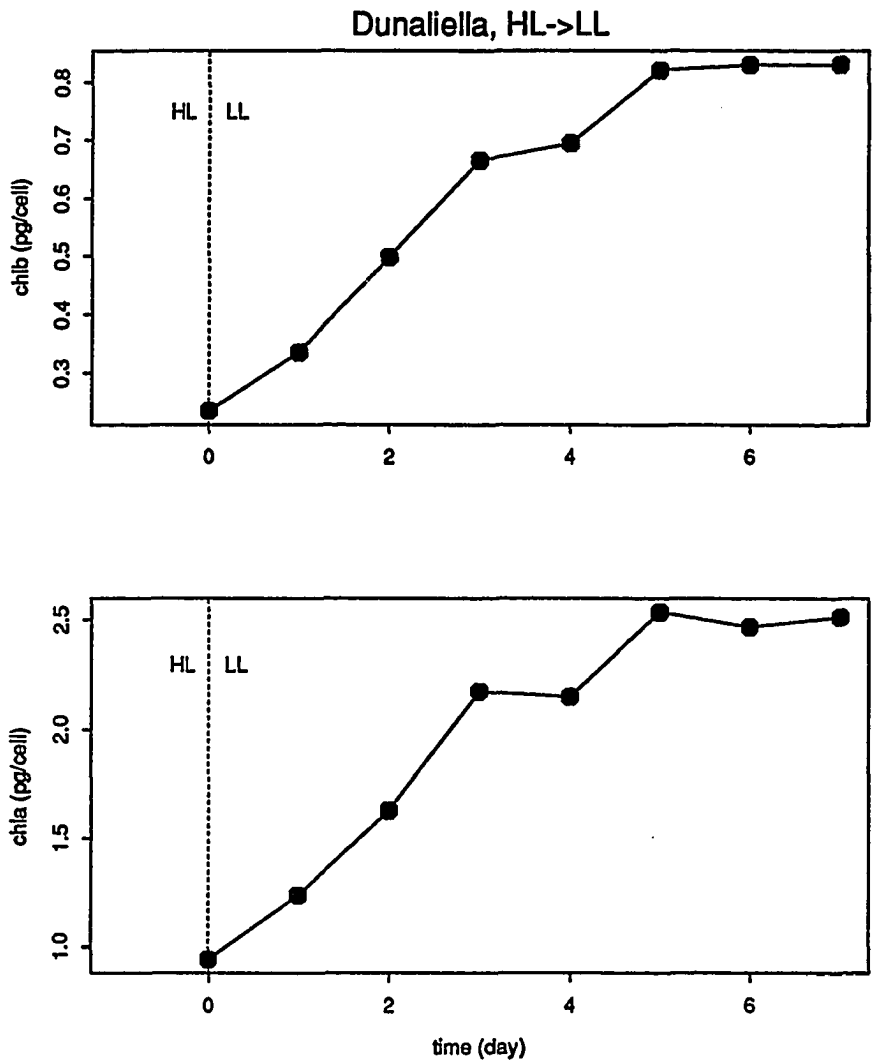


Fig. 5C

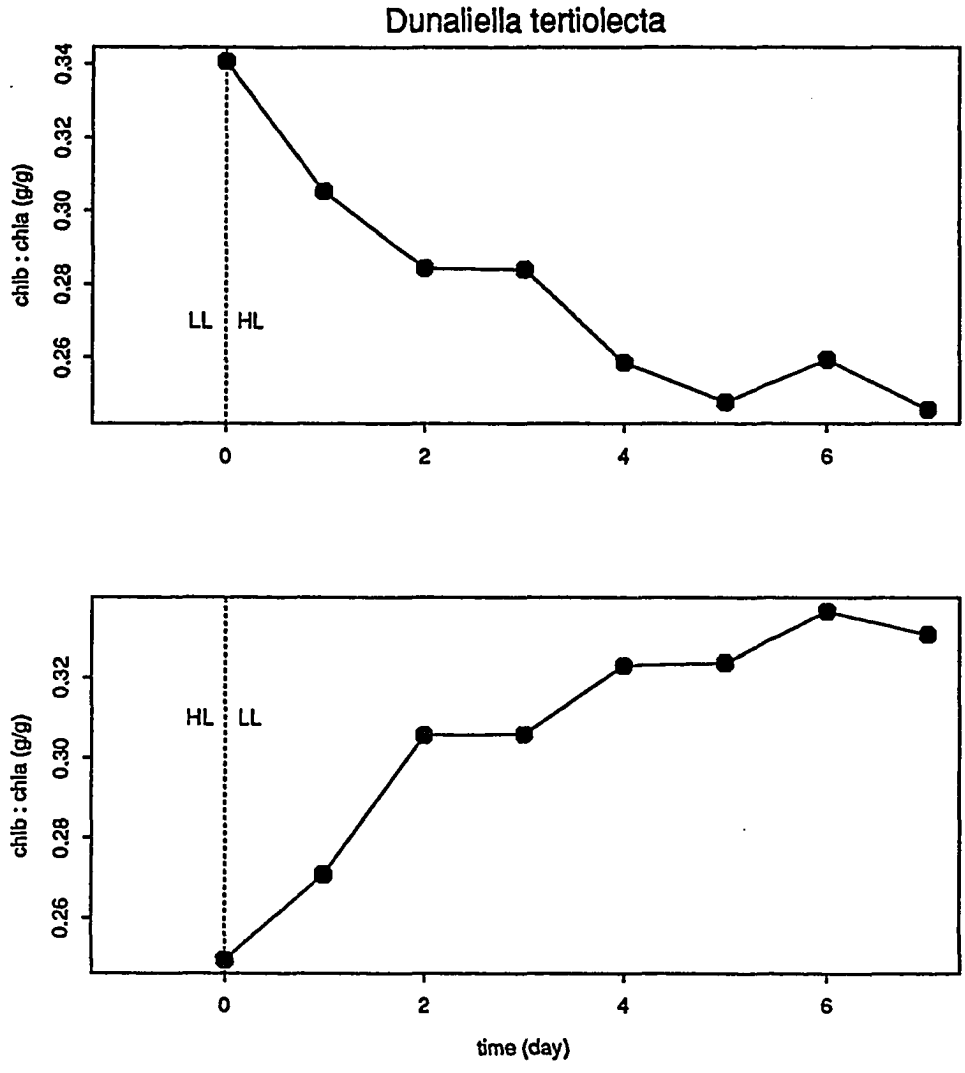


Fig. 6. Time course changes in cellular pigment contents and pigment ratios in *Tetraselmis levis*, following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in chl *b* and chl *a* contents (pg cell^{-1}) during LL–>HL (Fig. 6A) and during HL–>LL (Fig. 6B), and changes in chl *b* : chl *a* ratios (g g^{-1}) following each light transition (Fig. 6C). Light transitions occurred at time zero, which coincided with the beginning of the light period. All sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

Fig. 6A

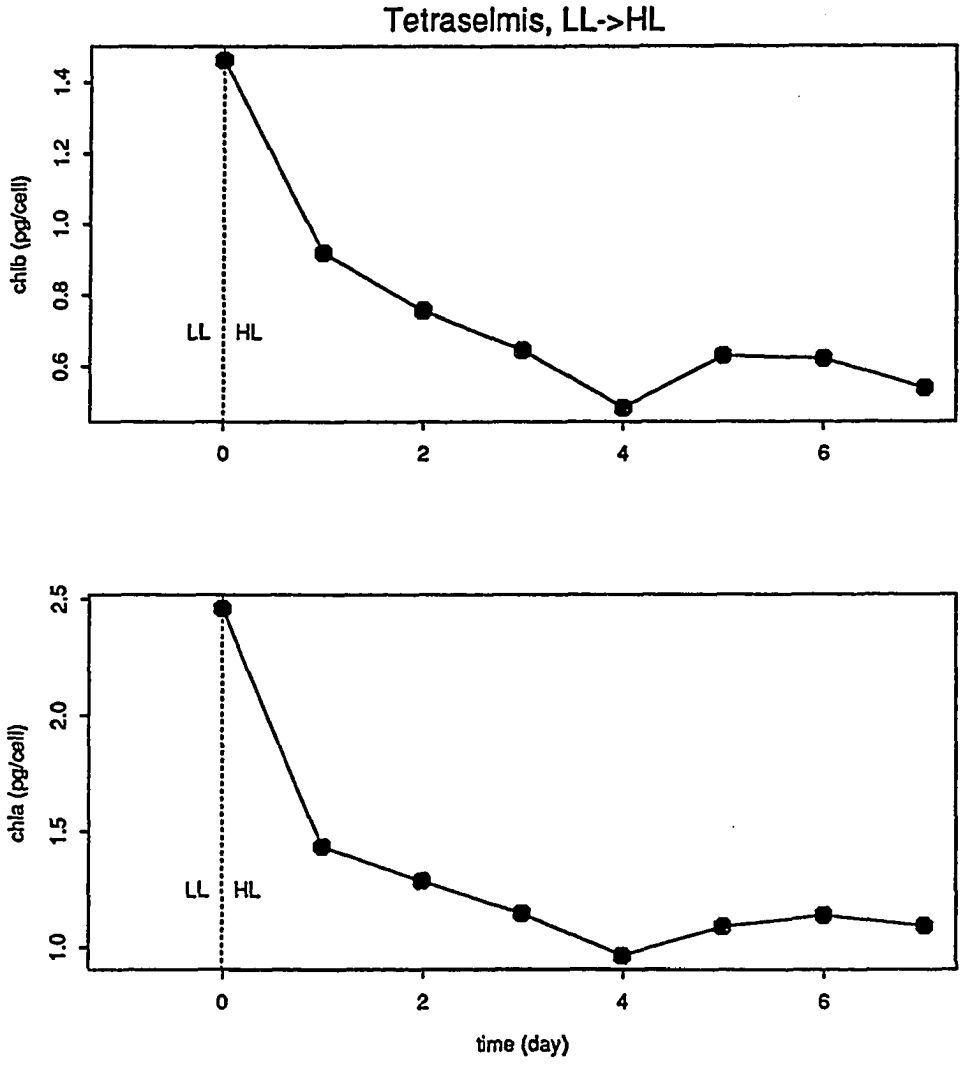


Fig. 6B

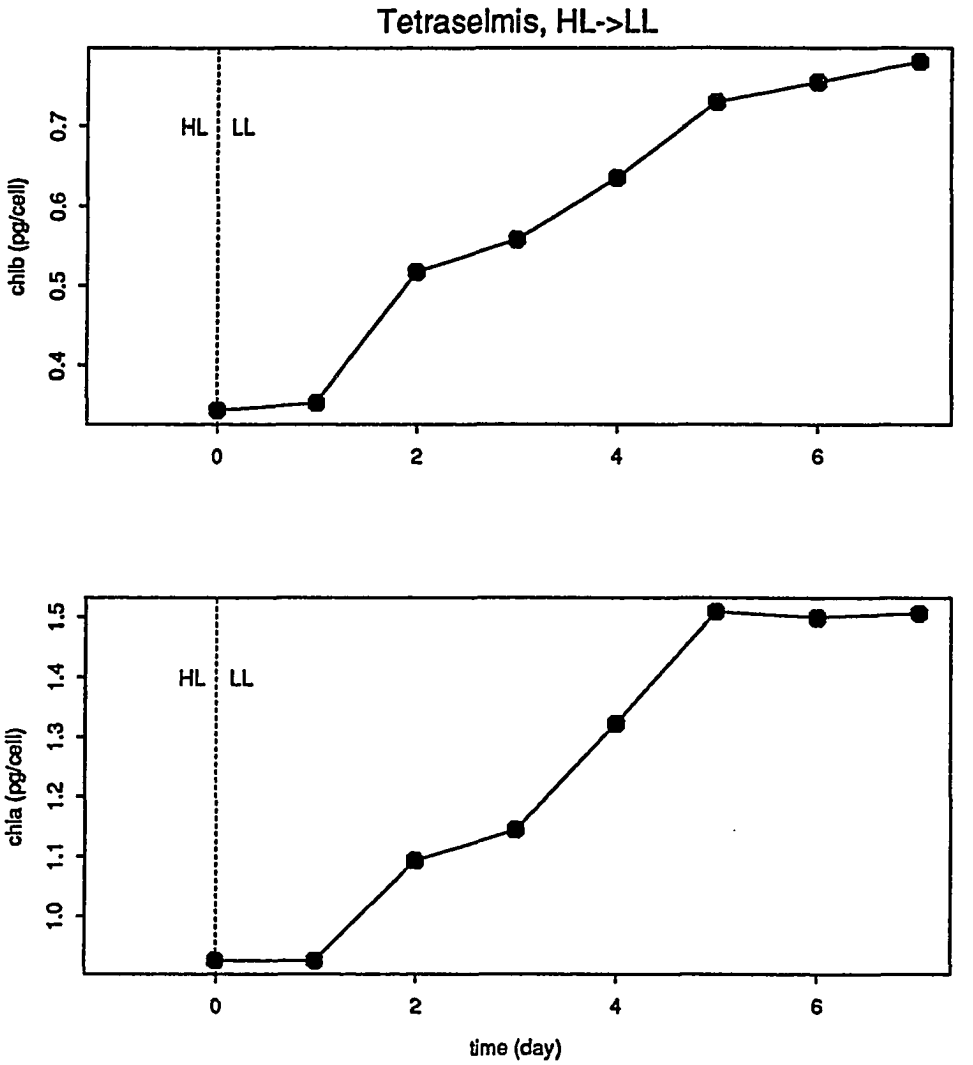
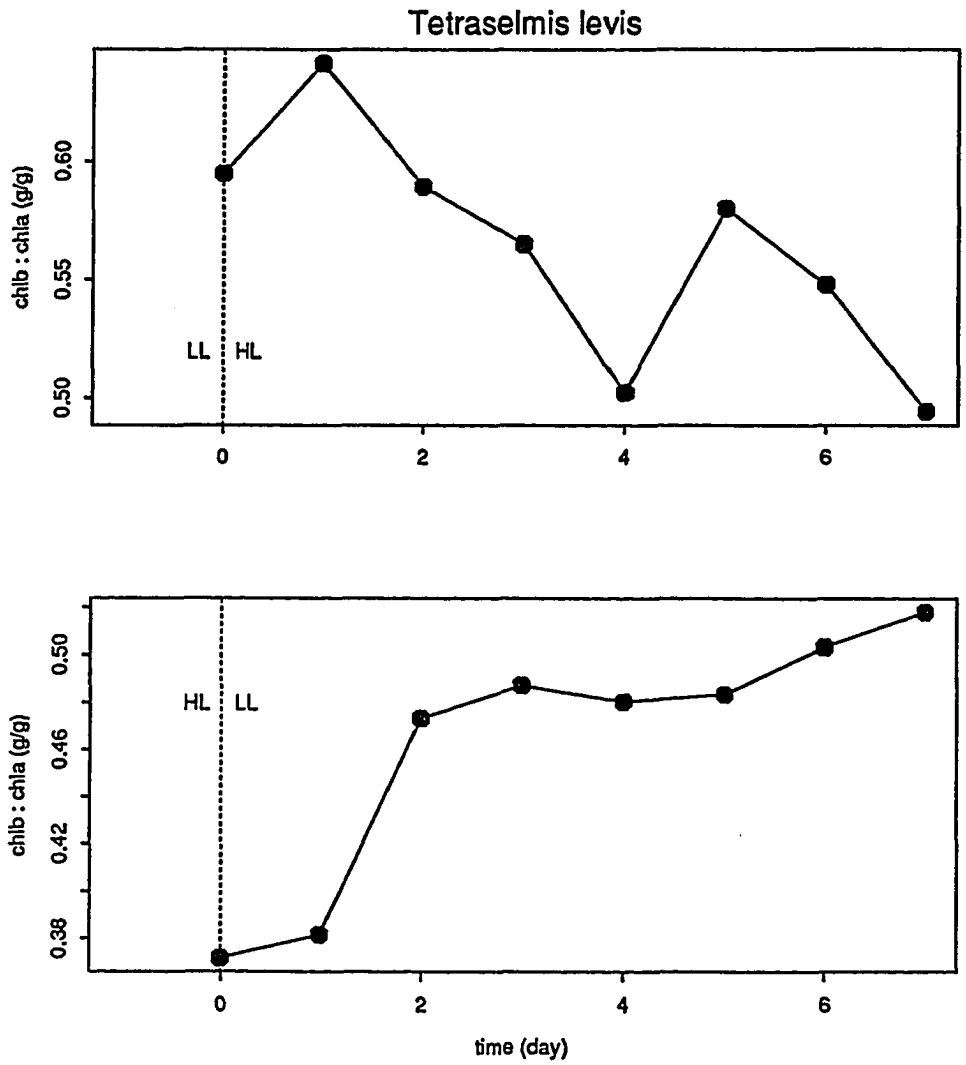


Fig. 6C



Carbon and Nitrogen Composition

Cellular carbon and nitrogen responded to light intensity changes in a way opposite of the response of light harvesting pigments (Fig. 7-9). In the LL->HL tansition, all three species increased their cellular concentrations of carbon and nitrogen. In both *S. costatum* and *T. levis,* carbon and nitrogen content increased slowly and reached equilibrium after four days (Fig.7, 9). In *D. tertiolecta,* however, carbon and nitrogen contents increased sharply during the first two days,overshooting the equilibrium value, followed by a slow decline to the new steady state level (Fig. 8). The C : N ratio increased from 7.3 to 8.5 g g^{-1} for *S. costatum,* but increased only slightly for *D. tertiolecta* and *T. levis.*

In the HL->LL transition, carbon and nitrogen contents decreased in *S. costatum* and *D. tertiolecta. T. levis* did not exhibit much change in cellular carbon and nitrogen contents in response to the light transition. C : N ratio decreased from 8.5 to 7.0 g g^{-1} for *S. costatum,* but decreased only slightly in *D. tertiolecta* and *T. levis.*

Carbon : Pigment Ratios

Because the light harvesting pigments and the cell carbon pool respond differently to changes in growth irradiance, C : pigment ratios appear to have a higher dynamic range of change (Fig. 10-12). In *S. costatum* (Fig. 10), C : fucox-

anthin ratio increased dramatically from 50 g g^{-1} at low light to a steady state value of 250 g g^{-1} at high light intensity, a factor of five fold. C : chl *a* ratio changed by a factor of three from 20 at low light to 60 g g^{-1} at high light intensity.

In *D. tertiolecta,* (Fig. 11), C : chl *b* ratio was about 20 g g^{-1} at LL, but inreased to 80 g g^{-1} at HL. The C : chl *a* ratio was about 6 g g^{-1} at LL but increased to about 20 g g^{-1} at HL. The changes were very rapid, and new equilibriums can be reached in two days.

Similarly, *T. levis* experienced a three fold change in both C : chl *b* and C : chl *a* ratios following transition of light regime from LL to HL (Fig. 12). During the reciprocal light shift, the ratios changed by only two fold. The discrepancy may be due to differences in culture conditions which will be discussed later.

Fig. 7. Time course changes in cellular carbon, nitrogen contents and C : N ratios in *Skeletonema costatum,* following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in carbon and nitrogen contents (pg $cell^{-1}$) during LL–>HL (Fig. 7A), during HL–>LL (Fig. 7B), and changes in C : N ratios (g g^{-1}) following each light transition (Fig. 7C). Light transitions occurred at time zero, which coincided with the beginning of the light period. All sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

Fig. 7A

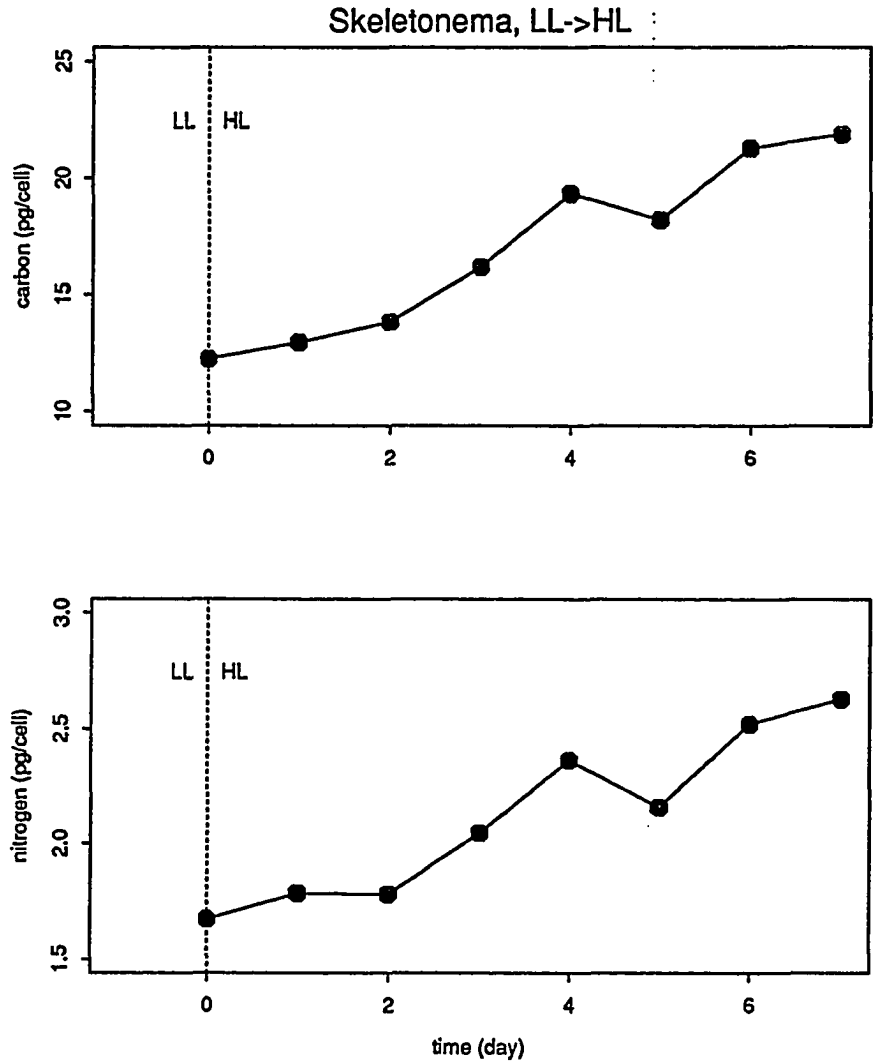


Fig. 7B

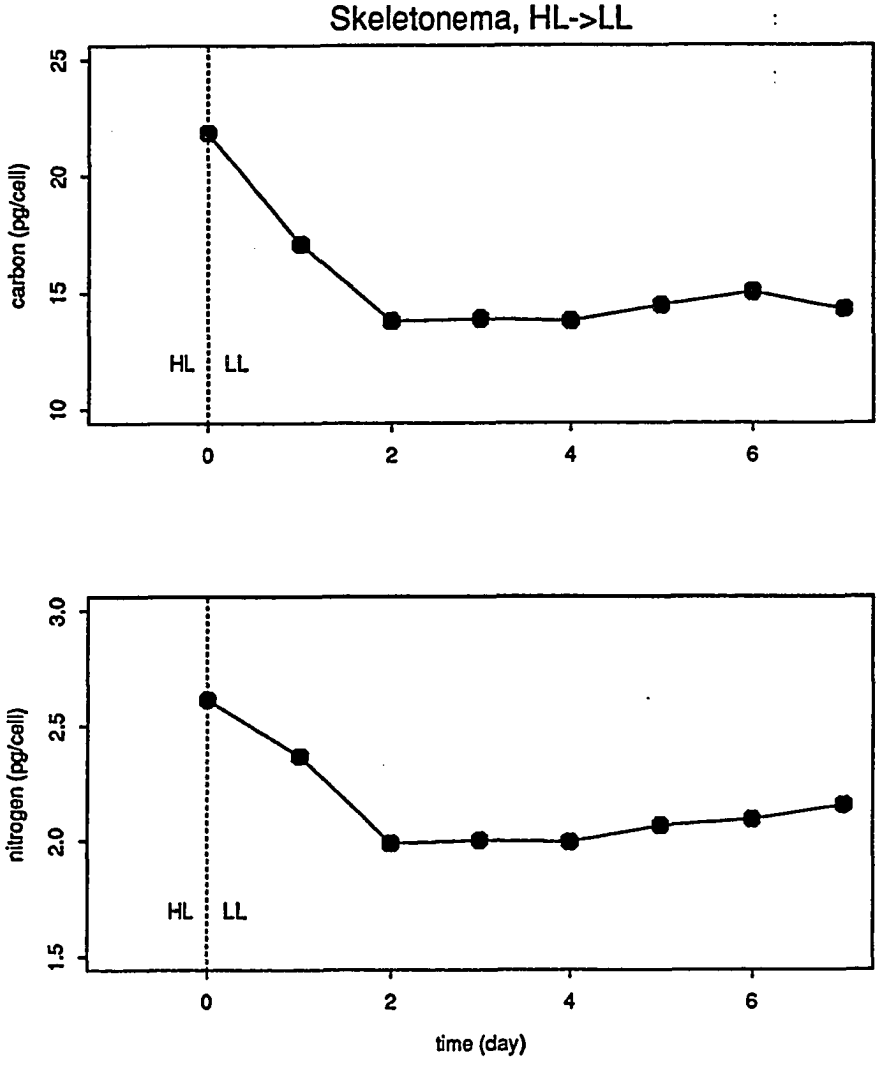


Fig. 7C

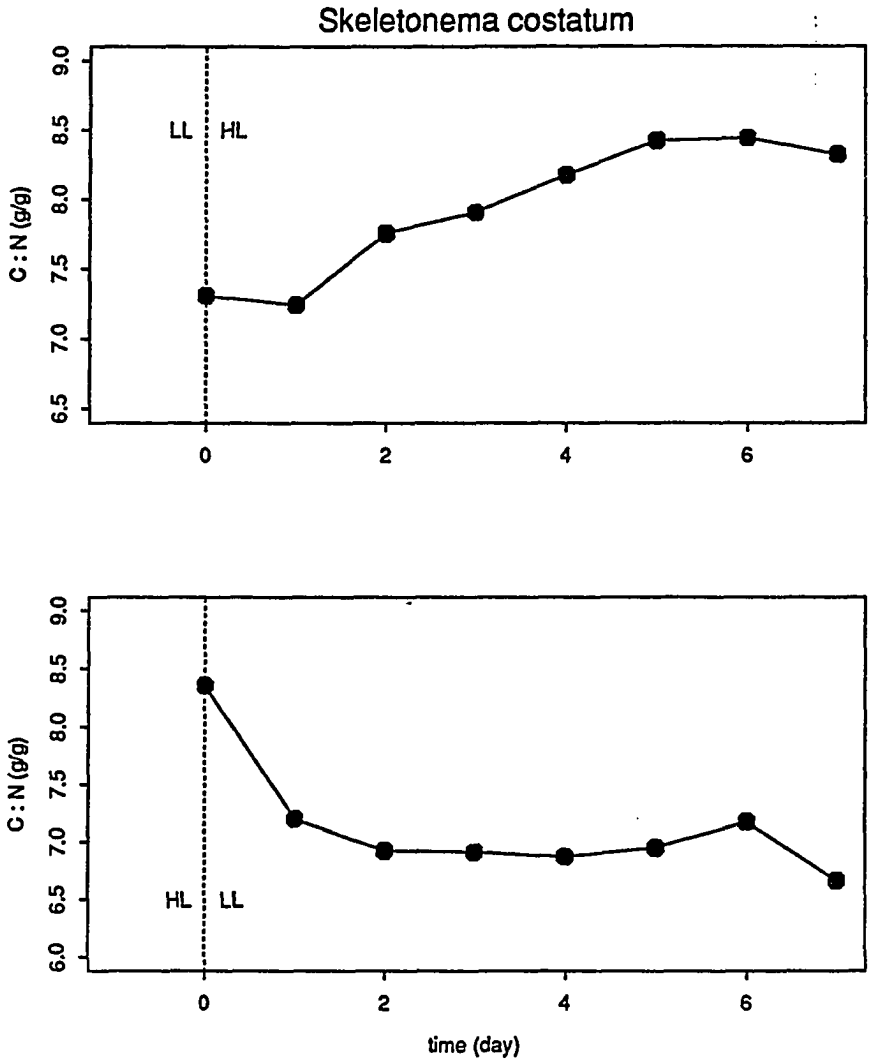


Fig. 8. Time course changes in cellular carbon, nitrogen contents and C : N ratios in *Dunaliella tertiolecta,* following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in carbon and nitrogen contents (pg $cell^{-1}$) during LL–>HL (Fig. 8A), during HL–>LL (Fig. 8B), and changes in C : N ratios (g g^{-1}) following each light transition (Fig. 8C). Light transitions occurred at time zero, which coincided with the beginning of the light period. All sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

Fig. 8A

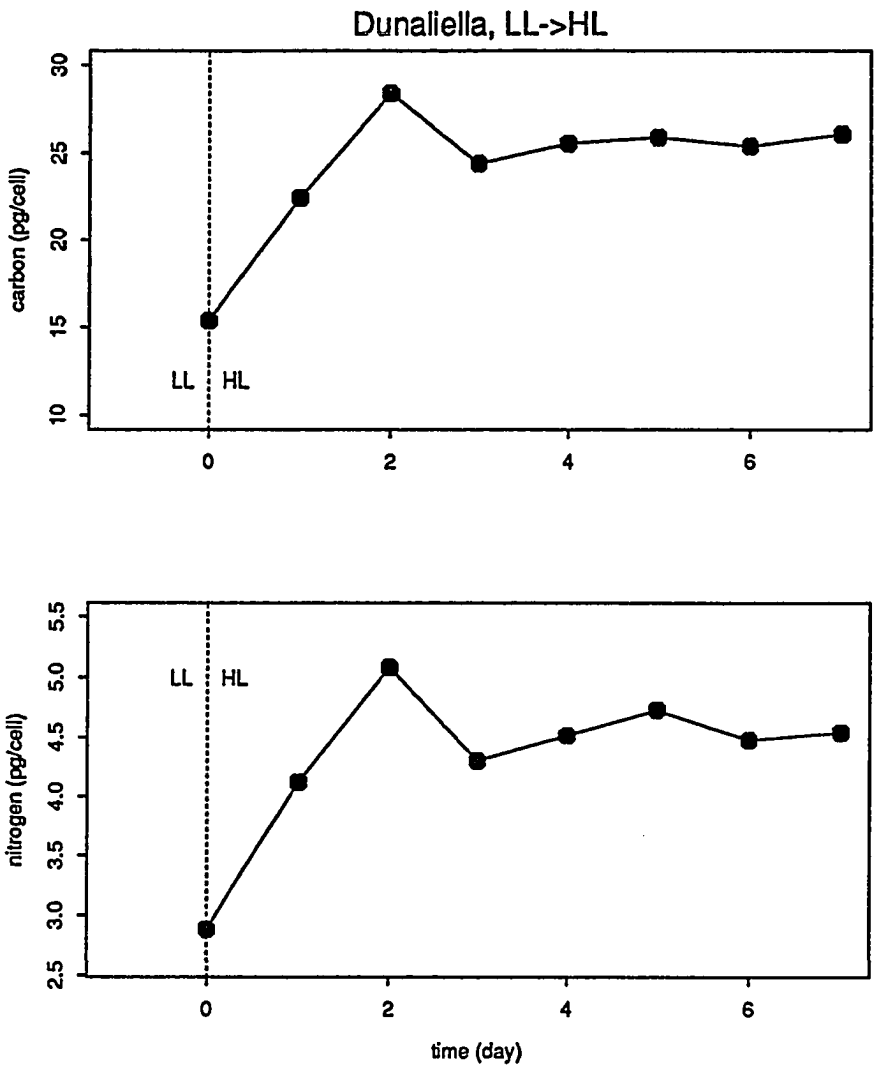


Fig. 8B

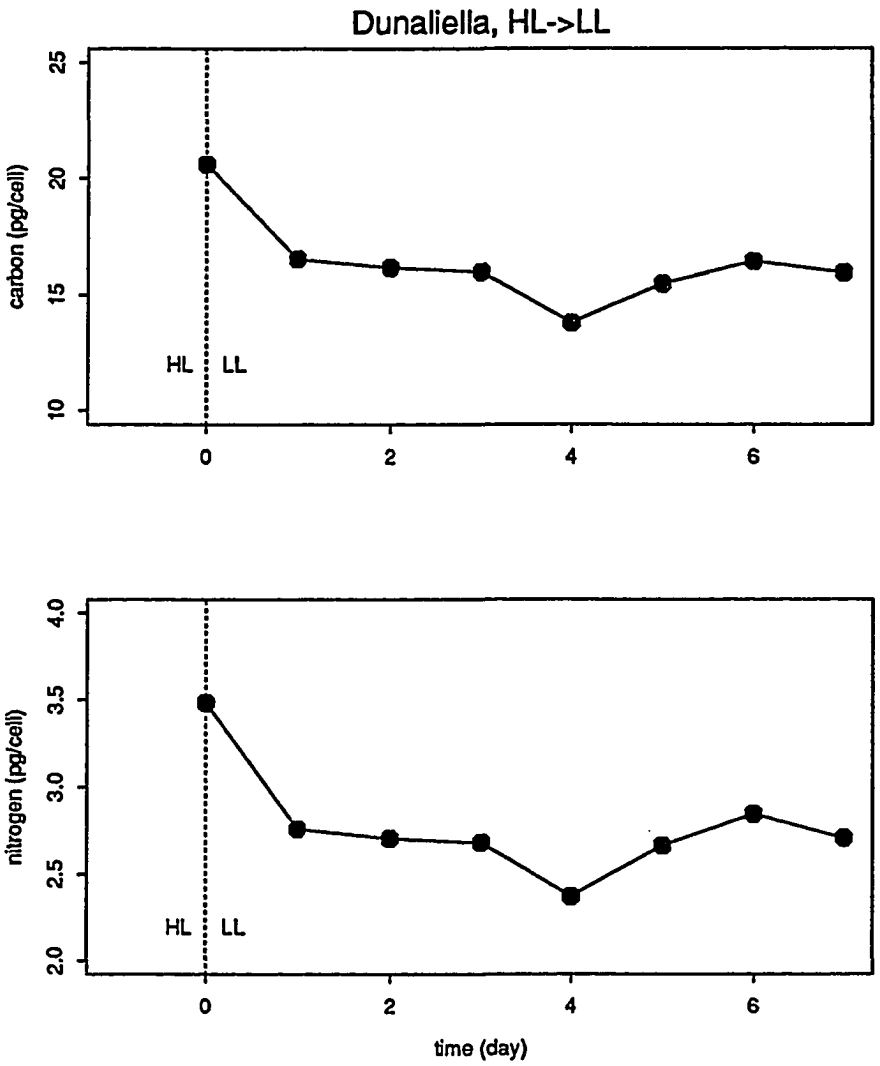


Fig. 8C

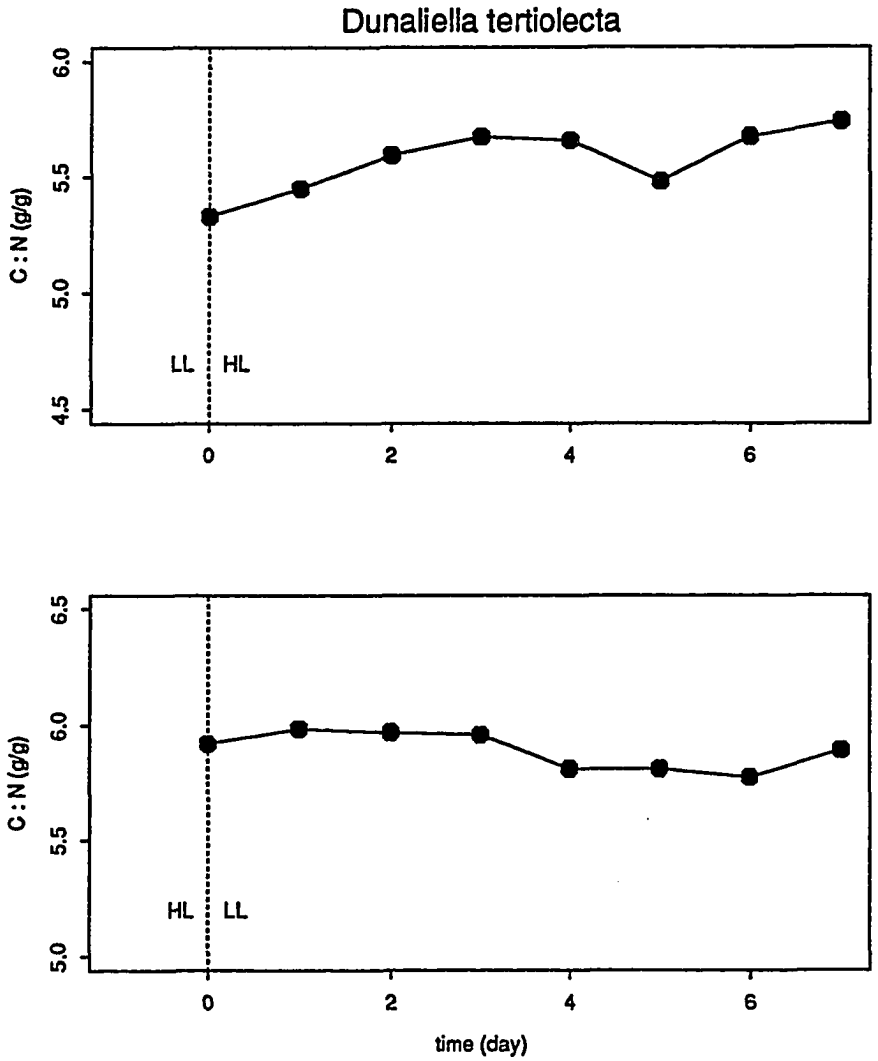


Fig. 9. Time course changes in cellular carbon, nitrogen contents and C : N ratios in *Tetraselmis levis,* following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in carbon and nitrogen contents (pg $cell^{-1}$) during LL–>HL (Fig. 9A), during HL–>LL (Fig. 9B), and changes in C : N ratios (g g^{-1}) following each light transition (Fig. 9C). Light transitions occurred at time zero, which coincided with the beginning of the light period. All sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

Fig. 9A

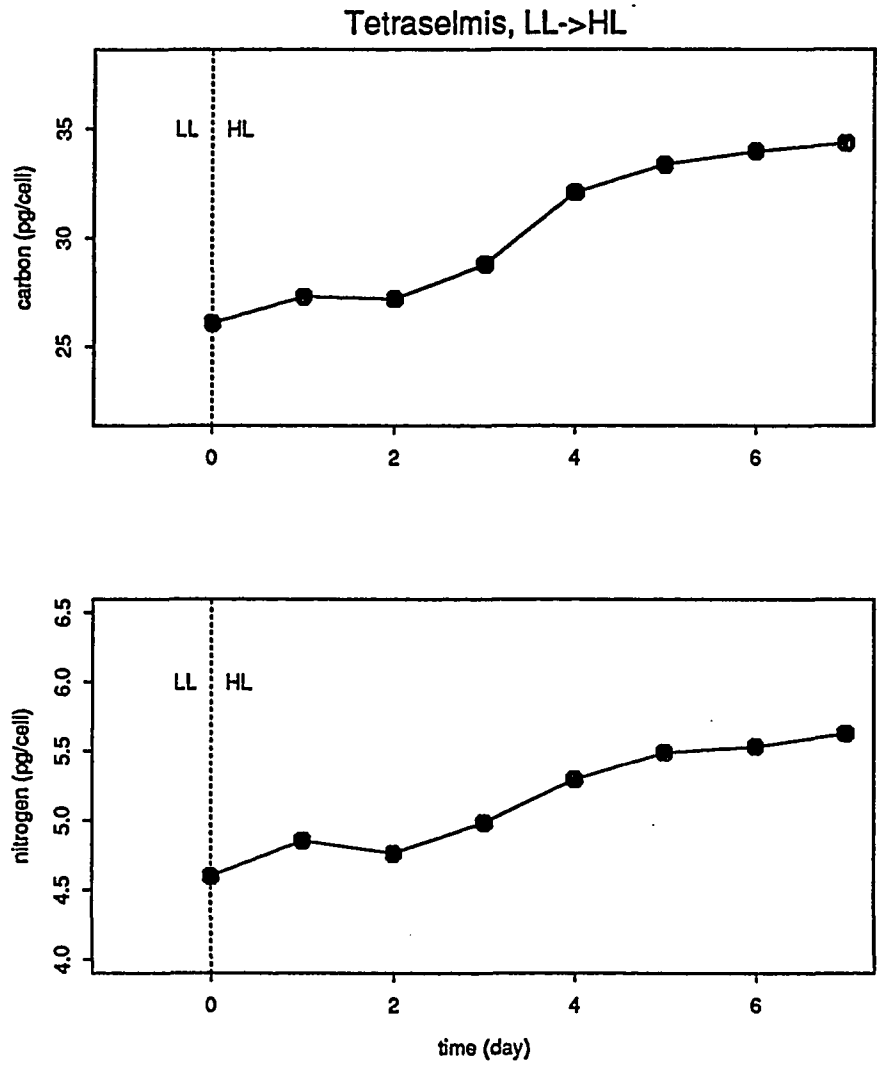


Fig. 9B

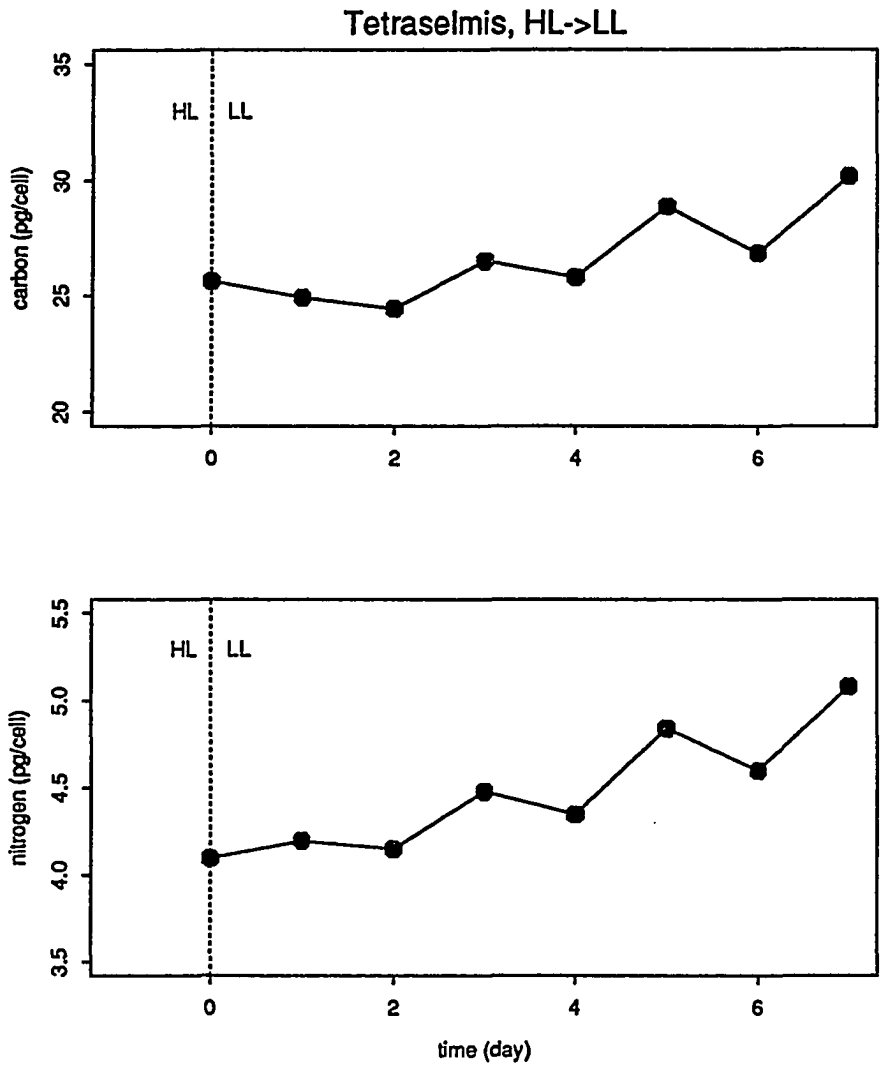


Fig. 9C

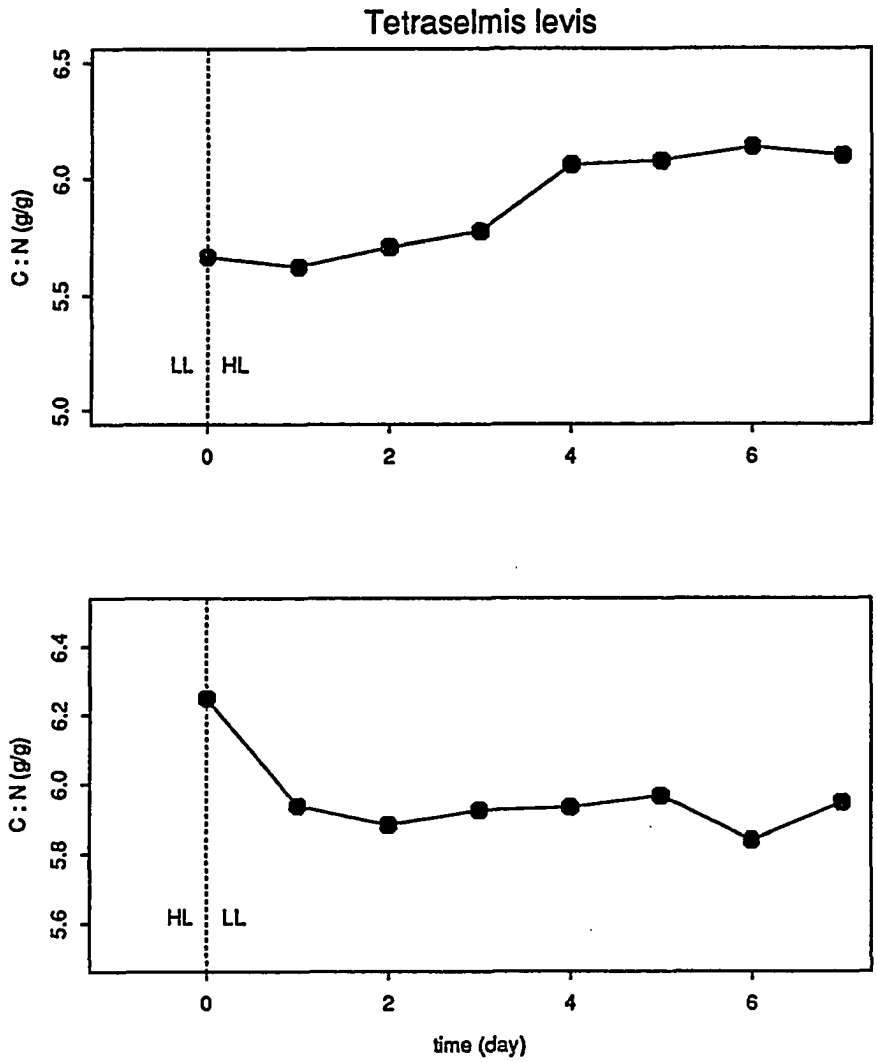


Fig. 10. Time course changes in C : pigment ratios in *Skeletonema costatum,* following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in C : fucoxanthin ratios (Fig. 10A) and C : chl *a* ratios (Fig. 10B) for each light transition. Light transitions occurred at time zero, which coincided with beginning of the light period. All sampling took place at the beginning of each light period. The ratios are in g g^{-1}. X–axis is time (day) elapsed following changes in light intensity.

Fig. 10A

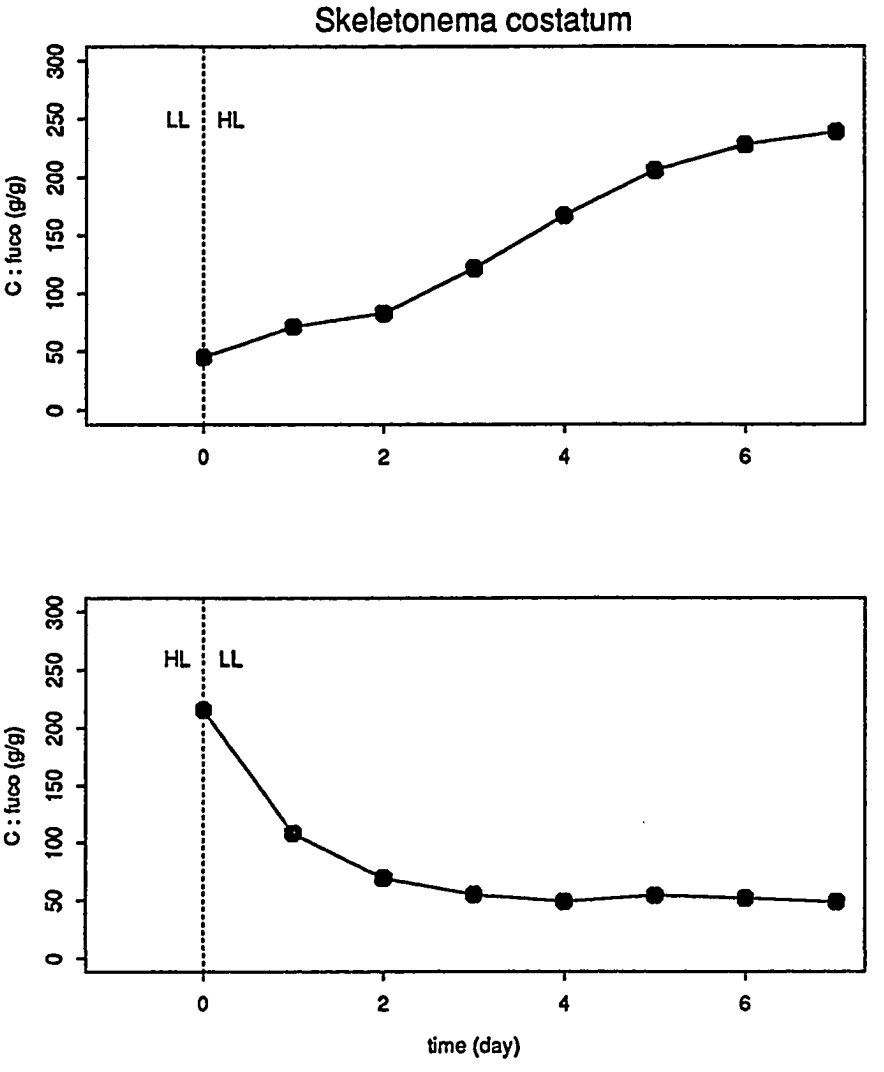


Fig. 10 B

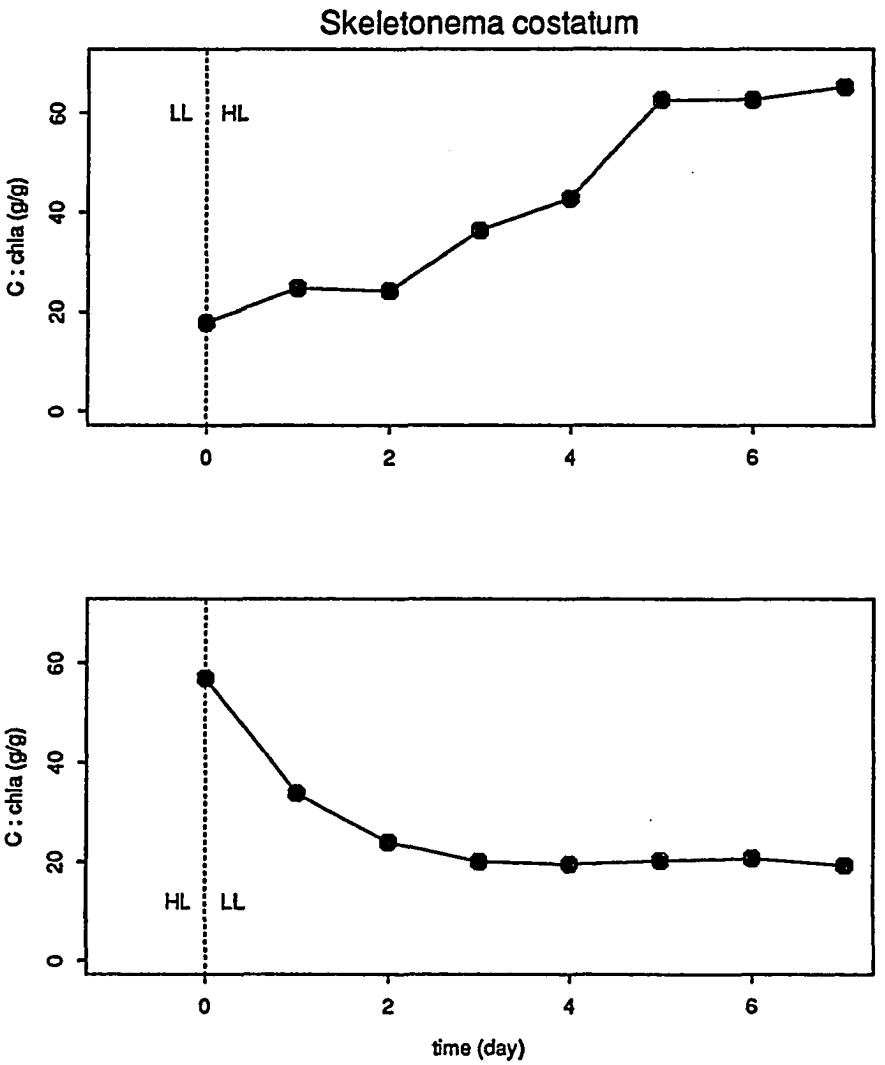


Fig. 11. Time course changes in C : pigment ratios in *Dunaliella tertiolecta,* following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in C : chl *b* ratios (Fig. 11A) and C : chl *a* ratios (Fig. 11B) for each light transition. Light transitions occurred at time zero, which coincided with beginning of the light period. All sampling took place at the beginning of each light period. The ratios are in g g^{-1}. X–axis is time (day) elapsed following changes in light intensity.

Fig. 11A

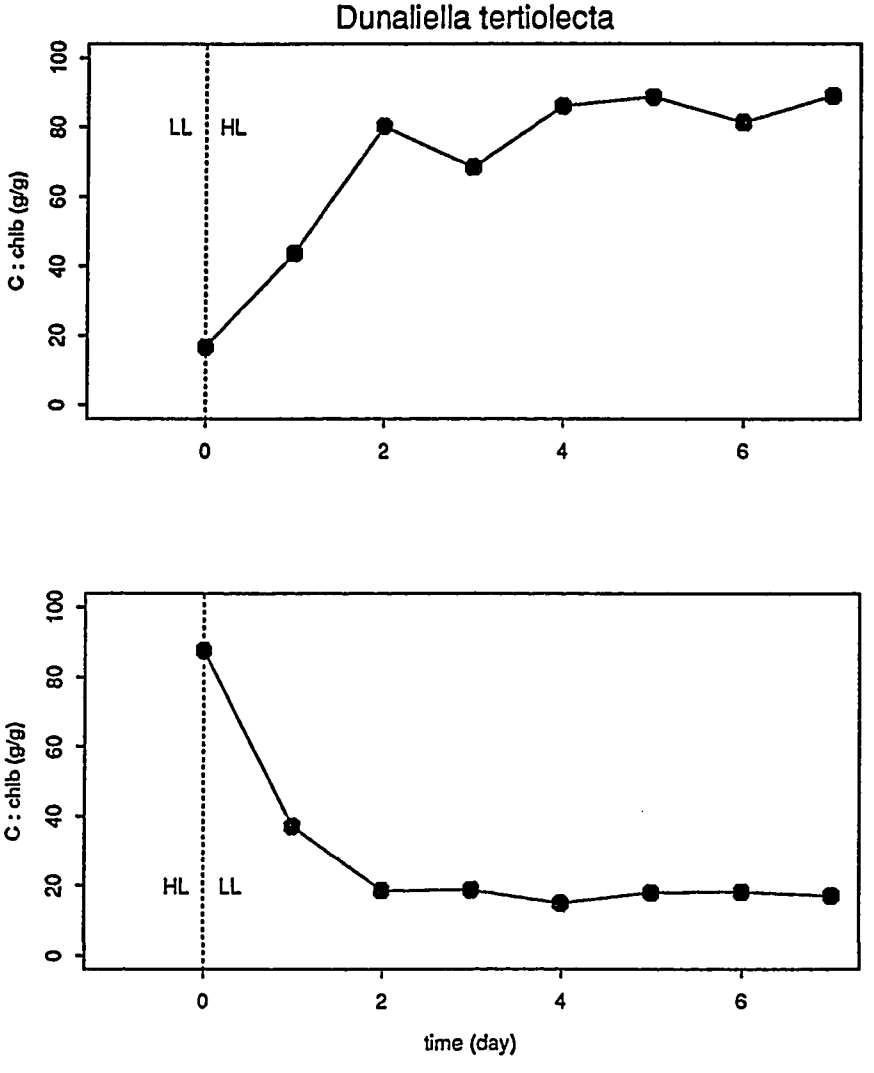


Fig. 11B

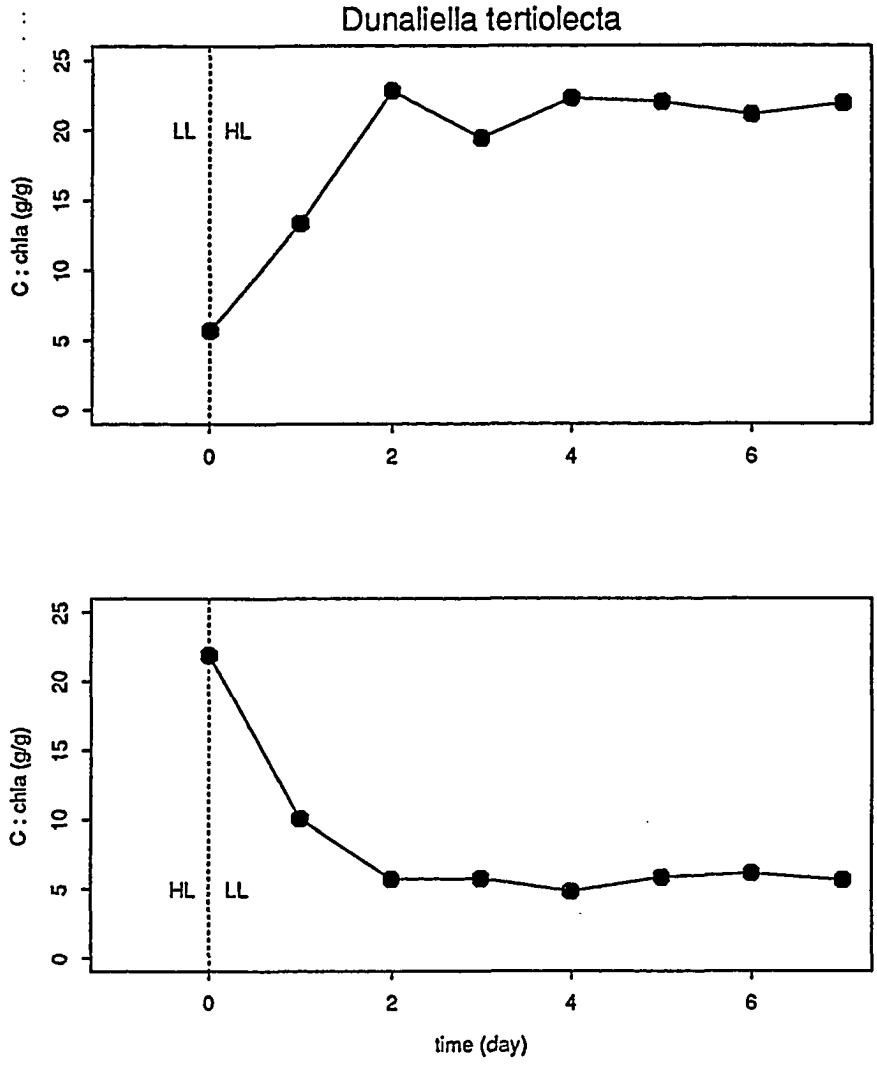


Fig. 12. Time course changes in C : pigment ratios in *Tetraselmis levis*, following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) during the continuous culture experiments. Shown are changes in C : chl *b* ratios (Fig. 12A) and C : chl *a* ratios (Fig. 12B) for each light transition. Light transitions occurred at time zero, which coincided with beginning of the light period. All sampling took place at the beginning of each light period. The ratios are in g g^{-1}. X–axis is time (day) elapsed following changes in light intensity.

Fig. 12A

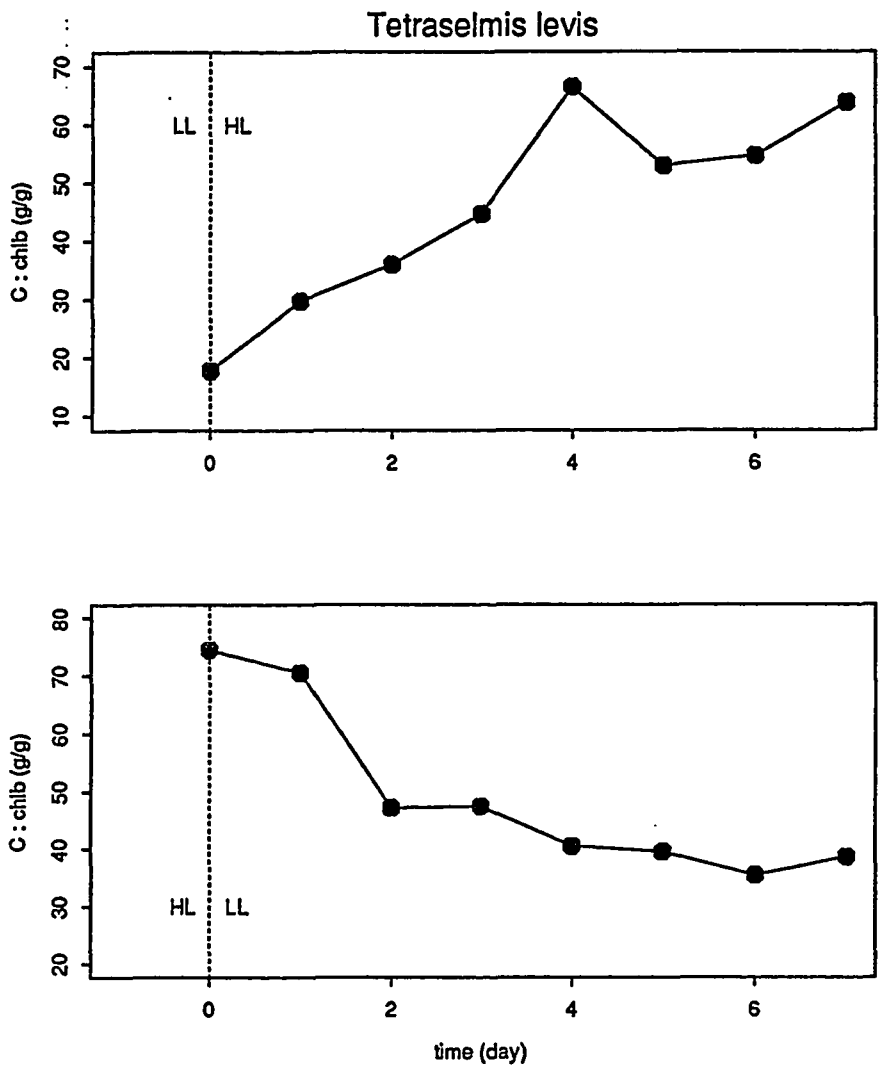
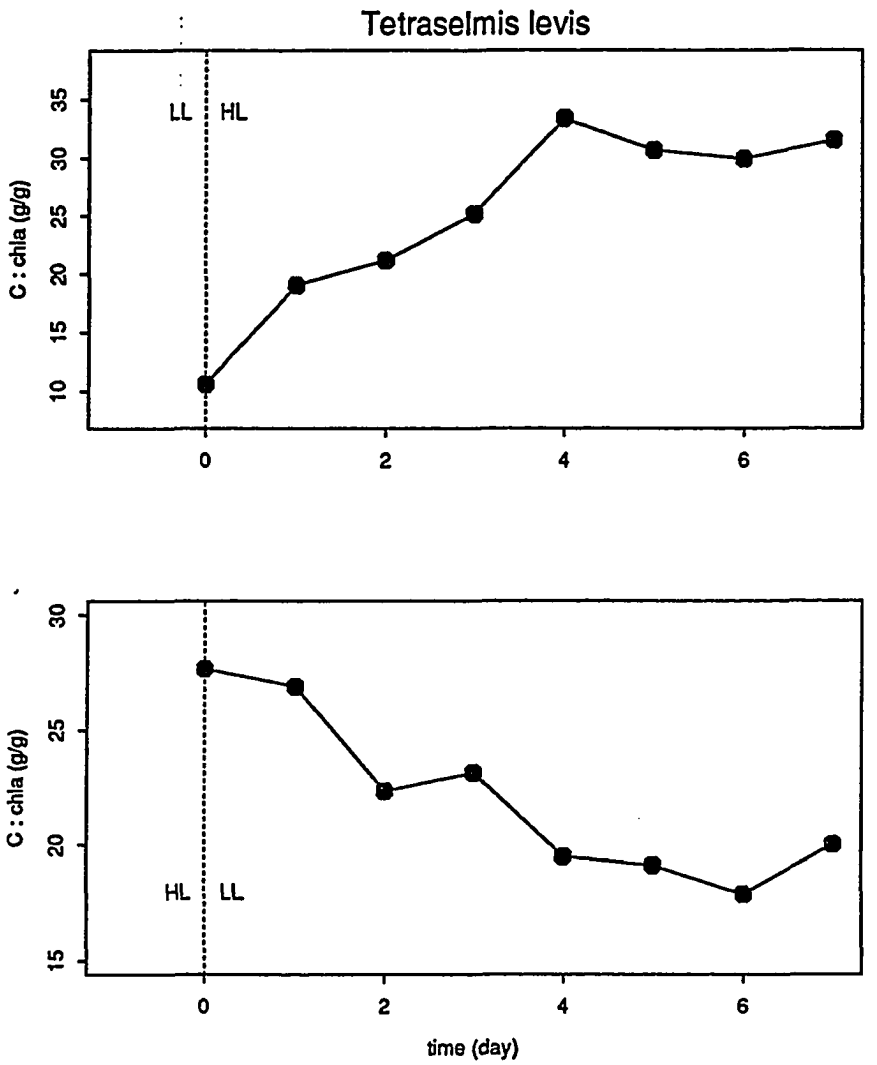


Fig. 12B



3.2 The Rate Constants Of Photoadaptation

Pigment Composition

The rate constants of photoadaptation of the above parameters were estimated from the nonlinear regression procedure discussed in Chapter2.4, assuming first order kinetics of the time course data. The results were listed in Table 1.

In the LL->HL transition, the cellular fucoxanthin content of *Skeletonema costatum* decreased at a first order rate of 0.63 d^{-1}, cellular chl *a* decreased at 0.55 d^{-1}, and fucoxanthin : chl *a* ratio decreased at a rate of 0.52 d^{-1}. In contrast, during the HL->LL transition, fucoxanthin $cell^{-1}$ increased at a first order rate of 0.37 d^{-1}, chl *a* $cell^{-1}$ at 0.44 d^{-1}, and fucoxanthon : chl *a* ratio at 0.42 d^{-1}. Thus, it seems that for photosynthetic pigments, the reciprocal changes due to light transition is not a simple mechanical reverse process.

This appears to be true also for *Dunaliella tertiolecta*. In this species, following transfer from LL to HL, chl *b* $cell^{-1}$ decreased at a first order rate of 1.08 d^{-1}, and chl *a* $cell^{-1}$ at 1.18 d^{-1}. The chl *b* : chl *a* ratio decreased at a rate of 0.49 d^{-1}. In the reciprocal light shift, HL->LL, cellular chl *b* , chl *a* contents and the chl *b* : chl *a* ratio all increased, but at much lower first order rates of 0.69, 0.86 and 0.44 d^{-1}, respectively.

In *Tetraselmis levis,* transition from LL to HL caused the cellular chl *b* and chl *a* to decrease. The rates of the decrease were about identical for either variable. As a result, chl *b* : chl *a* ratio did not show significant decrease over time. The rate constant was not quantified because the time course data did not exhibit strong patttern of first order kinetics. In the HL->LL transition, chl *b* $cell^{-1}$, chl *a* $cell^{-1}$, and chl *b* : chl *a* ratio all increased, at first order rates of 0.11, 0.06 and 0.39 d^{-1}, respectively. Although the changes following HL->LL transition may be better described by logistic curves (e.g., Fig. 4B, 5B, 6B), for consistency and comparative purpose, only first order kinetics model was used to estimate the rate constants.

Carbon And Nitrogen Composition

In all three species, cellular contents of carbon and nitrogen increased during LL->HL and decreased during HL->LL transition. Again, for consistency and comparative purpose, the rate constants were estimated assuming first order kinetics model, even though in certain cases a logistic model may be more appropriate (e.g., Fig 7A, 9A). These rate constants are listed in Table 1.

For *Skeletonema costatum,* cellular carbon and nitrogen increased very slowly following a LL->HL transition. The rate constants were not quantified because the time course data could not be described by first order kinetics model. C : N ratio also changed slowly, increasing at a first order rate of only 0.19 d^{-1}. Higher rates were observed during the HL->LL transition experiments, in which cellular carbon,

nitrogen contents, and C : N ratio decreased at modest rates of 1.26, 1.08 and 1.67 d^{-1}, respectively.

Cellular carbon and nitrogen contents increased rapidly in *Dunaliella tertiolecta* following light changes from LL to HL. Carbon $cell^{-1}$and nitrogen $cell^{-1}$ increased at 1.31 and 1.73 d^{-1} respectively. In the reverse light transition (HL->LL), cellular carbon and nitrogen decreased even faster, with first first order rate constants of 1.63 and 2.04 d^{-1}. In both cases of light regime changes, C : N ratio for *D. tertiolecta* changed only slightly, with rate constants not significantly different from zero.

In *Tetraselmis levis,* cellular carbon and nitrogen increased slowly following light shift from LL to HL. Time course changes in C : N ratio can not be fitted with the first order kinetics model. Interestingly, light changes from HL to LL failed to cause a decline in either carbon or nitrogen content of the cell (Actually there was a slight increase, though not statistically significant). This behavior is peculiar, and may be due to factors other than light regime changes. Like in *D. tertiolecta,* C : N ratio in *T. levis* did not show any significant changes over time.

C : Pigment Ratios

As mentioned earlier, C : pigment ratios change more dramatically than pigment ratios or C : N ratio. The first order rate constants, estimated from nonlinear regression analysis, are listed in Table 1.

The C : pigment ratios in *Skeletonema costatum* changed dramatically as the growth irradiance changed from LL to HL. However, the rates cannot be quantified because the pattern of change cannot be described by first order kinetics (the non-linear regression procedure failed to converge). In the transition from HL to LL, the C : fucoxanthin ratio decreased at a rate of 1.06 d^{-1}. Similar rate was obtained for the C : chl *a* ratio, with 1.02 d^{-1}.

The C : pigment ratios in *Dunaliella tertiolecta* changed rapidly as was the case in *S. costatum.* C : chl *b* and C : chl *a* ratios in *D. tertiolecta* increased at 0.65 and 0.93 d^{-1}, respectively, following a LL-HL transition. Again, it is interesting to note that during the reverse light shift (HL->LL), the first order rates of decrease were much higher, with 1.33 and 1.39 d^{-1}.

Tetraselmis levis was not as responsive as *D. tertiolecta.* C : chl *b* and C : chl *a* ratios increased at rates of only 0.30 and 0.41 d^{-1} as growth irradiance changed from LL to HL. However, like in *D. tertiolecta, T. levis* showed higher rates of decrease during reciprocal light transition, in which the ratios declined at rates of 0.40 and 0.59 d^{-1}, respectively.

Table 1. Regression analysis of first order kinetics of photoadaptation—results from non–linear curve fitting. R_0 and R_∞ are initial and asymptotic values of appropriate photoadaptive variables (pg cell^{-1} for cellular properties, g g^{-1} for ratios); k is the first order rate constant (d^{-1}); s.e. is standard error; S—first order rate constant significantly different from zero; NS—first order rate constant not significantly different from zero; NP—no pattern of first order kinetics, hence no meaningful figures (nmf) were obtained for the regression parameters.

Table 1.1 *Skeletonema costatum*

parameter	light shift	R_o	s.e.	R_{oo}	s.e.	k	s.e	r^2	Remarks
fuco	LL->HL	0.27	0.01	0.11	0.02	0.63	0.24	0.97	S
	HL->LL	0.10	0.01	0.31	0.02	0.37	0.08	0.99	S
chl *a*	LL->HL	0.68	0.06	0.43	0.13	0.55	0.28	0.82	S
	HL->LL	0.38	0.02	0.76	0.02	0.44	0.08	0.89	S
carbon	LL->HL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
	HL->LL	21.9	0.74	14.2	0.35	1.26	0.35	0.94	S
nitrogen	LL->HL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
	HL->LL	2.64	0.10	2.05	0.05	1.08	0.53	0.85	S
fuco : chl *a*	LL->HL	0.40	0.02	0.27	0.02	0.52	0.23	0.96	S
	HL->LL	0.27	0.01	0.40	0.01	0.42	0.12	0.96	S
C : N	LL->HL	7.17	0.15	8.95	0.74	0.19	0.14	0.72	S
	HL->LL	8.36	0.18	6.92	0.08	1.67	0.71	0.81	S
C : fuco	LL->HL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
	HL->LL	216	2.60	49.8	1.32	1.06	0.05	0.99	S
C : chl *a*	LL->HL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
	HL->LL	57.1	0.92	19.5	0.48	1.02	0.07	0.99	S

Table 1.2 *Dunaliella tertiolecta*

parameter	light shift	R_o	s.e.	R_{oo}	s.e.	k	s.e	r^2	Remarks
chl *b*	LL->HL	0.92	0.02	0.30	0.01	1.08	0.09	0.99	S
	HL->LL	0.20	0.08	0.93	0.05	0.69	0.22	0.93	S
chl *a*	LL->HL	2.71	0.05	1.17	0.02	1.18	0.10	0.99	S
	HL->LL	0.86	0.25	2.81	0.14	0.86	0.30	0.90	S
carbon	LL->HL	15.2	1.59	25.9	0.72	1.31	0.72	0.88	S
	HL->LL	20.6	0.93	15.6	0.42	1.63	0.91	0.83	S
nitrogen	LL->HL	2.87	0.30	4.59	0.13	1.73	1.05	0.65	S
	HL->LL	3.48	0.16	2.66	0.07	2.04	1.56	0.73	S
chl *b* : chl *a*	LL->HL	0.35	0.01	0.25	0.01	0.49	0.14	0.91	S
	HL->LL	0.25	0.01	0.34	0.01	0.44	0.10	0.97	S
C : N	LL->HL	5.32	0.39	5.67	1.47	0.29	0.44	0.41	NS
	HL->LL	5.97	0.56	5.61	3.21	0.06	0.50	0.35	NS
C : chl *b*	LL->HL	15.4	7.88	88.1	5.59	0.65	0.20	0.93	S
	HL->LL	88.0	2.05	16.8	0.96	1.33	0.11	0.99	S
C : chl *a*	LL->HL	5.32	1.96	22.0	1.07	0.93	0.30	0.92	S
	HL->LL	22.0	0.63	5.50	0.29	1.38	0.16	0.99	S

Table 1.3 *Tetraselmis levis*

parameter	light shift	R_o	s.e.	R_{oo}	s.e.	k	s.e	r^2	Remarks
chl *b*	LL->HL	1.46	0.06	0.57	0.04	0.88	0.17	0.97	S
	HL->LL	0.31	0.03	1.21	0.44	0.21	0.08	0.70	S
chl *a*	LL->HL	245	0.08	1.08	0.04	0.84	0.21	0.98	S
	HL->LL	0.87	0.06	2.78	2.74	0.16	0.12	0.38	NS
carbon	LL->HL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
	HL->LL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
nitrogen	LL->HL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
	HL->LL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
chl *b* : chl *a*	LL->HL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
	HL->LL	0.36	0.02	0.52	0.03	0.39	0.19	0.89	S
C : N	LL->HL	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
	HL->LL	6.25	0.24	5.91	0.32	0.14	0.16	0.31	NS
C : chl *b*	LL->HL	16.6	6.65	68.1	14.0	0.30	0.20	0.87	S
	HL->LL	77.1	4.55	33.2	6.09	0.40	0.16	0.92	S
C : chl *a*	LL->HL	10.5	2.31	33.3	2.93	0.41	0.16	0.93	S
	HL->LL	28.3	1.34	17.1	2.99	0.59	0.18	0.88	S

3.3 Discussion Of Continuous Culture Studies

Significance Of Changes

The preliminary experiments using continuous cultures showed that cellular carbon and nitrogen often do not respond to light changes according to first order kinetics, and that C : N ratio changes often are statistically nonsignificant. However, cellular pigment contents, pigment ratios and C : pigment ratios all exhibited significant changes following transitions of growth irradiance. The first order rate constants for these photoadaptive parameters, ranging from 0.16 to 2.04 d^{-1}, correspond to half-adaptation time scale of 0.3 to 4.3 days. Following a HL->LL transition, individual light harvesting pigments as well as accessary pigments to chl *a* ratios increased, whereas cellular carbon, nitrogen, C : N and C : pigment ratios decreased and vice versa. Of particular interest in this study is the pigment ratio changes. The data suggested that at the 5% level of significance, light changes caused significant changes in the pigment ratios examined in all three species.

Although the three species studied responded to light changes in a similar way, it is interesting to note the difference in their magnitiude of response. *Dunaliella tertiolecta* appear to be most responsive among three species, followed by *Skeletonema costatum,* and least responsive in *Tetraselmis levis. D. tertiolecta* consistently changed more rapidly than *T. levis* even though they have similar pigment composition.

Many of the time course data can not be described by first order kinetics. This is especially apparent in the carbon and nitrogen data from the LL->HL shift. Some of the time course data on pigments showed only weak patterns of first order kinetics, although they could still be fitted by the model. A logistic model often appears more appropriate to describe the time course changes in carbon and nitrogen content as well as C : N and C : pigment ratios during LL->HL transition. However, for the purpose of convenient rate comparison, only first order kinetics model was used (where it could be used).

Differences Between LL->HL and HL->LL Shifts

Considerable differences were observed in some photoadaptive parameters between reciprocal shifts of light intensity. The increase in cellular contents of both accessary pigments and chl *a* appeared to be faster in the LL->HL transition than the decrease in the HL->LL transition. For pigment ratios, however, no significant differences were found between the reciprocal light shifts. This means that the effects of hysteresis for either pigments have been canceled out, thus the pigment ratios may be better than other cellular properties as photoadaptation variables in studying vertical mixing processes.

In contrast to cellular pigmentation, carbon and nitrogen contents of the cells increase more slowly during LL->HL than they decrease during HL->LL transitions. Similarly, increases in C : N and C : pigment ratios are slower in the LL-

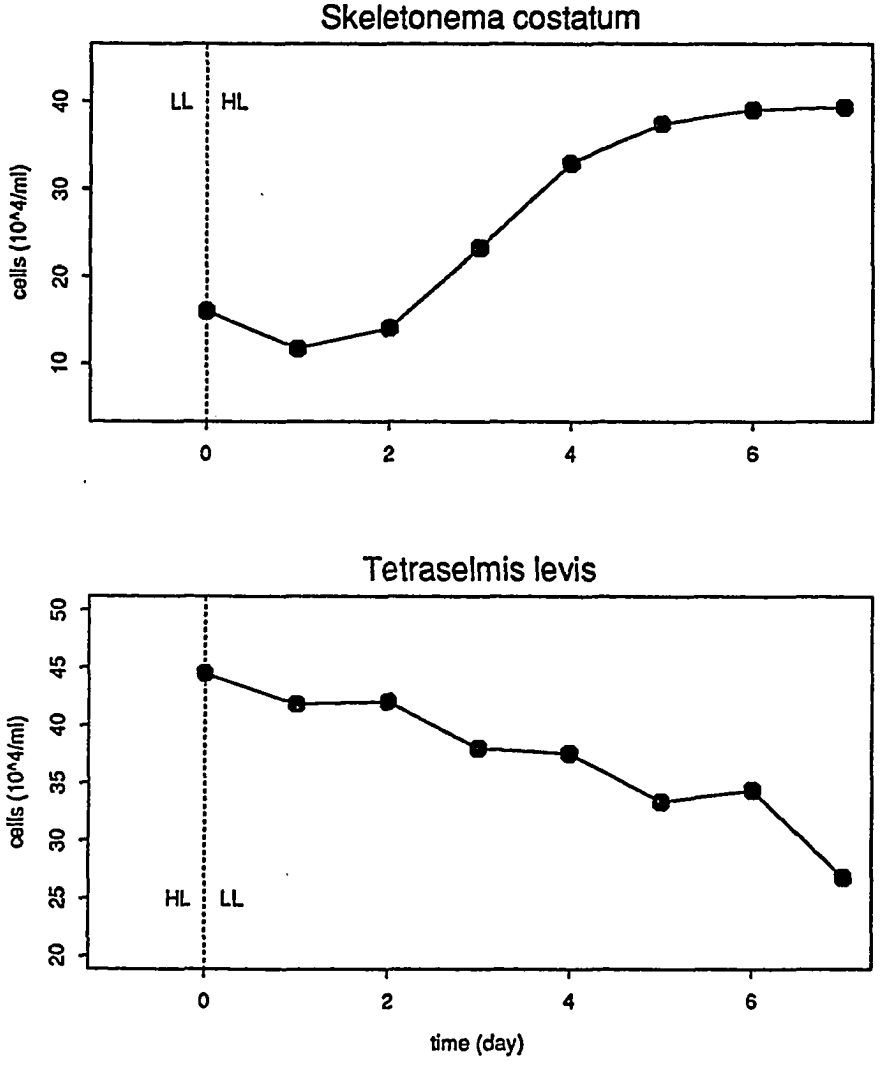
>HL shift than their decrease in the HL->LL shift. In any case, the decrease in a cellular property following a shift of growth irradiance is always faster than the increase in the same property in the reverse shift. The mechanisms regulating the response are clearly different for different directions of light transition. However, since this study is more concerned with the empirical behavior of the photoadaptive parameters rather than the physiological processes *per se,* the mechanisms leading to the differences in reciprocal light changes were left for future study.

Other Observations

It is not clear whether the irregularity in some of the time course data observed is related to the experimental conditions deviating from what were intended. Fig. 13 illustrates a typical problem encountered during the light transition experiment using continuous culture. It appeared that cell density did not remain relatively constant throughout the sampling period. This complicates our simulation of light regime changes as some degree of self-shading might have taken place. In particular, at high cell density, small changes in cell concentration due to perturbation of the system (sampling, light changes, etc.) could greatly affect the light attenuation, which tends to work against our hypothesis. For example, when light is switched from LL to HL, cell number increases immediately due to higher light availability, the increase in cell number in turn increases light extinction which could offset the light changes initially intended.

Under current continuous culture conditions, cell growth is controlled by the media dilution rate. At ca. 25% turnover rate, cells grow slowly, and thus may not be as responsive as they could be at higher growth rate. Further experiments were planned to allow higher growth rate so that comparison can be made.

Fig. 13. An illustration of changes in cell density following one–step changes in light intensity from low light to high light (LL–>HL) or vice versa (HL–>LL) in *Skeletonema costatum,* and *Tetraselmis levis* during the continuous culture experiments. Light transitions occurred at time zero. Cell density is in 10^4 cells ml^{-1}. X–axis is time (day) elapsed following changes in light intensity.



CHAPTER 4

RESULTS FROM SEMI-CONTINUOUS CULTURE STUDIES

4.1 Semi-Continuous Culture Experiments

Initial experiments indicated that it is difficult to achieve the ideal experimental conditions by using the chemostat continuous cultures. The continuous culture is best suited for studying physiological characteristics under the assumption of an environment in equilibrium. My primary interest is in examining the phytoplankton response to transitions in environmental conditions (in this case, light intensity). When a transition in light intensity is imposed, the culture equilibrium is disrupted and cell concentration will not remain constant. This effect is not desired because some degree of self-shading could have occurred. Further experiments were revised using periodic dilutions (semi-continuous culture). This will allow a better control of the constancy of cell density in the culture.

Basic sampling strategy is the same as in the initial experiments. In addition, these experiments will further address other fundamental questions: they are (1) short term changes, (2) diurnal variations, (3) influence of growth rate and nutrient limitation, and (4) species variability. Only the HL->LL transition will be examined in these experiments. To accomplish the above, diurnal sampling at 6-hour intervals will be carried out in the first day following light changes, to resolve shorter

term changes in pigment composition, and in the last day of sampling ("steady state") to find out if there are any systematic diurnal variations in the photoadaptive variables studied. To examine the influence of nutrient enrichment and growth rate on the time rate changes in photoadaptation, additional experiments with *Skeletonema costatum* were carried out using the semi-continuous culture with f/10 and f/60 media. The results can be compared to those obtained in the initial experiment for this species, which was grown under f/10 medium with a 25% turn over rate.

4.2 Cellular Response To Changes In Growth Irradiance

Pigment Composition

In the semi-continuous culture experiments, six species were studied. Results obtained with chl *b* containing (*Tetraselmis levis*) and fucoxanthin-containing (*Isochrysis galbana, Thalassiosira oceanica* and *Skeletonema costatum*) algae were consistent with previous results using continuous culture (see Appendix 1) In general, a light shift from HL to LL would lead to increases in the cellular pigment contents of fucoxanthin, chl *b* and chl *a* as well as in fucoxanthin : chl *a* and chl *b* : chl *a* ratios. There was one exception: when grown under f/60 medium *Skeletonema costatum* became visibly stressed during the light transition experiment. As a result, the time course data of pigment contents and ratio showed abrupt decreases after initial increases.

The other two species studied which represent two other distinct types of pigment composition were *Prorocentrum micans* and *Synechococcus sp.* (Fig. 14-15). *Prorocentrum micans* contains peridinin, a dinoflagellate marker, as major accessary pigment. *Synechococcus sp* contains zeaxanthin as its major carotenoid component.

In *P. micans,* (Fig. 14) after a transition of growth irradiance from HL to LL, cellular peridinin content more than tripled (from 0.6 pg cell^{-1} to 2.0 pg cell^{-1}) and chl *a* content about doubled (from 2.5 pg cell^{-1} to 5.5 pg cell^{-1}). The difference

resulted in an increase of peridinin : chl *a* ratio from 0.24 g g^{-1} to 0.37 g g^{-1}, a 54% rise.

In *Synechococcus sp,* (Fig. 15) following the light transition from from HL to LL, zeaxanthin content of the cell decreased slightly from 1.3 fg $cell^{-1}$ to 1.1 fg $cell^{-1}$, while chl *a* content doubled from 1.2 fg $cell^{-1}$ to 2.4 fg $cell^{-1}$. Zeaxanthin responds to light changes differently from chl *b,* fucoxanthin and peridinin, because its primary function is to protect thylakoid membrane from photooxidational damages rather than to harvest additional light for photosynthetic purpose. Although zeaxanthin $cell^{-1}$ did not change significantly following the light transition, a marked increase in chl *a* $cell^{-1}$ lead to a decrease in zeaxanthn : chl *a* ratio from 1:1 at high light to 0.4:1 (g g^{-1}) at low light.

Fig. 14. Time course changes in cellular pigment contents and pigment ratio in *Prorocentrum micans*, following one–step changes in light intensity from high light to low light (HL–>LL) during the semi–continuous culture experiments. Fig. 14A shows changes in peridinin and chl *a* contents (pg cell^{-1}); Fig. 14B shows changes in peridinin : chl *a* ratio (g g^{-1}). Light transitions occurred at time zero, which coincided with the beginning of the light period in a 12:12 L:D light regime. The interval of diurnal sampling during the first day (time 0~1) and last day (time 6~7) after light transition and one day before it (time –1~0), was 0.25 d (6 hr). All other sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

Fig. 14A

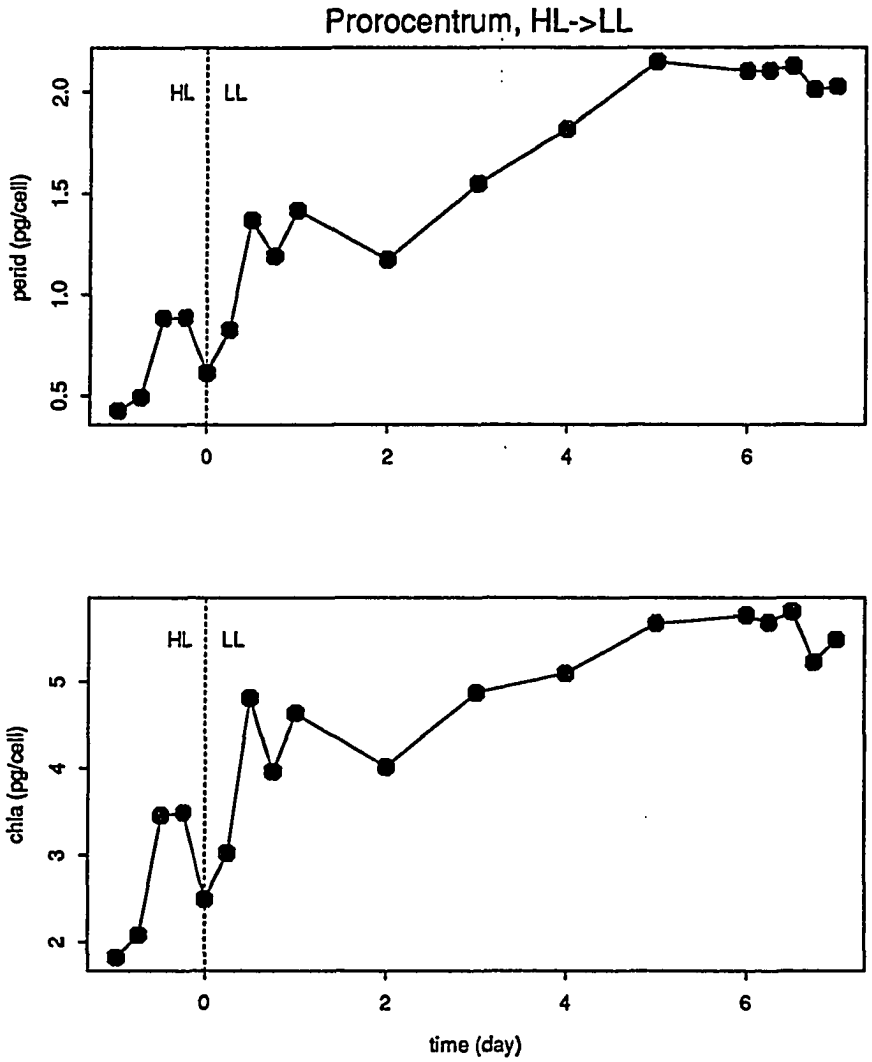


Fig. 14B

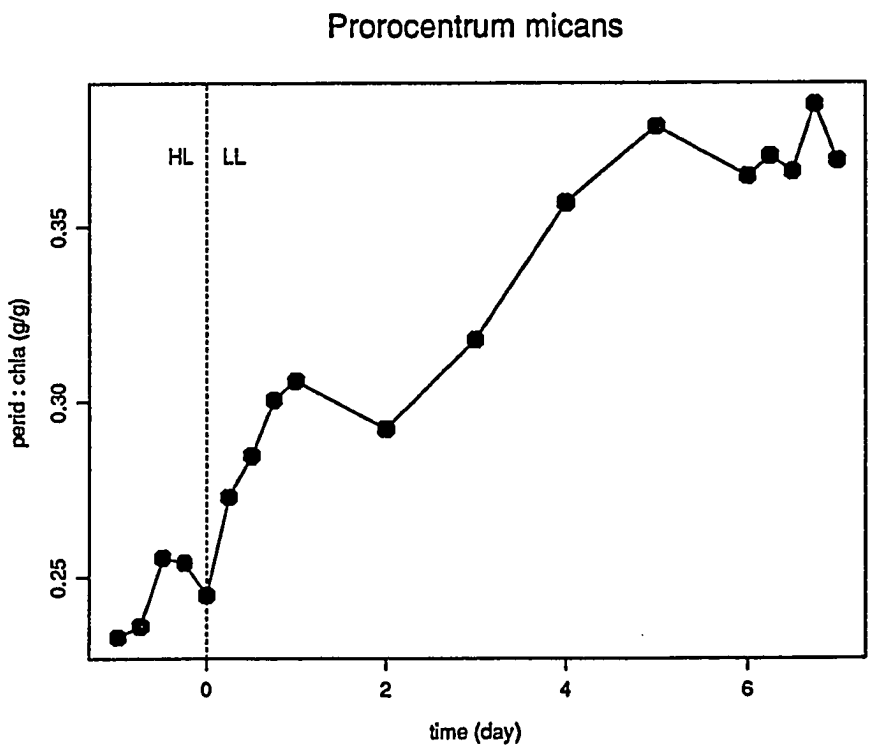


Fig. 15. Time course changes in cellular pigment contents and pigment ratio in *Synechococcus sp*, following one–step changes in light intensity from high light to low light (HL–>LL) during the semi–continuous culture experiments. Fig. 15A shows changes in zeaxanthin and chl *a* contents (fg $cell^{-1}$); Fig. 14B shows changes in zeaxanthin : chl *a* ratio (g g^{-1}). Light transitions occurred at time zero, which coincided with the beginning of the light period in a 12:12 L:D light regime. The interval of diurnal sampling during the first day (time 0~1) and last day (time 6~7) after light transition and one day before it (time –1~0), was 0.25 d (6 hr). All other sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

Fig. 15A

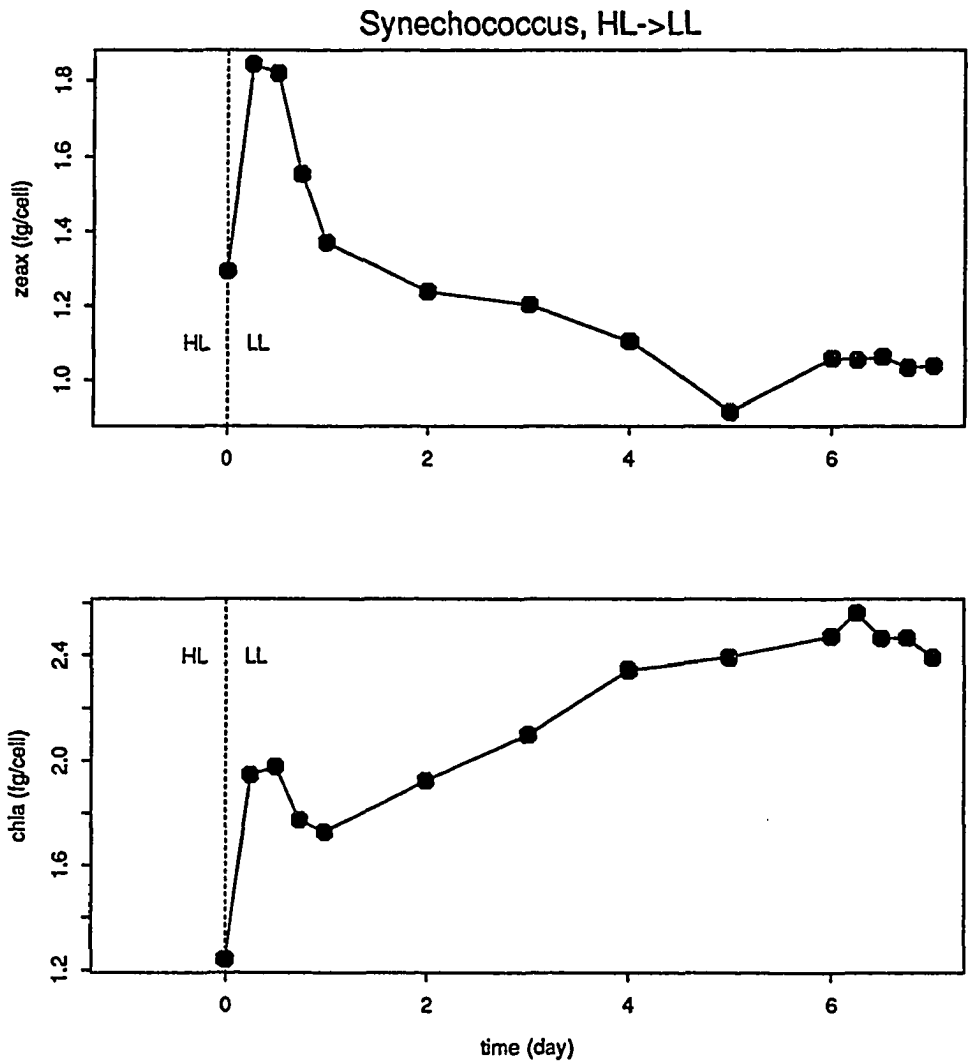
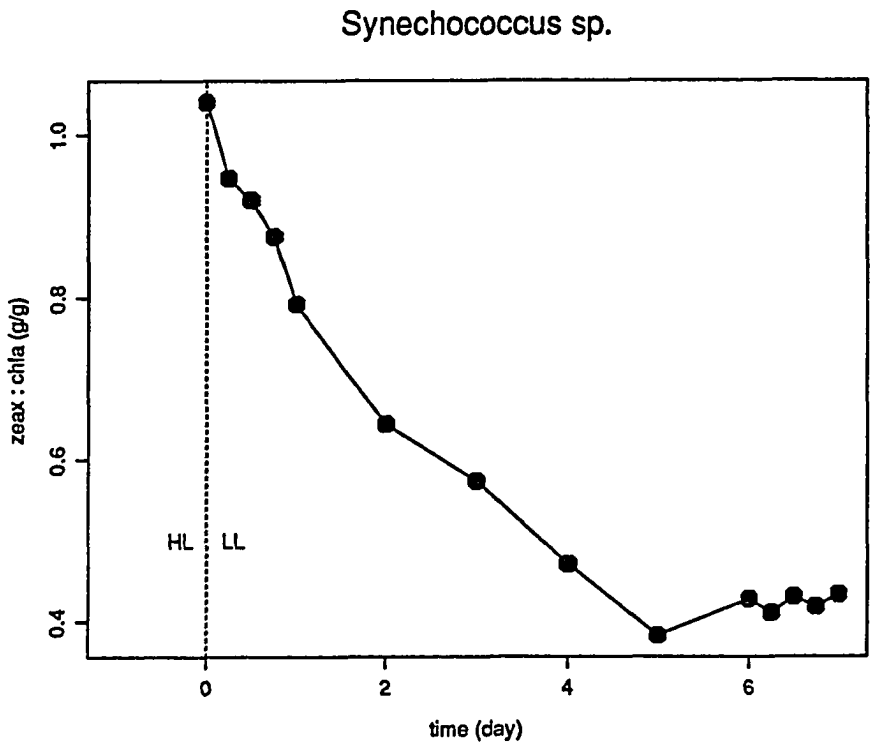


Fig. 15B



Carbon And Nitrogen Composition

Changes in carbon, nitrogen contents and C : N ratio following light transition from HL to LL in the semi-continuous culture studies are consistent from those obtained in the preliminary continuous culture studies in that no dramatic changes were observed (see Appendix 2). In *Tetraselmis levis,* carbon and nitrogen contents decreased from 35 and 6 pg $cell^{-1}$ at high light to 23 and 4.0 pg $cell^{-1}$ at low light, respectively; both declined about 34%, leading to a relatively unchanged C : N ratio. In *Synechococcus sp,* carbon and nitrogen contents both decreased about 15% from 334 and 56 fg $cell^{-1}$ to 283 and 48 fg $cell^{-1}$ respectively; little changes in C : N ratio were observed. In *Prorocentrum micans,* carbon and nitrogen contents decreased slightly during the first two days but increased gradually back to their original level; C : N ratio followed similar pattern. Except for the nutrient-stressed *Skeletonema costatum,* which showed substantial decreases in both carbon and nitrogen contents, no significant changes in carbon and nitrogen $cell^{-1}$ or C : N ratios were observed in all other fucoxanthin-containing species (Appendix 2.D-F).

C : Pigment Ratios

Changes in C : pigment ratios were very apparent in all species studied. Similar to the initial experiments for the chl *b*- and fucoxanthin-containing species, C : chl *a,* C : chl *b,* and C : fucoxanthin ratios all decreased rapidly following a

transition of growth irradiance from HL to LL (see Appendix 3). Results for *Prorocentrum micans* and *Synechococcus sp* are shown in Fig. 16-17. In *P. mican* (Fig. 16), C : peridinin ratio exhibited similar pattern, decreasing from 1500 at HL to 400 g g^{-1} at LL. In *Synechococcus sp* (Fig. 17), C : zeaxanthin ratio did not show much daily changes after the light shift because both carbon and zeaxanthin contents decreased slightly and in similar magnitude in response to the decrease in growth irradiance.

Fig. 16. Time course changes in C : pigment ratios in *Prorocentrum micans*, following one–step changes in light intensity from high light to low light (HL–>LL) during the semi–continuous culture experiments. Shown are changes in C : peridinin ratio (upper panel) and C : chl *a* ratio (lower panel). The ratios are in g g^{-1}. Light transitions occurred at time zero, which coincided with the beginning of the light period in a 12:12 L:D light regime. The interval of diurnal sampling during the first day (time 0~1) and last day (time 6~7) after light transition and one day before it (time –1~0), was 0.25 d (6 hr). All other sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.

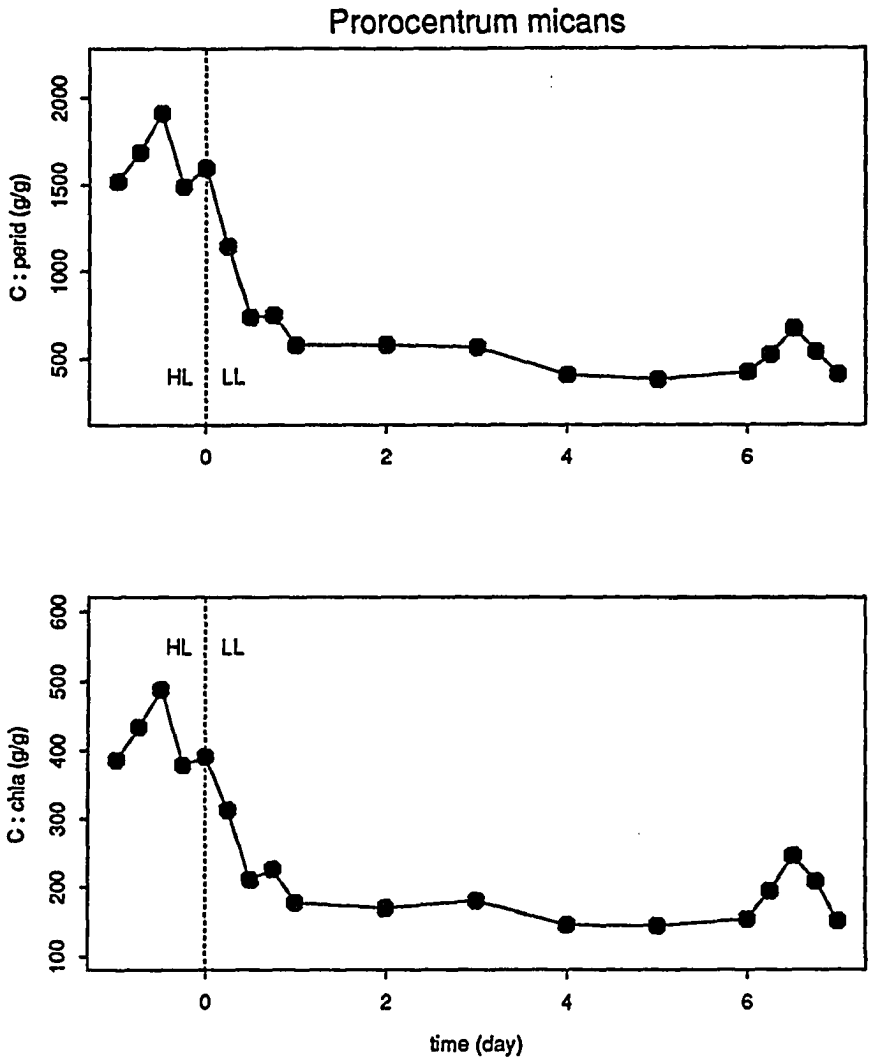
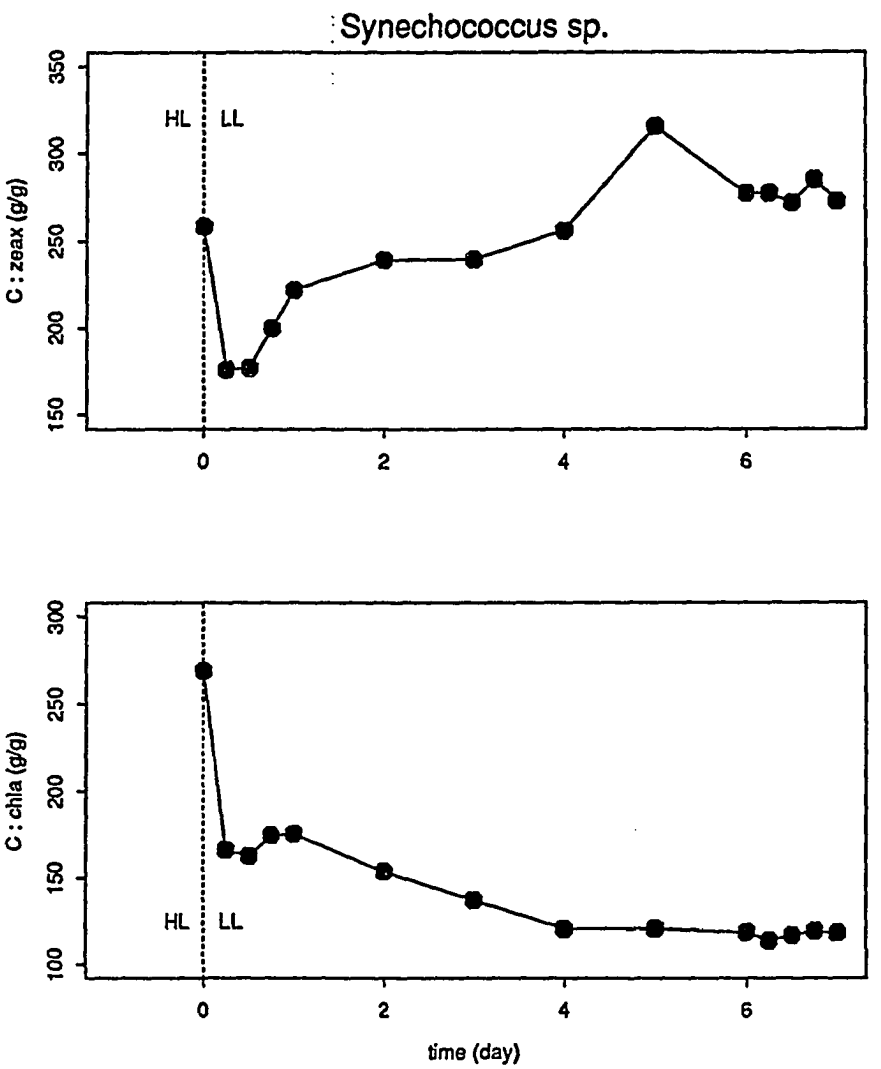


Fig. 17. Time course changes in C : pigment ratios in *Synechoccocus sp*, following one–step changes in light intensity from high light to low light (HL–>LL) during the semi–continuous culture experiments. Shown are changes in C : zeaxanthin ratio (upper panel) and C : chl *a* ratio (lower panel). The ratios are in g g^{-1}. Light transitions occurred at time zero, which coincided with the beginning of the light period in a 12:12 L:D light regime. The interval of diurnal sampling during the first day (time 0~1) and last day (time 6~7) after light transition and one day before it (time –1~0), was 0.25 d (6 hr). All other sampling took place at the beginning of each light period. X–axis is time (day) elapsed following changes in light intensity.



4.3 Rate Constants Of Photoadaptation

To be comparable to the rate constants obtained from the preliminary experiments, diurnal samples were not used for the calculation of first order rate constants. However, the effect of light/dark cycle on the photoadaptation kinetics will be discussed in a separate section. The rate constants of photoadaptation following HL->LL transition are listed in Table 2.

In *Tetraselmis levis,* regression of first order kinetics are significant for all photoadaptive variables examined except C : N ratio. In terms of cellular contents, chl *a* changed most quickly, with a first order rate constant of 1.09 d^{-1}. Chl *b*, carbon and nitrogen have slower rates of 0.68, 0.57 and 0.55 d^{-1} respectively. In terms of ratios, C : pigment ratios (C : chl *a*, C : chl *b*) changed most quickly with rates as high as 1.8-2.0 d^{-1}; pigment ratio (chl *b* : chl *a*) changed at a rate of 0.68 d^{-1}; and C : N ratio did not show significant changes.

In *Synechococcus sp,* the cellular content of zeaxanthin did not show much change, with a first order rate constant of 0.07 d^{-1}, which is not significantly different from zero. Chl *a* content, on the other hand, doubled with a first order rate constant of 0.41 d^{-1}. Both carbon and nitrogen contents decreased slightly, reaching a new equilibrium quickly with first order rate constants of 0.99 and 0.96 d^{-1} respectively. Zeaxanthin : chl *a* ratio increased at a rate (0.46 d^{-1}) similar to the decrease of chl *a* content (0.41 d^{-1}). C : N ratio did not show significant changes

Table 2 Regression analysis of first order kinetics of photoadaptation—results from non–linear curve fitting. R_o and R_∞ are initial and asymptotic values of appropriate photoadaptive variables; s.e. is standard error; cellular properties are in unit of pg $cell^{-1}$, except in *Synechococcus sp* where fg $cell^{-1}$ is used; ratios are in g g^{-1}; k is the first order rate constant (d^{-1}); S—first order rate constant significantly different from zero; NS—first order rate constant not significantly different from zero; NP—no pattern of first order kinetics, hence no meaningful figures (nmf) were obtained for the regression parameters.

Table 2.1 *Tetraselmis levis, Synechococcus sp.*

parameter	R_o	s.e.	R_{oo}	s.e.	k	s.e.	r^2	remarks
			Tetraselmis levis					
chl *b*	0.28	0.08	1.20	0.06	0.68	0.17	0.96	S
chl *a*	0.84	0.14	1.88	0.08	1.09	0.44	0.91	S
carbon	34.2	1.56	22.8	1.29	0.57	0.23	0.89	S
nitrogen	6.03	0.29	4.09	0.25	0.55	0.26	0.87	S
chl *b* : chl *a*	0.32	0.01	0.63	0.01	0.68	0.06	0.99	S
C : N	5.68	0.25	5.54	0.13	0.26	0.30	0.39	NS
C : chl *b*	131.8	4.47	22.1	2.27	2.03	0.37	0.99	S
C : chl *a*	41.9	2.14	13.3	1.04	1.82	0.51	0.97	S
			Synechococcus sp.					
zeax	1.26	0.58	0.85	1.02	0.07	0.26	0.46	NS
chl *a*	1.25	0.06	2.53	0.07	0.41	0.07	0.98	S
carbon	334.0	4.35	286.8	2.29	0.99	0.25	0.95	S
nitrogen	55.7	1.15	49.0	0.62	0.96	0.46	0.84	S
zeax : chl *a*	1.04	0.03	0.38	0.03	0.46	0.08	0.98	S
C : N	6.00	0.14	5.85	0.07	0.99	2.55	0.15	NS
C : zeax	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
C : chl *a*	266.9	5.62	118.9	3.27	0.83	0.09	0.99	S

Table 2.2 *Prorocentrum micans, Isochrysis galbana*

parameter	R_o	s.e.	R_{oo}	s.e.	k	s.e.	r^2	remarks
			Prorocentrum micans					
perid	0.62	0.13	2.48	0.40	0.25	0.11	0.95	S
chl *a*	2.73	0.45	5.72	0.46	0.48	0.24	0.86	S
carbon	975	80.3	807	33.2	3.40	15.5	0.43	NS
nitrogen	162	12.9	141	5.40	2.75	10.8	0.30	NS
perid : chl *a*	0.25	0.02	0.40	0.04	0.27	0.17	0.89	S
C : N	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
C : perid	1592	83.0	451	36.0	1.94	0.55	0.97	S
C : chla	390	14.0	156	5.93	2.30	0.65	0.98	S
			Isochrysis galbana					
fuco	0.033	0.005	0.128	0.003	0.61	0.09	0.98	S
chl *a*	0.10	0.006	0.20	0.003	0.97	0.18	0.97	S
carbon	11.7	0.44	10.5	0.20	1.50	1.77	0.45	NS
nitrogen	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
fuco : chl *a*	0.33	0.03	0.64	0.02	0.55	0.14	0.96	S
C : N	8.86	0.15	8.16	0.07	0.28	0.80	0.19	NS
C : fuco	348	5.10	86.4	2.34	1.45	0.09	0.99	S
C : chl *a*	114	3.10	53.2	1.42	1.45	0.23	0.98	S

Table 2..3 *Skeletonema costatum.*

parameter	R_o	s.e.	R_{oo}	s.e.	k	s.e.	r^2	remarks
				Skeletonema costatum				
fuco	0.07	0.03	0.32	0.01	2.29	1.43	0.91	S
chl *a*	0.21	0.03	0.66	0.01	0.87	0.14	0.98	S
carbon	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
nitrogen	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
fuco : chl *a*	0.34	0.01	0.52	0.01	0.85	0.16	0.97	S
C : N	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
C : fuco	292	6.50	65.4	2.75	2.37	0.33	0.99	S
C : chl *a*	98.3	2.31	33.0	0.98	2.23	0.36	0.99	S
				Skeletonema costatum, f/60				
fuco	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
chl *a*	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
carbon	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
nitrogen	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
fuco : chl *a*	0.21	0.01	0.30	0.01	0.74	0.31	0.88	S
C : N	10.36	0.57	9.41	0.47	1.37	2.51	0.31	NS
C : fuco	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
C : chl *a*	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP

Table 2.4 *Thalassiosira oceanica*

parameter	R_o	s.e.	R_{oo}	s.e.	k	s.e.	r^2	remarks
			Thalassiosira oceanica					
fuco	0.066	0.009	0.26	0.007	0.61	0.09	0.98	S
chl *a*	0.20	0.013	0.40	0.007	0.98	0.18	0.97	S
carbon	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
nitrogen	2.69	0.16	2.51	0.13	0.77	1.25	0.40	NS
fuco : chl *a*	0.33	0.03	0.64	0.02	0.55	0.14	0.95	S
C : N	nmf	nmf	nmf	nmf	nmf	nmf	nmf	NP
C : fuco	193	3.16	52.3	1.45	1.45	0.10	0.99	S
C : chl *a*	63.3	1.93	32.2	0.87	1.53	0.31	0.98	S

while changes in C : zeaxanthin ratio can not be fitted by the first order kinetics model. However, C : chl *a* ratio decreased significantly, with a first order rate constant of 0.83 d^{-1}.

In *Prorocentrum micans,* cellular peridinin and chl *a* contents as well as peridinin : chl *a* ratio showed significant increases over time; however, these photoadaptive variables approached new equilibrium slowly, with first order rate constants of 0.25, 0.48 and 0.27 d^{-1}, respectively. The slower increase in cellular pigmentation of *P. micans* may be a direct consequence of its much slower growth rate (0.14 d^{-1}) in comparison with other species. Carbon and nitrogen contents as well as C : N ratio either did not show significant changes or can not be fitted by first order kinetics model. However, C : peridinin and C : chl *a* ratios all exhibited large changes, with first order rate constants of 1.94 and 2.30 d^{-1} respectively.

In *Isochrysis galbana,* fucoxanthin, chl *a* contents and fucoxanthin : chl *a* ratio all increased significantly following transfer from high to low growth irradiance, with first order rate constants of 0.61, 0.98 and 0.55 d^{-1}, respectively. As in *Prorocentrum micans,* carbon, nitrogen contents and C : N ratio in *Isochrysis galbana* either showed insignificant changes or irregular patterns of change which can not be fitted by the first order kinetics model. However, both C : fucoxanthin and C : chl *a* ratios decreased dramatically, with a identical rate constant of 1.45 d^{-1}.

First order rate constants of photoadaptation in *Thalassiosira oceanica* are very close to those observed in *Isochrysis galbana.* Fucoxanthin, chl *a* contents

increased at rates of 0.61 and 0.98 d^{-1}. Fucoxanthin : chl *a* ratio increased somewhat more slowly, with a rate of 0.55 d^{-1}. As in some previous species, carbon, nitrogen contents and C : N ratio either exhibited patterns that can not be described by the first order kinetics model or showed only minimal changes over time. However, there was a large decrease in C : fucoxanthin and C : chl *a* ratios following the HL->LL transition, with first order rate constants reaching 1.45 and 1.53 d^{-1}, respectively.

Photoadaptation kinetics of *Skeletonema costatum* examined under different nutrient enrichment (f/10, f/60) conditions during HL->LL transition showed different responses (see Table 2.3). Under f/10 enrichment, *S. costatum* showed definite patterns of first order kinetics of photoadaptation in its cellular fucoxanthin, chl *a* contents, fucoxanthin : chl *a* and C : chl *a* ratios (see also Appendix 1D, 3D). These variables all changes significantly with rate constants ranging from 0.85 to 2.37 d^{-1}. First order kinetic response pattern was not discernable for cellular carbon and nitrogen contents as well as C : N ratio (see also Appendix 2F)

Under f/60 enrichment, severe nutrient stress became apparent during the time course study of photoadaptation. Results showed that changes in fucoxanthin, chl *a* contents and C : chl *a* ratios did not exhibit monotonic response. Initially, fucoxanthin, chl *a* contents increased in response to a decrease in light intensity, but as nutrient stress became severe, they all decreased rapidly (Appendix 1.E1). C : fucoxanthin and C : chl *a* ratios showed the opposite response (Appendix 3.E).

Cellular carbon and nitrogen decreased steadily and did not stablized (Appendix 2.G1). Consequently, regression of their time course data using first order kinetics model failed to converge. The change in C : N ratio (Appendix 2.G2) is statistically insignificant. The only variable that showed significant monotonic response which can be described by first order kinetics model was fucoxanthin : chl *a* ratio (Appendix 1.E2) having a rate constant of 0.74 d^{-1}. However, the continued (monotonic) increase in this ratio after day four was a manifestation of chl *a* degrading faster than fucoxanthin, not a result of photoadaptation in which an increase in fucoxanthin : chl *a* ratio results from a faster increase in fucoxanthin relative to chl *a*.

4.4 Effect Of Growth Rate On Photoadaptation Rate

To examine the effect of growth rate on photoadaptation rate, *Skeletonema costatum* and *Tetraselmis levis* were each subject to the same light transitions but under different growth rates as controlled by nutrient dilution. For each species, the first order rate constants of photoadaptation at two different growth rates during the HL->LL transition are shown in Table 3.

In *S. costatum,* at a specific growth rate of 0.26 d^{-1}, photoadaptation rate constants for the variables examined range from 0.37 to 1.67 d^{-1}. In contrast, at a specific growth rate of 0.42 d^{-1}, photoadaptation rates range from 0.85 to 2.37 d^{-1}. Similarly, there is a general trend of increase in the photoadaptation rates of *T. levis* as growth rate increases. Specifically, at a growth rate of 0.22 d^{-1}, first order rate constants of photoadaptation range from 0.16 to 0.59 d^{-1}; however, when growth rate increased to 0.43 d^{-1}, photoadaptation rates increased to a range from 0.26 to 2.03 d^{-1}.

For those variables that can be directly compared, photoadaptation rates appear to be higher at higher specific growth rate for all variables (with the exception of C : N ratio in *T. levis* which did not exhibit rates statistically different from zero under both growth conditions). In both species, the increase in photoadaptation rate is not proportional to the increase in growth rate. In addition, the magnitude of increase is different for different variables within the same species, as well as for

the same variables between the two species. These results suggest that growth is unbalanced during photoadaptation and that there exist differences between species in their sensitivity to light intensity changes.

Table 3. Comparison of first order rate constants (K) under different growth rates for *Skeletonema costatum* and *Tetraselmis levis*. Growth rates (μ, d^{-1}) controlled by nutrtient dilution rate. S.E.= standard error. Data shown are from HL–>LL transitions under f/10 media enrichment.

Table 3.1 *Skeletonema costatum*

variables	fuco	chl *a*	carbon	nitrogen	fuco : chl*a*	C : N	C : fuco	C : chl*a*
$K_{(\mu=0.26)}$	0.37	0.44	1.26	1.08	0.42	1.67	1.06	1.02
S. E.	0.08	0.08	0.35	0.53	0.12	0.71	0.05	0.07
$K_{(\mu=0.42)}$	2.29	0.87	nmf	nmf	0.85	nmf	2.37	2.23
S.E.	1.43	0.14	nmf	nmf	0.16	nmf	0.33	0.36

Table 3.2 *Tetraselmis levis*

variables	chl *b*	chl *a*	carbon	nitrogen	chl*b* : chl*a*	C : N	C : chl*b*	C : chl*a*
$K_{(\mu=0.22)}$	0.21	0.16	nmf	nmf	0.39	0.14	0.40	0.59
S.E.	0.08	0.12	nmf	nmf	0.19	0.16	0.16	0.18
$K_{(\mu=0.43)}$	0.68	1.09	0.57	0.55	0.68	0.26	2.03	1.82
S.E.	0.07	0.44	0.23	0.26	0.06	0.30	0.37	0.51

4.5 Diurnal Variations

During the semi-continuous culture experiments, diurnal sampling were carried out at 0.25 d intervals during the first day and last day of sampling following light transition (i.e., time 0-1 day and 6-7 day in the Appendices). For some species, diurnal sampling include the day just before light changes occur (i.e., time -1-0 day in the Appendices).

As can be seen, large diurnal variations are common in the cellular contents of all pigments examined. In general, pigment synthesis exceeds degradation during the light period, resulting in a net accumulation. Because none of the cultures were synchronized, the decrease in pigment contents during the dark period must be due to degradation rate exceeding the rate of synthesis, rather than cell division. The diurnal variations are especially pronouced at high light and during the first day immediately following the transfer from high light to low light, but become somewhat damped at the end of the sampling period as the algae became adapted to low light conditions. As a result of diurnal variations, the response curves of cellular pigment contents following the HL->LL transition become non-monotonic. It is interesting to note that diurnal variations in accessary pigments : chl *a* ratios are not as apparent as in individual pigment contents, because the diurnal patterns are similar for accessary pigments and chl *a*.

Carbon and nitrogen content underwent diurnal rhythms similar to pigment content. Both carbon and nitrogen $cell^{-1}$ increased during the light period but

decreased during the dark period. Beause the rate of change is always greater for cell carbon than nitrogen, there is a concomitant daytime increase and night time decrease in C : N ratio. Although the diurnal variation in C : N ratio is most pronouced at high light, it is also very apparent at low light. In most cases, the magnitude of diurnal changes in carbon and nitrogen contents as well as C : N ratio greatly exceeds changes due to transitions of light intensity.

C : pigment ratios have large diurnal variations at high light, but variations at low light are not severe. Immediately following the HL->LL transition, no apparent diurnal changes were found in the direction of response in C : pigment ratios, resulting in relatively smooth, monotonic curves. The dynamic range of the ratios in response to light changes is comparable to the range of diurnal variations at high light, and larger than that at low light.

4.6 Species Differences

To examine differences of photoadaptation rates between species of different taxonomic groups, results, obtained under same experimental conditions of light transition and nutrient enrichment, from *Tetraselmis levis, Synechococcus sp, Prorocentrum micans* and *Isochrysis galbana* were compared. These species are representatives of Chlorophyta, Cyanophyta, Pyrrophyta and Chrysophyta. The variables studied that are common to all species include cellular chl *a,* carbon, nitrogen contents, C : N and C : chl *a* ratios. For carbon, nitrogen contents and C : N ratio, it is clear that there are species differences in the first order rate constants--For some species, the rates were significantly different from zero, for others they were not (see Table 2). For chl *a* content and C : chl *a* ratio, the photoadaptation rates were significantly different from zero for all species. However, statistical analysis (simultaneous multiple comparison) showed that at least one of the species had a different rate of photoadaptation in either variables from other species (Table 4)

Similarly, to examine the species difference within the same taxonomic group, results, obtained under identical experimental light transitions and nutrient enrichment, from *Isochrysis galbana, Thalassiosira oceanica* and *Skeletonema costatum* were compared. These species all belong within the Chrysophyta. As can be seen from Table 2, results of carbon, nitrogen contents and C : N ratios were not comparable because meaningful numbers on their rate constants often could not be

obtained (i.e., the nonlinear regression procedure failed to converge). For those variables that can be compared, the results of multiple comparison are shown in Table 5. At the 5% level of significance, it appears that there is at least one species that has a different rate of photoadaptation in one of the variables (except chl *a* $cell^{-1}$) from other species.

Table 4. Statistical comparison of first order rate constants of photoadaptation in cellular chl *a* content and C : chl *a* ratios among species representing different taxonomic groups (see text). SSW is the sum of squares within, SSB is the sum of squares between; df is degree of freedom; S means that according to the F–statistic there is at least one species that has significantly different rate constant from others; the level of significance is chosen at 5% (α=0.05).

variables	SSW	SSB	df	F	remarks
chla	0.082	0.938	3, 28	11.44	S
C : chla	0.213	3.077	3, 28	14.45	S

Table 5. Statistical comparison of first order rate constants of photoadaptation in cellular fucoxanthin, chl *a* contents as well as fucoxanthon : chl *a*, C : fucoxanthin and C : chl *a* ratios among species within the same taxon of Chrysophyta (see text). SSW is the sum of squares within, SSB is the sum of squares between; df is degree of freedom; S means that according to the F–statistic there is at least one species that has significantly different rate constant from others; the level of significance is chosen at 5% (α=0.05). NS means that there is no significant species difference.

variables	SSW	SSB	df	F	remarks
fuco	0.685	7.50	2, 21	10.95	S
chla	0.028	0.027	2, 21	0.96	NS
fuco : chla	0.022	0.240	2, 21	11.11	S
C : fuco	0.042	2.257	2, 21	53.74	S
C : chla	0.093	1.473	2, 21	15.84	S

CHAPTER 5

DISCUSSION

5.1 Dynamics Of Photoadaptation

The results of these experiments show that although photoadaptive response of phytoplankton is generally predictable, the rate, pattern and magnitude of response are highly variable depending on a number of environmental, phylogenetic and physiological factors. The first order kinetics model is not adequate in describing all types of response of photoadaptive parameters. In particular, cell carbon and nitrogen often do not respond to light changes according to first order kinetics; C : N ratio changes are often statistically insignificant. Although pigment contents, pigment ratios and C : pigment ratios exhibited behavior that can be approximated by first order kinetics, there is considerable difference between the shift-up and shift-down processes.

When algae are brought from photosyntheticallly-saturating levels of irradiance (HL) to subsaturating levels of irradiance (LL), the cells undergo an energy crisis. They need to harvest more light in order to maintain optimal growth rate under the low growth irradiance condition. As a result, additional light-harvesting pigment-protein complexes are synthesized, leading to the increase in cellular contents of chl *a* and other accessary pigments such as chl *b*, fucoxanthin and

peridinin. Data on pigment ratio changes are consistent with the prediction based on structural information on the photosynthetic apparatus, i.e., a decrease in growth irradiance leads to an increase in accessary pigment : chl *a* ratios due to disproportionate increase in specific light-harvesting components which contain higher accessary pigment : chl *a* ratios relative to the whole plant (e.g., Prezelin 1971, 1976, Larkum and Barret 1983, Anderson 1986, Owens and Wold 1986).

The above changes during shift-down can be reversed during the shift-up process. However, the rates of change are different between the two reciprocal light shifts. For the photosynthetic pigments, the rates of increase during HL->LL shift are always lower than the rates of decrease during LL->HL. For cellular carbon and nitrogen, the direction of response to light changes is just the opposite, but the rates of increase during LL->HL are also always lower than the rates of decrease during HL->LL. The effects of hysteresis in the cellular components has potential consequences for the application of photoadaptation kinetics in estimating vertical mixing rates. For pigment ratios, however, no significant differences in the rate constants of photoadaptation were found between the reciprocal light shifts, suggesting that the hysteresis effect for photosynthetic pigments has been canceled out when expressed as ratios. In this regard, pigment ratios may be considered more favorable as photoadaptive variables in studying vertical mixing processes of similar time scales.

Falkowski (1984) studied the photoadapation kinetics of *Dunaliella tertiolecta* and suggested that during HL->LL, 50% of chl *a* was synthesized to support cell

growth while 50% could be considered shade-adaptation. He further suggested that during LL->HL transition, the decrease of chl *a* is the consequence of dilution due to growth. That is , as cell number increases, there is no net increase in chlorophyll hence for individual cell in the culture the chl *a* concentration is "diluted". His kinetic analysis was based on the assumption that under steady-state, the rate of change in cellular pigment content equals the rate of change in growth. This assumption may not be valid, because the rate of change in a certain cellular property measures "flux", whereas the rate of change in growth measures "acceleration". Therefore, the results from the kinetic analysis based on the above assumption may need re-interpretation. If the decrease in cellular chl *a* content during LL->HL is a consequence of dilution due to growth, one might expect to see the same rate of decrease for other photosynthetic pigments as for chl *a*. Our data showed that accessary pigments typically decrease more rapidly than chl *a* during LL->HL, suggesting that it is not a simple mechanical dilution. In addition, the first order rate constants of photoadaptation were twice as high as the rates of cell growth, indicating that only 50% of the decrease may be attributed to dilution due to growth, with other degradation processes accounting for the rest of the decline. We propose photodegradation as a potential mechanism responsible for some of the decline in cellular photosynthetic pigments during LL->HL transitions. On the other hand, the present study does show that there is an intrinsic difference between the reverse light shifts, hence supporting Falkowski's assertion that changes in the cellular property of algae during HL->LL and LL->HL transitions

are not simply mechanistically reverse processes.

Post et al (1984) pointed out that the first order kinetics model may not have any inherent physiological meaning, yet several studies indicated that the model predicted quite well the photoadaptation processes of phytoplankton under continuous illumination (Falkowski 1984, Hoffmann and Senger 1988, Sukenik et al 1990). On the other hand, Cullen and Lewis (1988) found that large differences in the time scales for adaptation from low to high light as compared to high to low light and proposed an alternative model to describe the photoadaptive response, i.e., the logistic model. Our observation under the 12:12 hour of light:dark cycle suggests that the difference lies in the direction of change of the cellular component in question. While decreases in photoadaptive cellular properties following a light transition may be well approximated by the first order kinetics, increases in the same cellular properties following a reverse light transition are better approximated by the logistic model. In other words, the exponential decay model (first order) more satisfactorily describes the decrease, while allometric growth model (logistic) more satisfactorily describes the increase in cellular properties in response to changes in light intensity.

5.2 Strategies Of Photoadaptation

As discussed above, phytoplankton respond to decreased light intensity by increasing pigment concentration. This can be achieved either by synthesizing addi-

tional pigment molecules within the existing photosynthetic units (PSUs) so as to increase the size of each unit without changing the number of reaction centers, or by building up completely new PSUs with all the reaction center components so as to increase the number of PSUs without changing the size of each photosynthetic unit. These responses are considered to represent two basic strategies of light intensity adaptation in marine phytoplankton (e.g., Prezelin and Sweeney 1979, Falkowski and Owens 1980, Prezelin 1981).

The two strategies of alternations in photosynthetic units are reflected in the photosynthetic pigment composition of phytoplankton. Since the pigment composition of each PSU is the same, a change in PSU number will not change the photosynthetic pigment composition; although the concentrations of individual pigments do change, they change in direct proportion to one another, thus the molar ratios remain constant. Changes in PSU size, on the other hand, result in disproportionate changes in cellular photosynthetic pigment contents. This is because changes in PSU size are achieved by changing specific components of the light harvesting part of the PSU which has a characteristically different pigment composition. Thus, with other components of the PSU remaining fairly constant (e.g., Thornber et al 1977), changing the light harvesting component leads to a change in whole cell photosynthetic pigment ratios.

Falkowski and Owens (1980) showed that the chl *a* : P700 ratio changed in *Skeletonema costatum* following changes in growth irradiance but the ratio remained relatively constant for *Dunaliella tertiolecta.* They thus proposed that

diatoms photoadapt by altering the size of the PSUs, whereas chlorophytes alter the number of PSUs. Subsequent studies have shown that both strategies can be found within the same algal group (Perry et al 1981, Falkowski etal 1985, Fisher et al 1989), even within clones of the same species (Gallagher et al 1984). From the standpoint of pigment composition, the present results support the conclusion that *Skeletonema costatum* photoadapts by changing the size of the PSUs. On the other hand, pigment ratio changes in *Dunaliella tertiolecta* suggest that this species also changes the size of the PSUs during photoadaptation. This is in conflict with Falkowski and Owen's (1980) conclusion. The difference may be in part due to the fact that changes in pigment composition primarily measure the photoadaptation occurring within the Photosystem II (PSII), while the chl *a* : P700 ratio measures only the size of PSU in Photosystem I (PSI). As Falkowski and LaRoche (1991) pointed out, if the ratio of PSI reaction centers (P700) and PSII reaction centers (P680) changes during photoadaption, an alga which appears to change the number of PSUs as determined by the chl *a* : P700 ratio, may also appear to change the size of existing numbers of PSUs as determined by the chl *a* : P680 ratio.

5.3 Diurnal Variations And Species Difference

Diurnal variations in photoadaptive parameters are not a consequence of physiological acclimation to a light:dark cycle (Post et al 1984). However, for processes with time scales on the order of days, diurnal periodicity can have a sub-

stantial effect on the kinetics of photoadaptation (Harding et al 1987). Our observations showed that photosynthetic pigments (chl *a*, as well as other accessary light harvesting pigments) increased during the light period and decreased during the dark period (e.g., Appendix 1). This cycle is not related to synchronized cell division and is the opposite of photoadaptation, hence supporting the contention of Post et al (1984) that phytoplankton do not shade-adapt at night; rather, they adapt to the average growth irradiance experienced during the photoperiod.

Except for pigment ratios, all other photoadaptive parameters examined in this study showed substantial diurnal variations. Such variations violate the requirement that in order for a photoadaptive parameter to be useful in a model of photoadaptation and vertical mixing, the response of the parameter to changes in growth irradiance must be monotonic. As a result, these parameters will be of limited use quantitatively in studies of vertical mixing because of the non-monotonic nature of change caused by diurnal cell cycles. This is especially true for cellular carbon, nitrogen or C : N ratio, whose magnitude of diurnal changes usually greatly exceed the changes due to the transition of light intensity. An exception to this is the accessary pigment : chl *a* ratios. No apparent diurnal variations were observed in the pigment ratios following transitions in growth irradiance. Accessary pigments and chl *a* have similar patterns of change during the light:dark cycle; when expressed as a ratio, changes due to the cycle become insignificant. In this sense, the pigment ratio may again be considered a better indicator to study the relationship between photoadaptation and vertical mixing.

However, it should be noted that there is substantial variability in the reponsiveness of different algal species to changes in growth irradiance. For example, Sakshaug et al (1987) found that *Thalassiosira pseudonana* was much more responsive to bright light than the oceanic clone *T. oceanica.* Our study also showed that there are significant differences in the photoadaptation rate constants between and within taxonomic groups of phytoplankton. Consequently, to use vertical distribution of photoadaptive parameters to infer vertical mixing, one must examine potential difference in the vertical distribution of phytoplankton assemblages and interpret the results accordingly. Since in natural systems phytoplankton often occur in mixed assemblages of diverse species groups, and that there is reason to expect more variations in nature than observed in the lab, it is advisable that rate constants of photoadaptation be derived from field incubations of the study region, which should provide more reliable estimate than would be generalized from culture studies.

5.4 Effect Of Growth Rate And Nutrient Limitation

Growth rate of phytoplankton has previously been implicated as one of the determinants of the time scales of photoadaptation in the contents of certain celllular components (Falkowski 1984, Post et al 1985) as well as in the quantum yield response (Morel et al 1987). Although implicit in the photoadaptation kinetic models (Falkowski 1983, Cullen and Lewis 1988), it is generally believed that,

other things being equal, slowly growing algae will take longer to acclimate to new light conditions than more rapidly growing algae. For example, if dilution due to cell growth is one of the factors causing the decline in cellular chl *a* content during LL->HL transitions (Falkowski 1984, Post et al 1985), then one might expect that other things being equal, with higher growth rate the cellular chl *a* content will decrease more rapidly and reach a new steady state in a shorter period of time. Thus far, there are few experiments that would allow direct comparison of the effect of growth rate on the rate of photoadaptation. Present experiments with *Skeletonema costatum* and *Tetraselmis levis,* each studied under different growth rates (as controlled by nutrient dilution) but otherwise same conditions during HL->LL transitions, support the hypothesis that for each individual species, as growth rate increases, photoadaptation rate increases. However, the result showed that the increase in photoadaptation rate is not proportional to the increase in growth rate; it further showed that the magnitude of relative rate increase is not homogeneous among different photoadaptive variables. These results suggest that growth is unbalanced and the synthesis of cellular materials is uncoupled with cell division during photoadaptation. Therefore, the extent to which the rate of photoadaptation depends on the rate of growth varies with different photoadaptive parameters. This concept is important and must be remembered when attempting to apply "correction factors" on photoadaptaion rate to account for differences in growth conditions (e.g., growth temperature). Finally, when growth is retarded due to severe nutrient stress, alternations in cellular pigment and chemical composition following

changes in light intensity may not result from photoadaptation, but from differential rates of degradation among individual cellular components.

The effects of growth rate and nutrient limitation, along with the hysteresis effect and diurnal variations as well as species difference, indicate that the kinetics of light intensity adaptation in marine phytoplankton is highly variable. The rate and pattern of algal adaptation to light regime changes are continuously being modified by changes in physiological and environmental factors other than light. Therefore, to use vertical distribution of photoadaptive variables to estimate the vertical displacement of phytoplankton in nature, the potential impacts of the above factors must be carefully examined.

5.5 Pigments As Biomarkers

The recent availability of HPLC method for separating and quantifying algal pigments (e.g., Montoura and Llewellyn 1983) has stimulated great interest in using taxon-specific accessary pigments as chemosystematic markers of natural phytoplankton populations (e.g., Guillard et al 1985, Gieskes and Kraay 1986, Hooks et al 1988). However, to use pigments as quantitative indicators of biomass, we must first understand the physiological plasticities associated with them, i.e., how environmental factors affect algal pigmentation. The present study demonstrated that fucoxanthin in diatoms and chrysophytes, chl *b* in green algae and peridinin in dinoflagellates, all changed remarkably in response to changes in light

intensity. The difference in cellular pigment contents between low-light and high-light acclimated cells may reach as high as three to five fold (e.g., Fig. 4A, 4B, 14A). These pigments are sensitive to light regime changes because they serve accessary light harvesting functions. When light availability decreases, the contents of these accessary pigments will increase just as chl *a* (though with different rate and magnitude). Consequently, to use these biomarkers as quantitative indicators of the standing stocks of specific taxa of phytoplankton, the light climate to which the phytoplankton population has been adapted must be taken into consideration when attempting to compare the spatial and temporal variations of phytoplankton distribution. An exception to this is the carotenoid zeaxanthin in the cyanobacteria *Synechoccucus sp.* Zeaxanthin content in *Synechoccocus sp* remained relatively constant after being subjected to transitions in growth irradiance. Similar results were obtained by Kana et al (1988). In this respect, zeaxanthin may be viewed as a better biomass indicator of field populations of *Synechoccocus sp.* However, the extent to which this indicator, or any other pigment markers, is reliable will also depend on the effect of other environmental factors such as nitrogen availability, which needs to be examined more thoroughly in the future.

In recent years, there has been increased use of Redalje and Law's (1981) method of incorporating ^{14}C into chl *a* to assess ocean primary productivity (Redalje 1983, Laws 1984, Welschmeyer and Lorenzen 1984). The method has been extended to estimate the contribution of different species groups to total photosynthesis through incorporation of ^{14}C into taxon-specific carotenoids (Gieskes

and Kraay 1989). This novel technique is a powerful tool in that it allows more precise determination of algal carbon biomass and carbon specific growth rate. However, the validity of this approach relies on the assumption that during incubation with ^{14}C, the specific activity of carbon in chl *a* or carotenoids is the same as the specific activity of carbon in the total cellular carbon pool. This assumption may be robust for chl *a* labelling under balanced algal growth conditions (Redalje 1983, Laws 1984). Gieskes and Kraay (1989) argued that according to Cook (1966) and Jorgensen (1966), synthesis of chlorophylls and carotenoids takes place simultaneously in the light period, hence it can be assumed that in each species group the specific activity in its characteristic carotenoid is similar to that in chl *a*, which in turn is similar to that of total cellular carbon (Redalje 1983, Laws 1984). However, the present study showed that during photoadaptation growth is unbalanced. As a result, the rate of pigment synthesis differs from the rate of carbon fixation. This should be an important consideration in interpreting pigment-labelling data from field incubations, since in nature phytoplankton are frequently subjected to changes in light regime due to turbulence and other vertical mixing processes. Even under constant light conditions, C : pigment ratios undergo diurnal cycles, as was also demonstrated by the present study. Therefore, short term incubations of a few hours may not be adequate to ensure the equality of carbon specific activity in the pigment carbon pool and that in the total cellular carbon pool. Pigment synthesis usually lags carbon fixation during the light period, possibly because of the extra metabolic steps required in forming the pigment-precursor pools. Considering

that pigment synthesis and carbon fixation are uncoupled over the diurnal cycle, and that unbalanced growth is characterisic of phytoplankton during photoadaptation, we support Goericke and Welschmeyer's (1992) recommendation that incubation of 24 hours be used for pigment labelling experiments and that every effort be made to ensure minimal perturbation of light regime during such experiments.

CHAPTER 6

GENERAL SUMMARY

This study investigated the changes in some photoadaptive variables of marine phytoplankton in response to changes in growth irradiance. The variables examined include chl *a*, the major accessary pigments, carbon and nitrogen, expressed either as individual cellular contents or as relative composition (ratios). Important conclusions from the study are summarized as follows.

Pigment contents, pigment ratios, as well as C : pigment ratios change significantly following changes in growth irradiance. Therefore, these parameters are useful as indicators of phytoplankton photoadaptation status. In general, as light intensity increases, photosynthetic pigment contents as well as the accessary pigment to chl *a* ratio will decrease; but the C : pigment ratio will increase. If light intensity decreases, the opposite will occur. In contrast, C : N ratios do not exhibit significant changes in response to light changes.

Some of the time course data of photoadaptive variables can be modelled according to first order kinetics. However, the first order kinetic model is inadequate in describing all types of photoadaptive response. It appeared that the decrease in the contents of cellular properties following a light transition is more rapid than the increase following the reverse light transition. As a result, the

decrease is better fitted by a first order kinetic model, while the increase is better fitted by a logistic model. The result suggests that changes in cellular properties of algae during low to high and high to low light shifts are not simply mechanically reverse processes.

Photosynthetic pigments increase during the light period and decrease during the dark period. This cycle is not related to synchronized cell division, and is the opposite of photoadaptation. The result suggests that phytoplankton do not shade-adapt at night, but adapt to average growth irradiance during the light period. Diurnal variations are significant in both accessary pigment and chl *a* contents, but are not apparent when expressed as accessary pigment : chl *a* ratios. On the other hand, carbon, nitrogen contents, as well as C : N ratios undergo strong diurnal variations.

Substantial variabilities exist in terms of the responsiveness of different algal species to changes in growth irradiance. For example, the dinoflagellate *Prorocentrum micans* typically photoadapts more slowly than species from other taxa (perhaps somewhat related to its slow-growing characteristc, see below). Within diatoms, coastal species (such as *Skeletonema costatum*) are more responsive to light changes than the oceanic species (such as *Thalassiosira oceanica*).

Phytoplankton growth rate, as controlled by nutrient limitation, has positive effects on photoadaptation. An increase in the rate of growth results in an increase in the rate of photoadaptation. However, the increase in photoadaptation rate is not proportional to the increase in growth rate. Moreover, the magnitude of increase in

photoadaptation rates resulting from growth rate increases is different for different photoadaptive variables. The results indicate that growth is unbalanced during photoadaptation, and that biosynthesis of cellular materials is uncoupled from cell division.

The hysteresis effect, diurnal variations, species differences and growth rate influence strongly indicate that the kinetics of photoadaptation is highly variable. Vertical distribution of photoadaptive variables reflects not only the light history of phytoplankton, but also the above factors. Therefore, in estimating vertical mixing with time scales comparable to photoadaptation of phytoplankton cellular components, these variables must be examined in the context of sampling time (diurnal cycle), species composition, and nutrient status (growth rate). In view that natural populations of phytoplankton would be expected to be more variable than the lab-cultured phytoplankton used in this study, it is concluded that the existing model of understanding vertical mixing through the measurements of photoadaptive characteristics is tenuous. In order to be used, very detailed "fine tuning" of photoadaptive characteristics is required.

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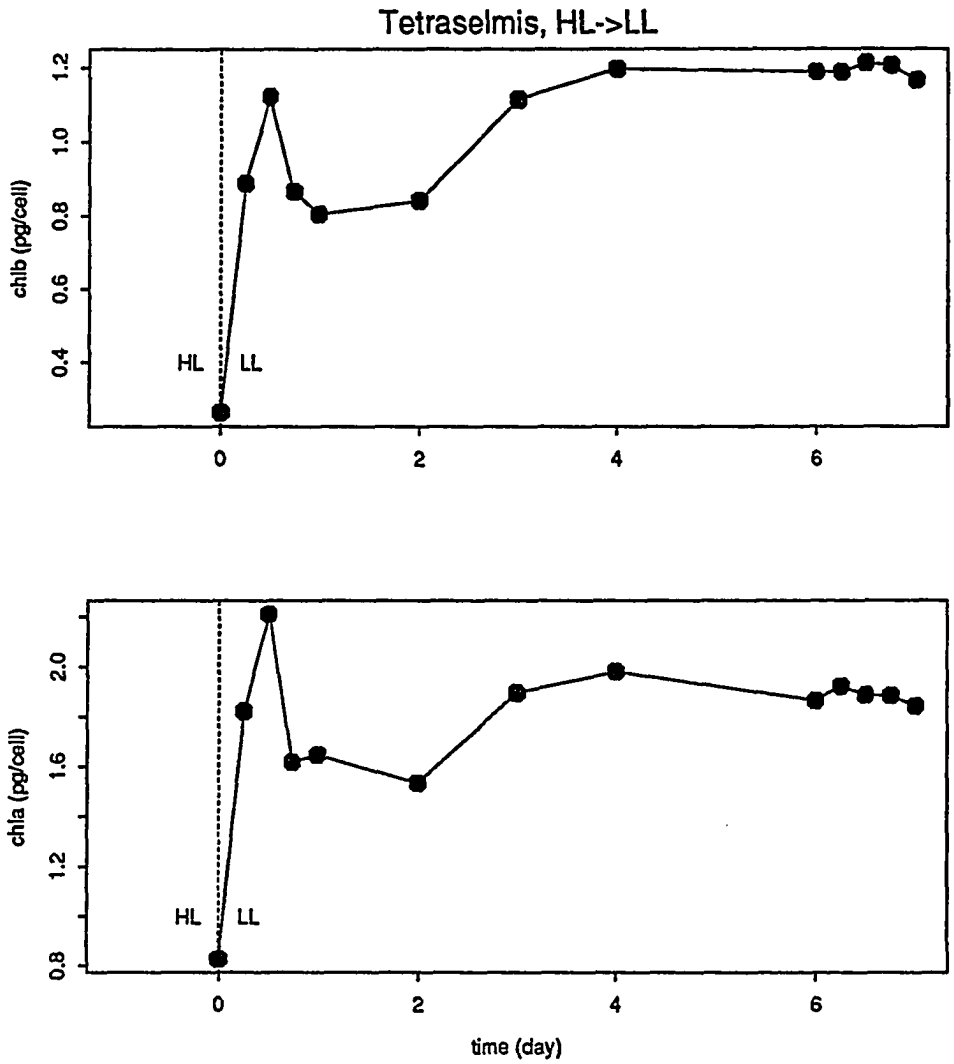
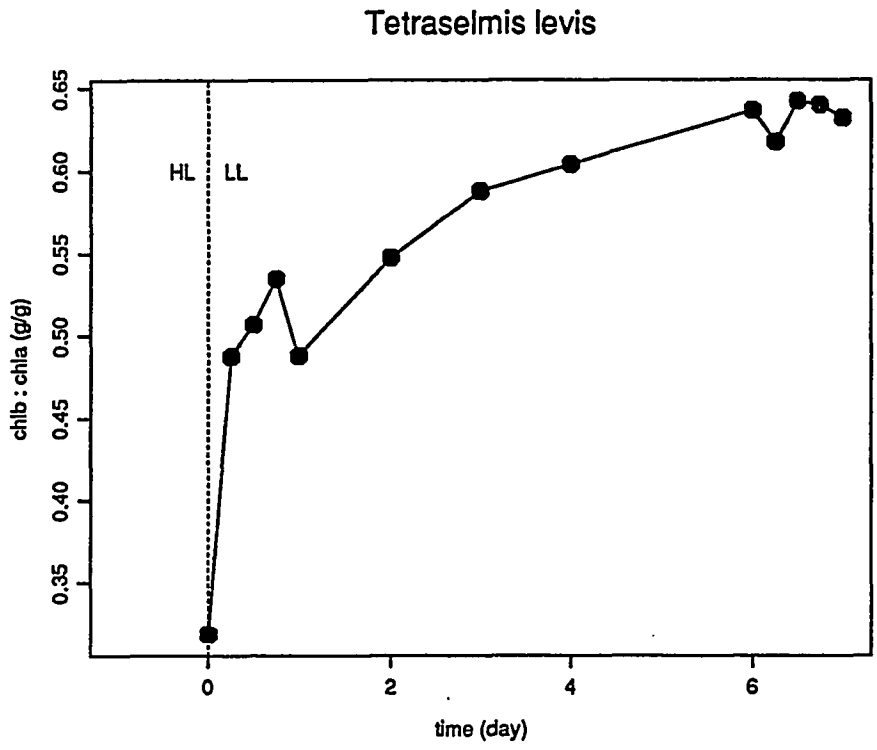
Appendix 1. Time course changes in pigment contents and pigment ratios in marine phytoplankton. (A) *Tetraselmis levis,* (B) *Isochrysis galbana,* (C) *Thalassiosira oceanica,* (D) *Skeletonema costatum*, and (E) *Skeletonema costatum* (f/60), following one–step changes in light intensity from high light to low light (HL–>LL) during the semi–continuous culture experiments. Nutrient enrichments are f/10 media unless otherwise noted. Light transitions occurred at time zero, which coincided with the beginning of the light period in a 12:12 L:D light regime. The interval of diurnal sampling during the first day (time 0~1) and last day (time 6~7) after light transition and one day before it (time –1~0), was 0.25 d (6 hr). All other sampling took place at the beginning of each light period. Pigment contents are in pg $cell^{-1}$; pigment ratios are in g g^{-1}.

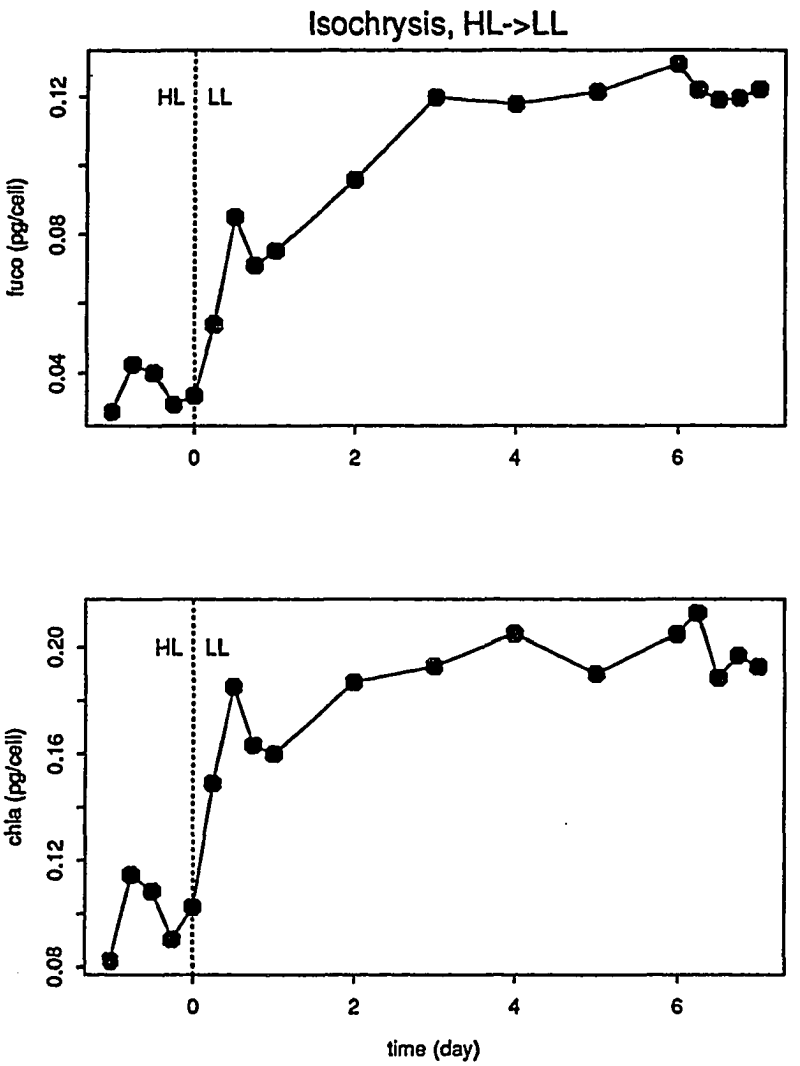
Appendix 1.A1



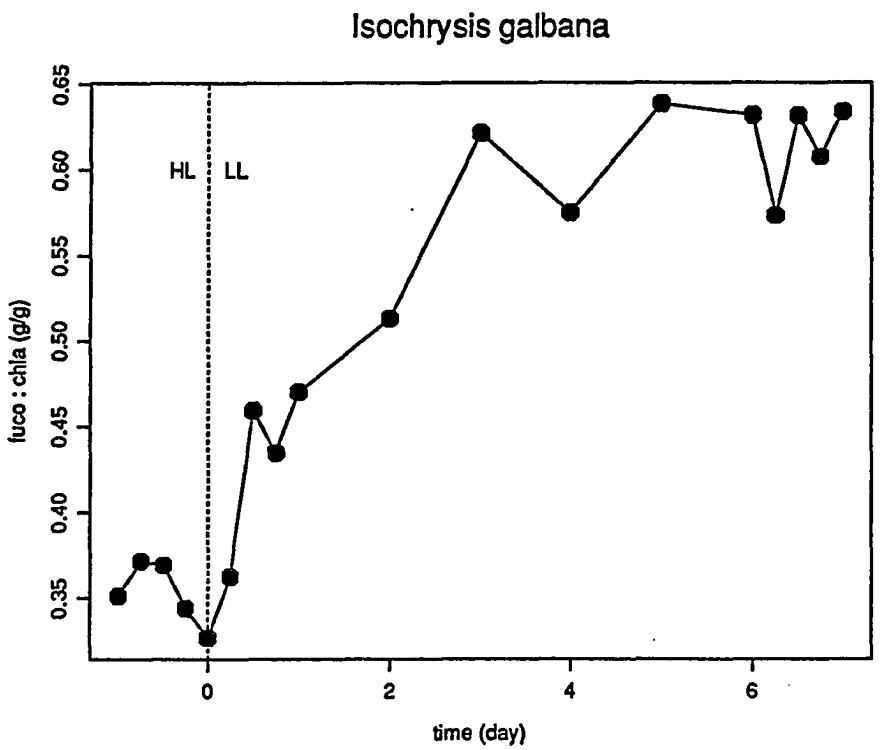
Appendix 1.A2



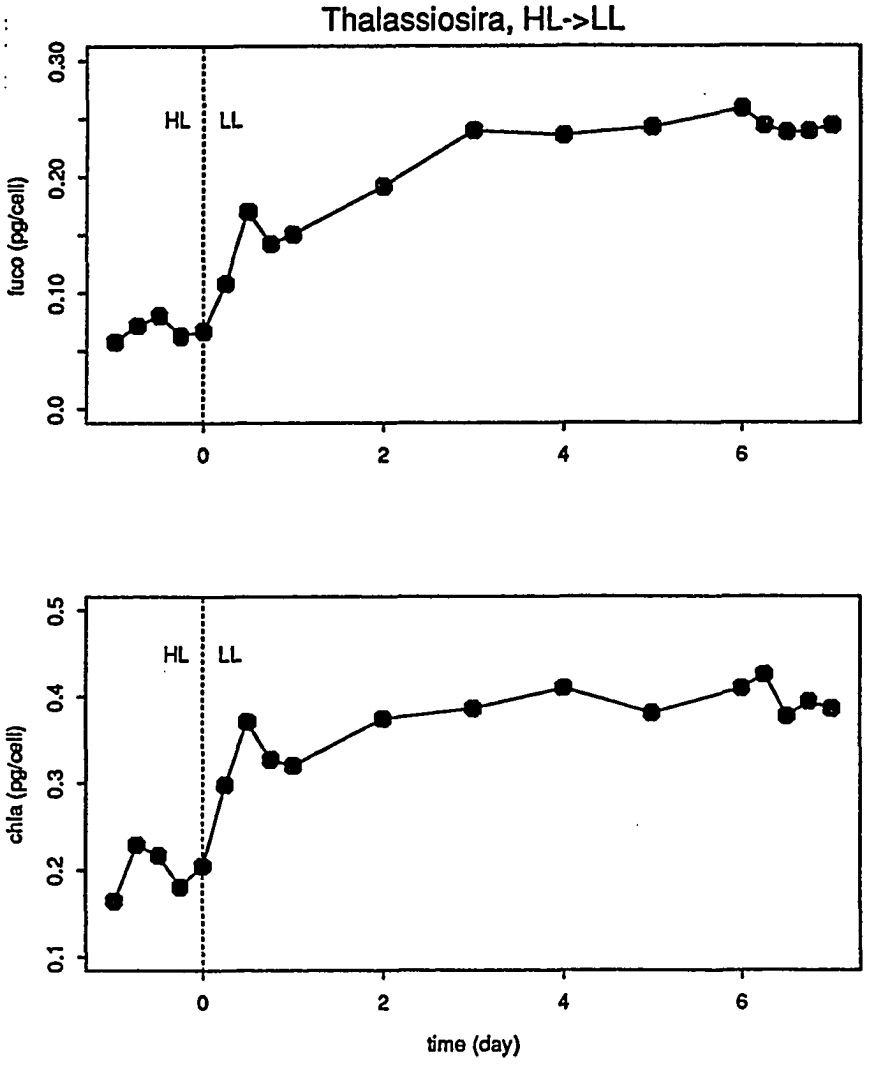
Appendix 1.B1



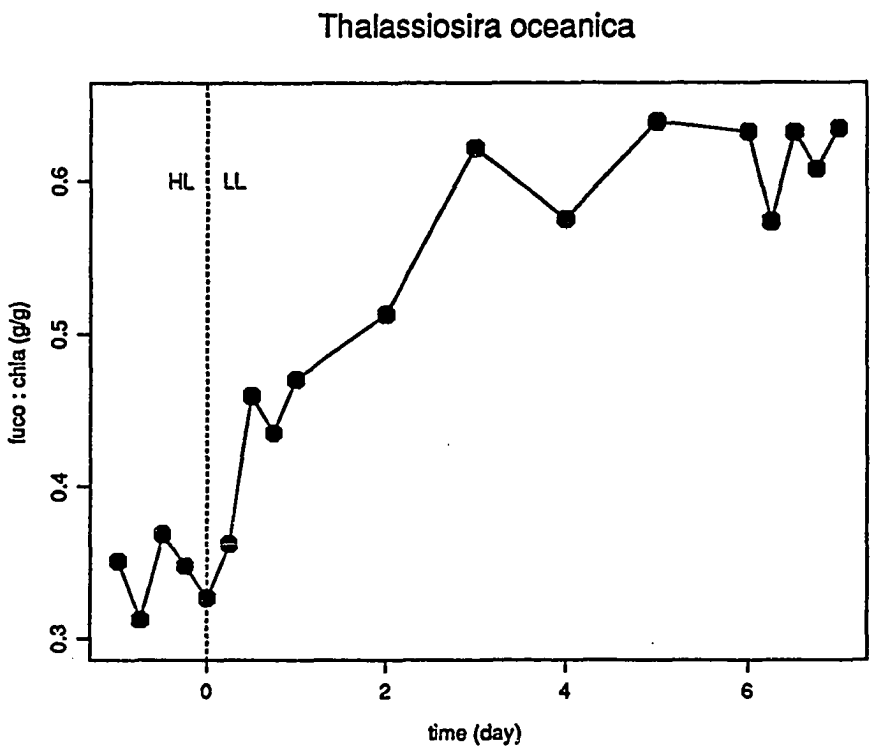
Appendix 1.B2



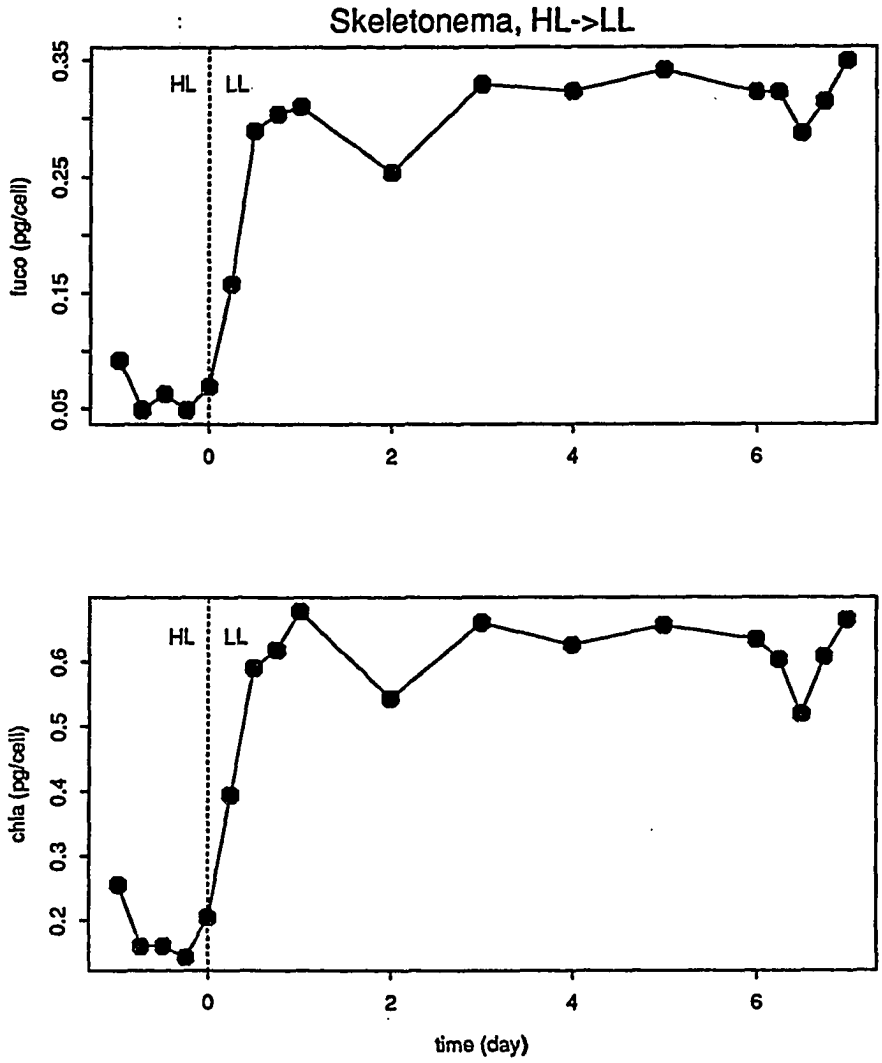
Appendix 1.C1



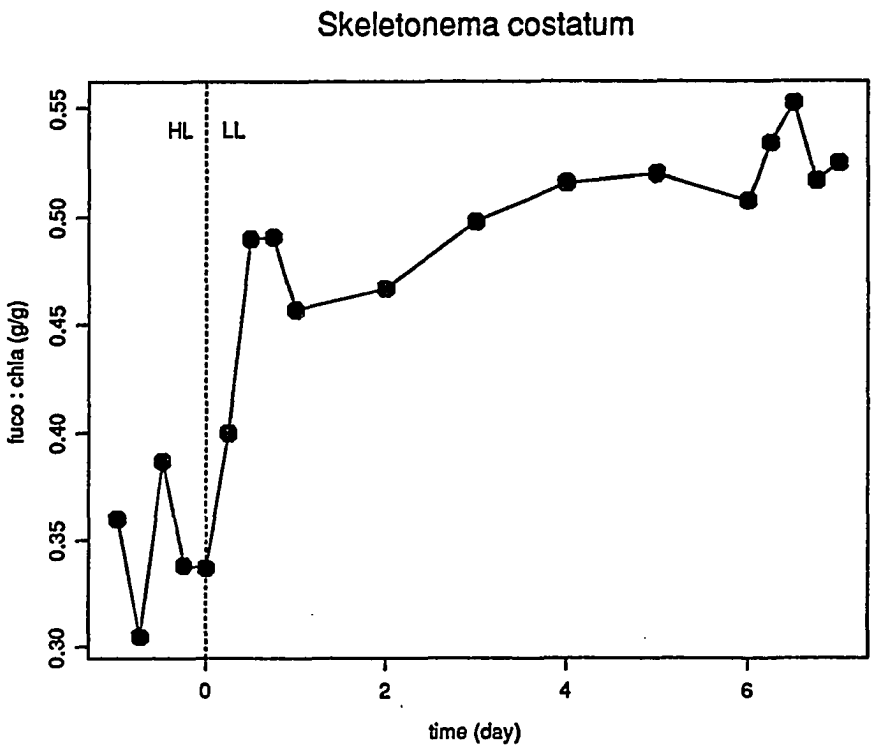
Appendix 1.C2



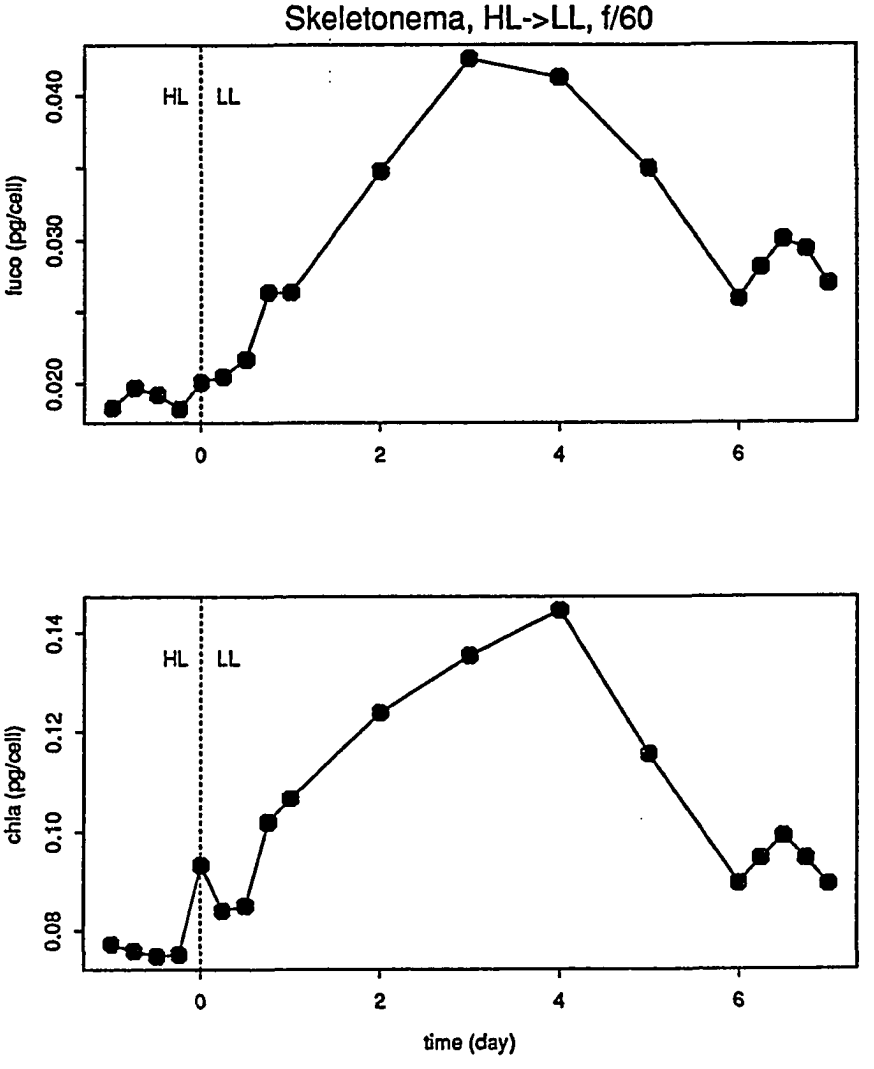
Appendix 1.D1



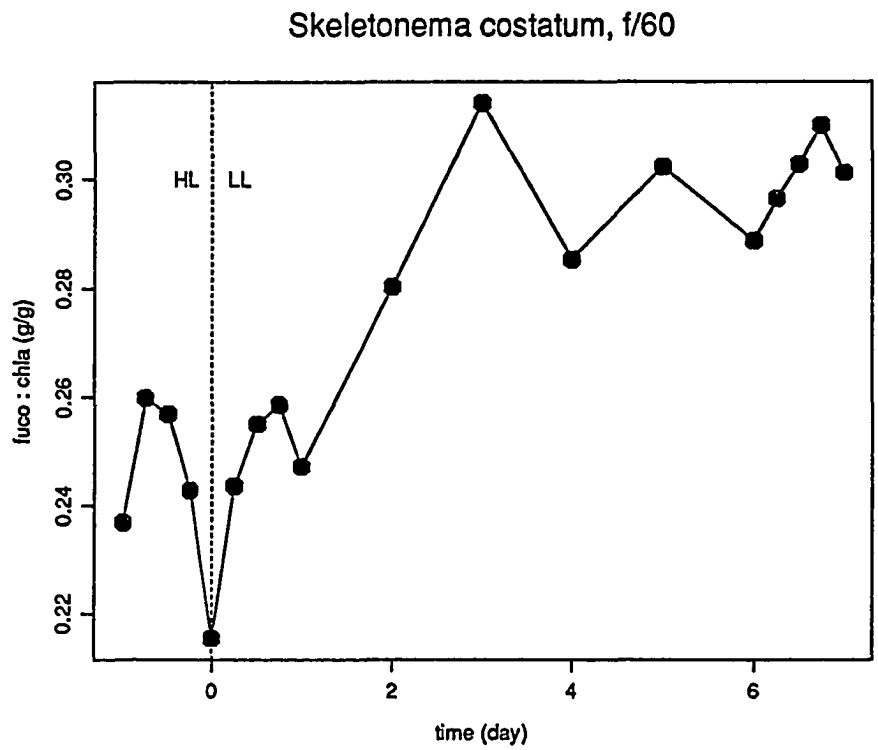
Appendix 1.D2



Appendix 1.E1

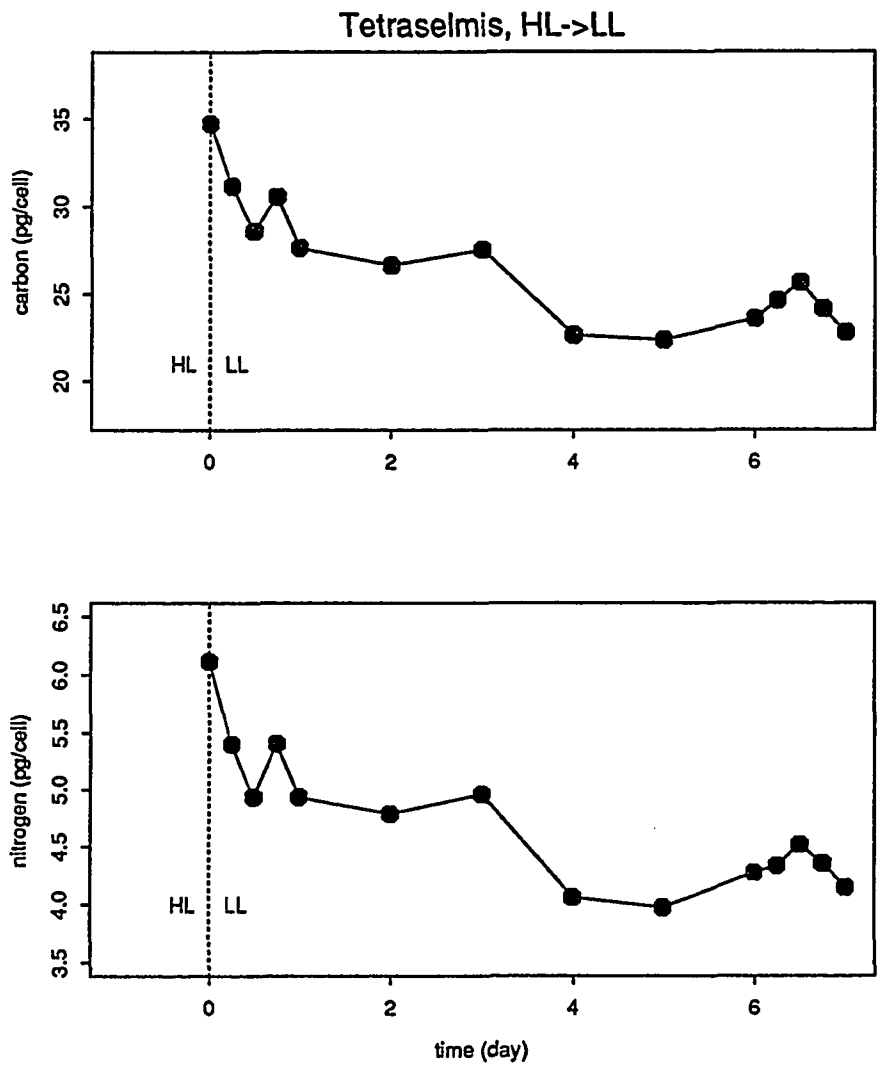


Appendix 1.E2

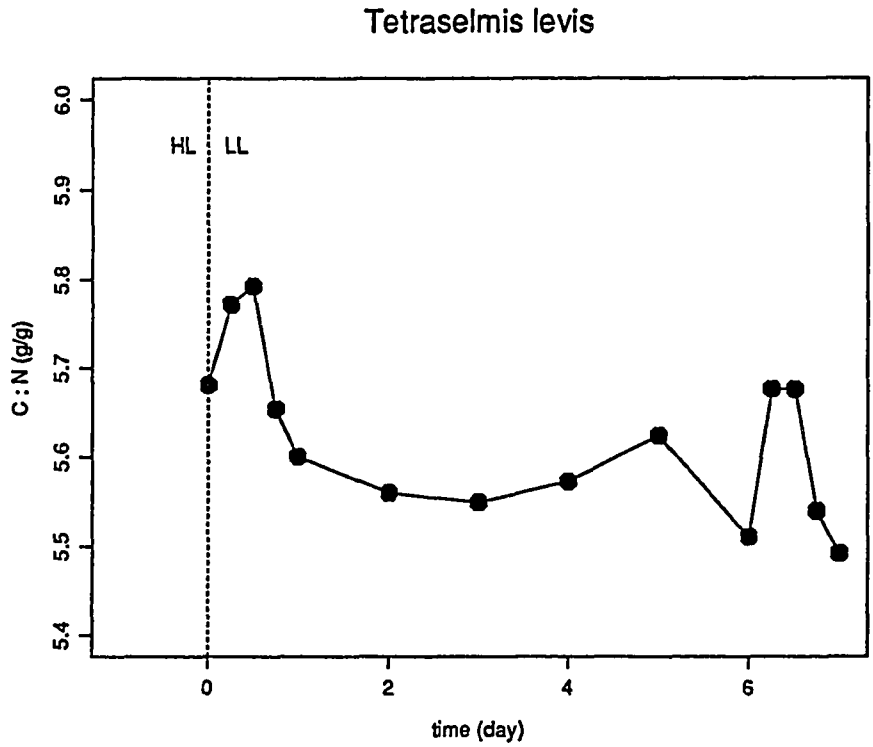


Appendix 2. Time course changes in carbon, nitrogen contents and C : N ratios in marine phytoplankton. (A) *Tetraselmis levis,* (B) *Synechococcus sp,* (C) *Prorocentrum micans,* (D) *Isochrysis galbana,* (E) *Thalassiosira oceanica,* (F) *Skeletonema costatum,* and (G) *Skeletonema costatum* (f/60), following one–step changes in light intensity from high light to low light (HL–>LL) during the semi–continuous culture experiments. Nutrient enrichments are f/10 media unless otherwise noted. Light transitions occurred at time zero, which coincided with the beginning of the light period in a 12:12 L:D light regime. The interval of diurnal sampling during the first day (time 0~1) and last day (time 6~7) after light transition and one day before it (time –1~0), was 0.25 d (6 hr). All other sampling took place at the beginning of each light period. Carbon, nitrogen contents are in pg $cell^{-1}$ (except in *Synechococcus sp* where fg $cell^{-1}$ is used); C : N ratios are in g g^{-1}.

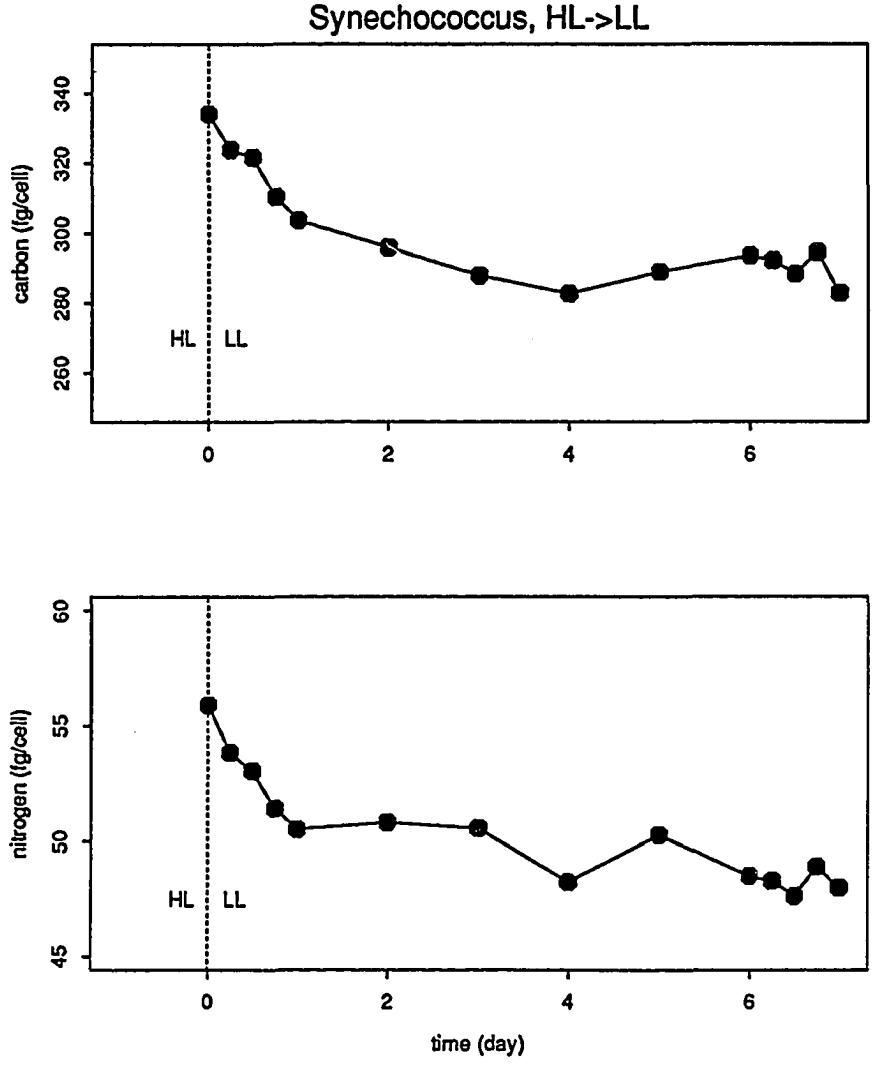
Appendix 2.A1



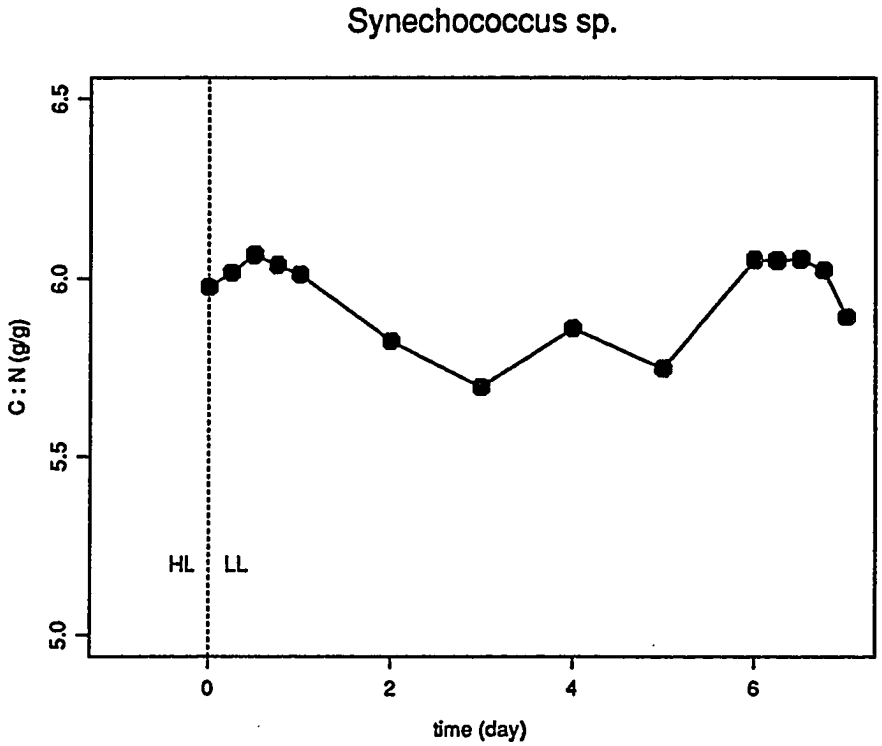
Appendix 2.A2



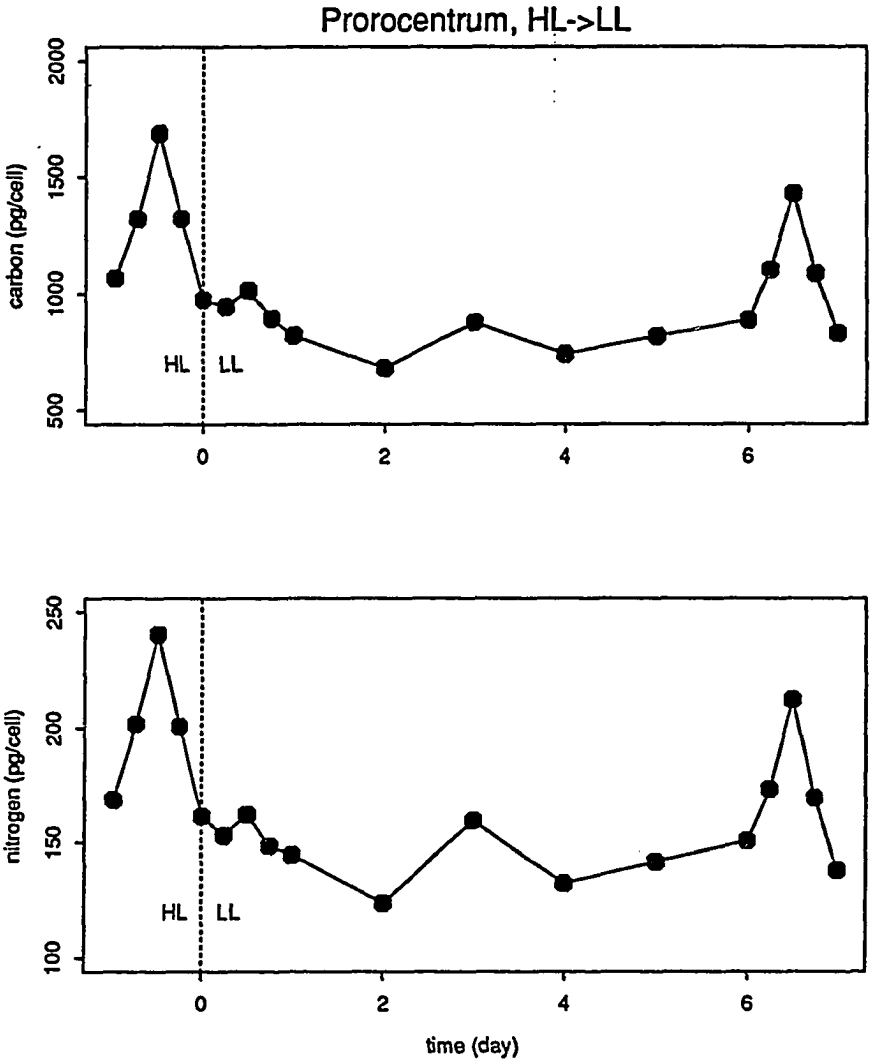
Appendix 2.B1



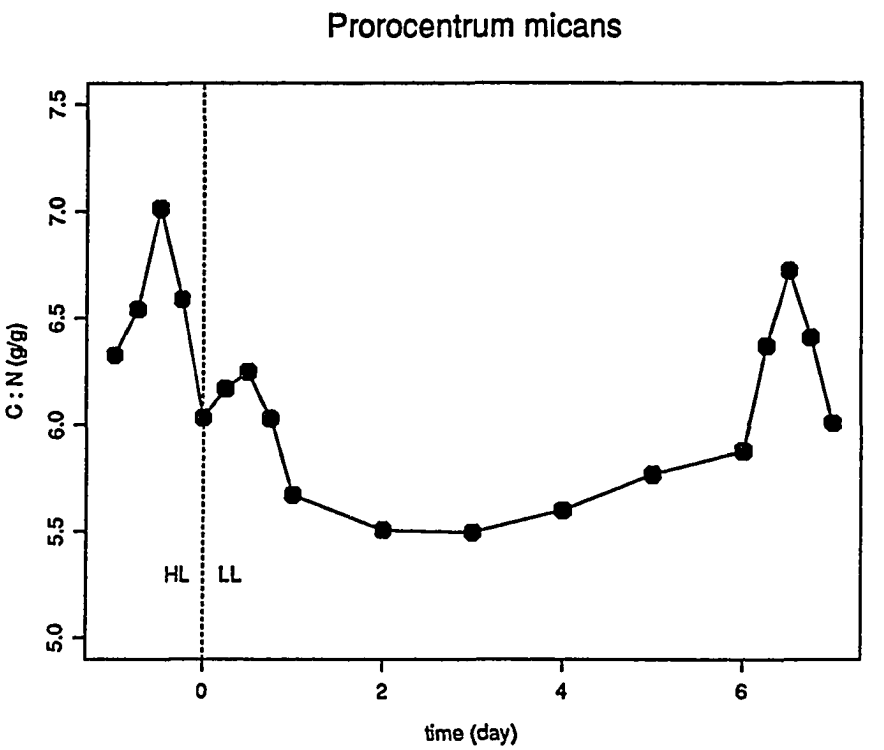
Appendix 2.B2



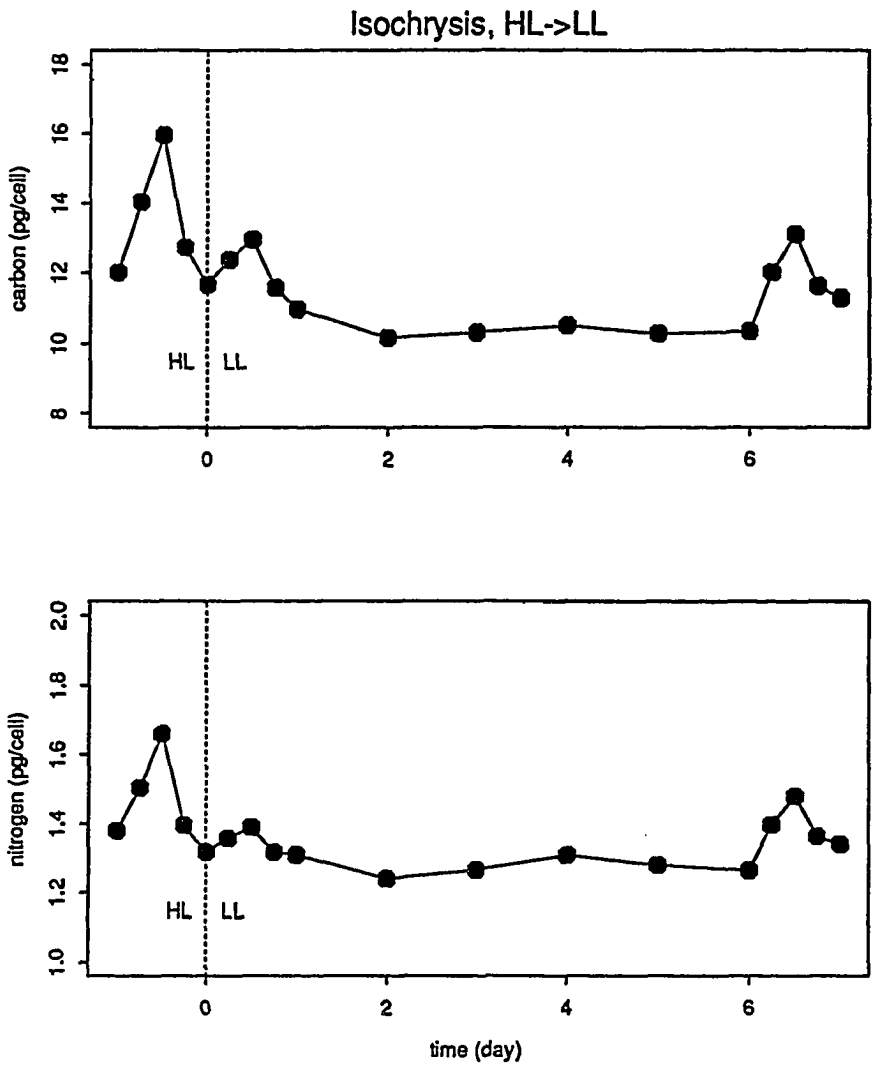
Appendix 2.C1



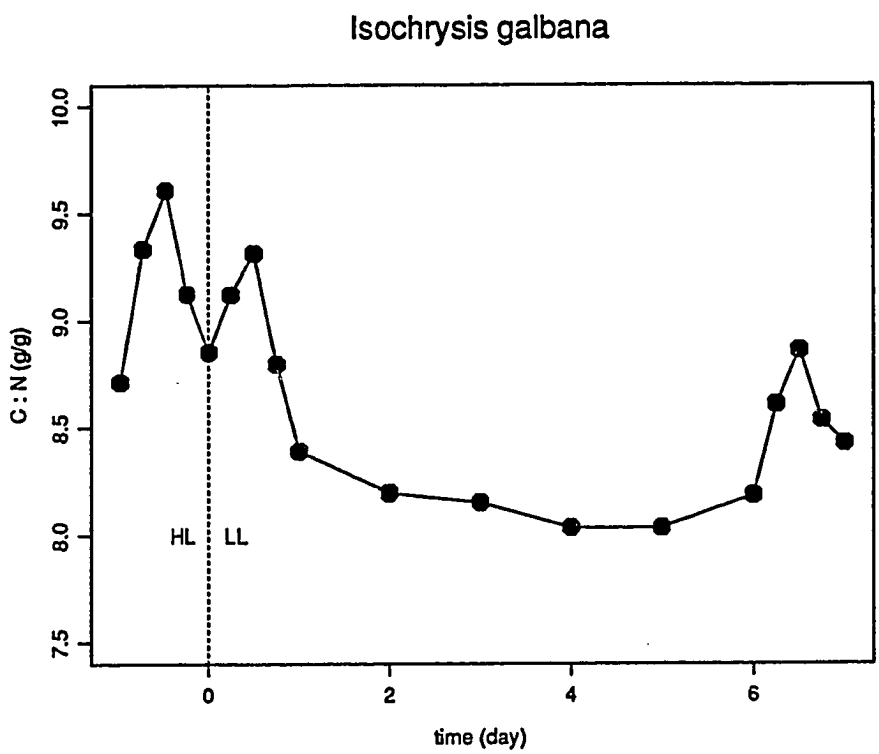
Appendix 2.C2



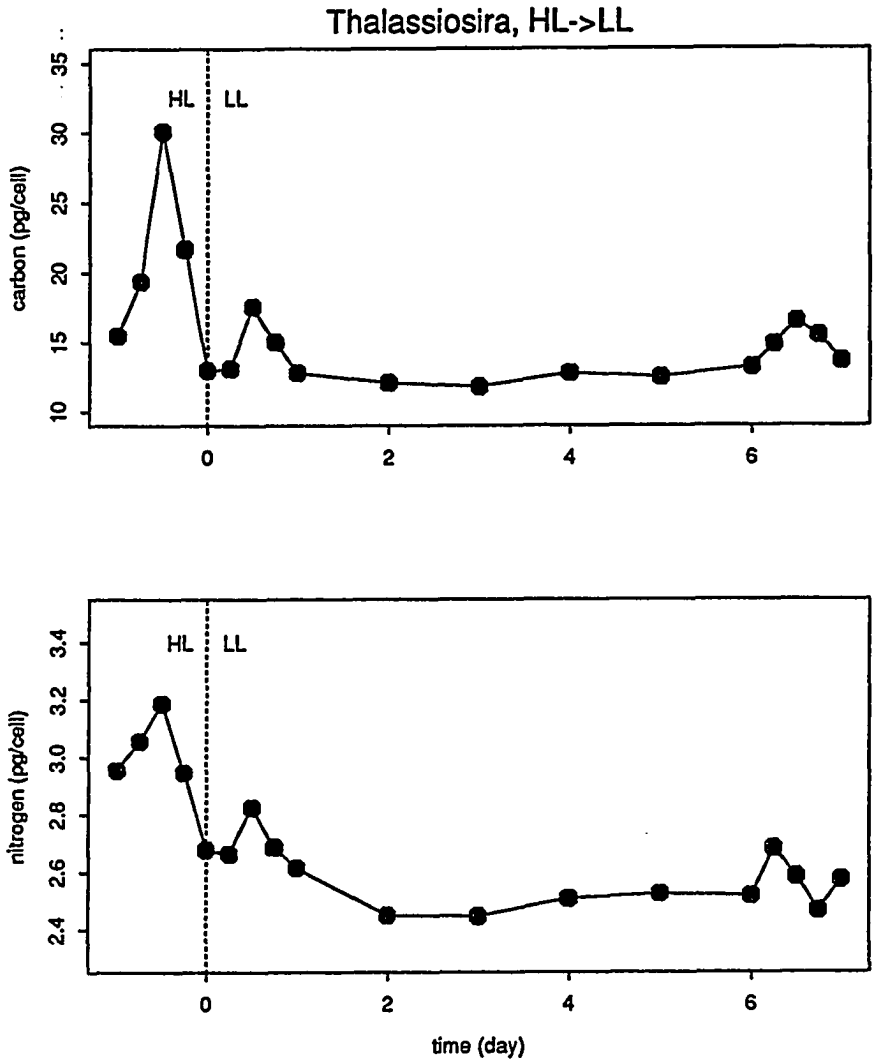
Appendix 2.D1



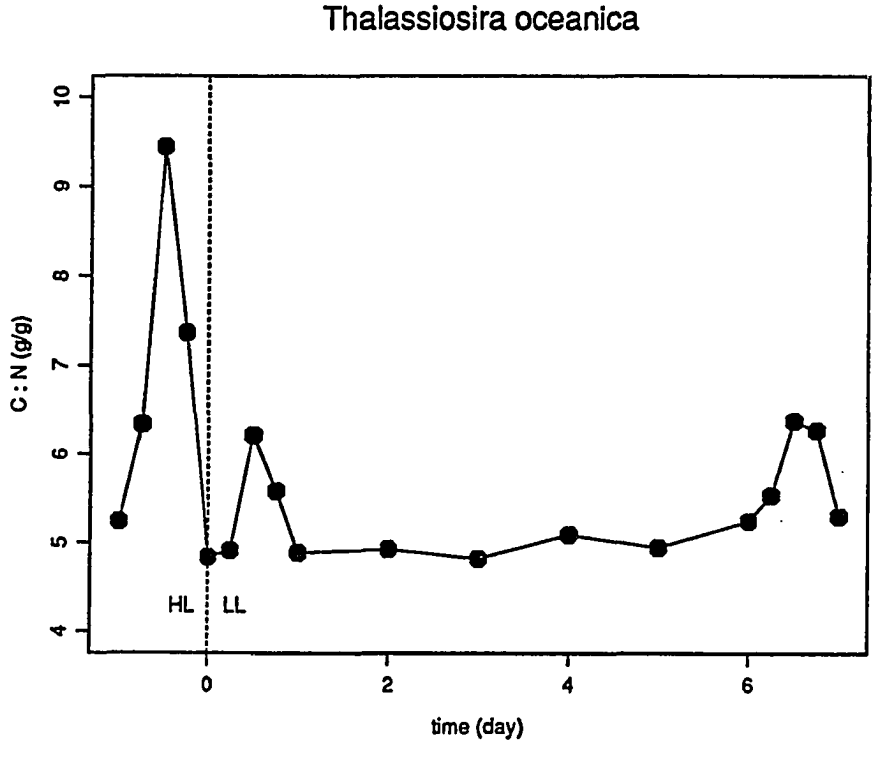
Appendix 2.D2



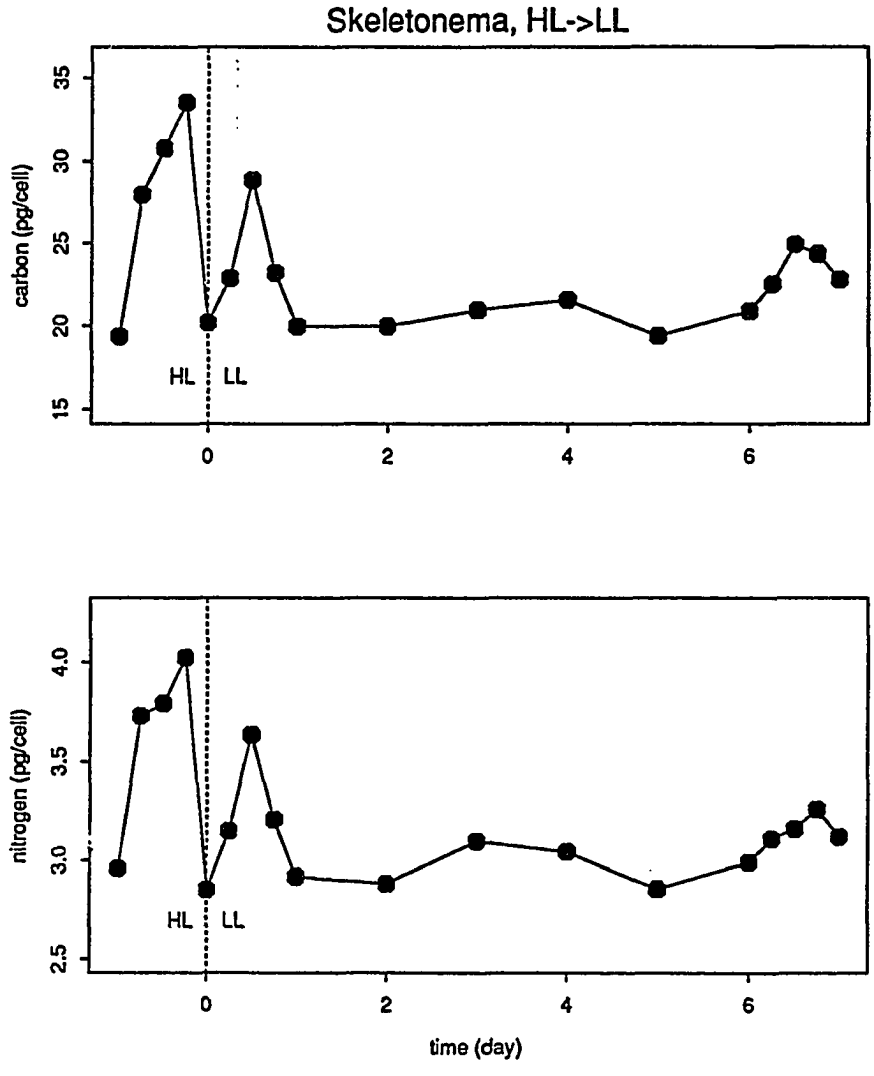
Appendix 2.E1



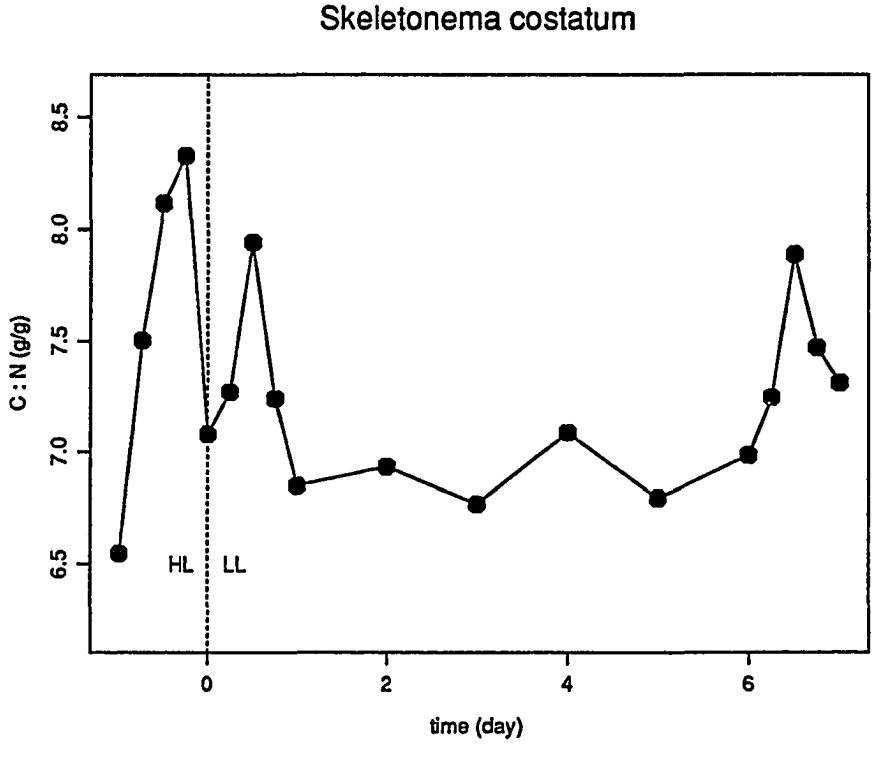
Appendix 2.E2



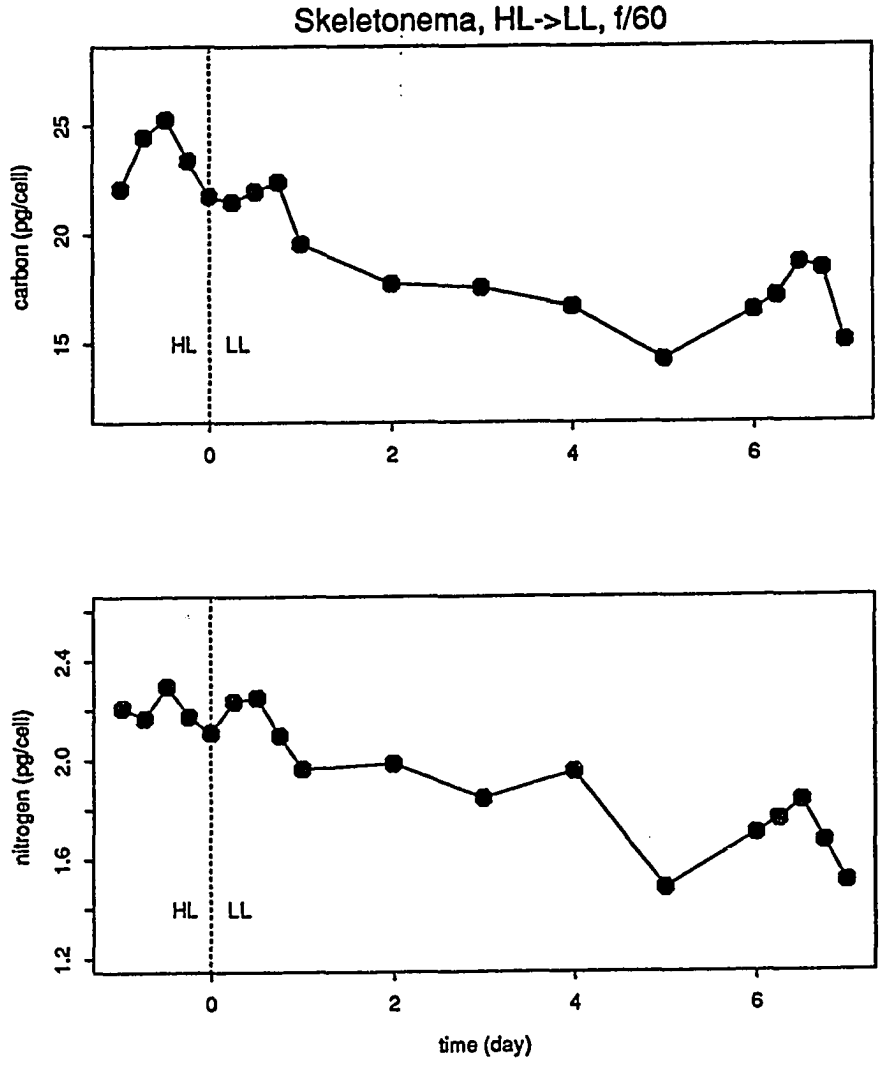
Appendix 2.F1



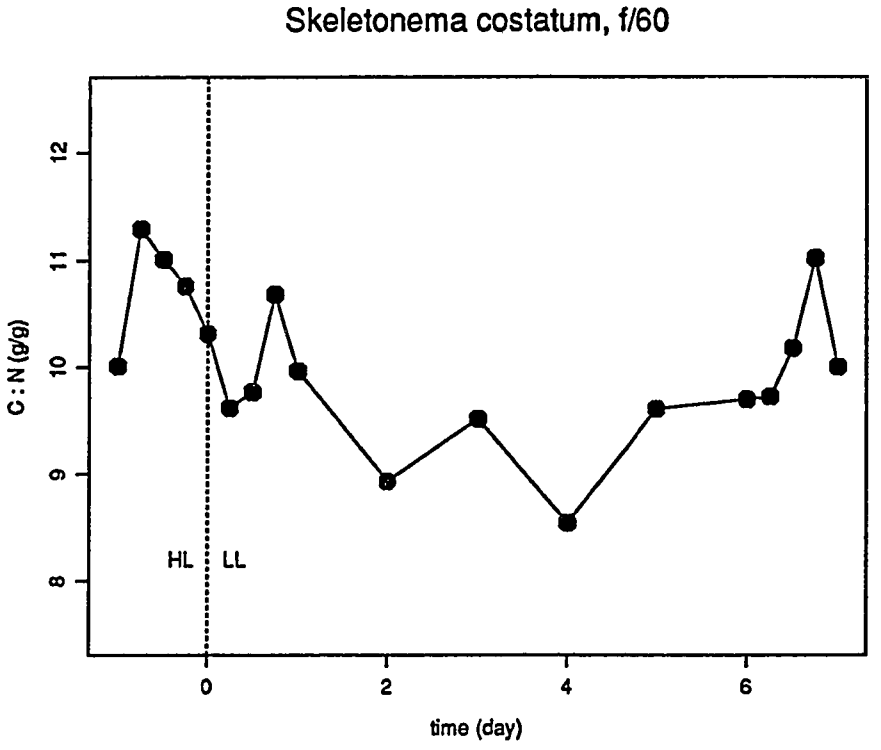
Appendix 2.F2



Appendix 2.G1

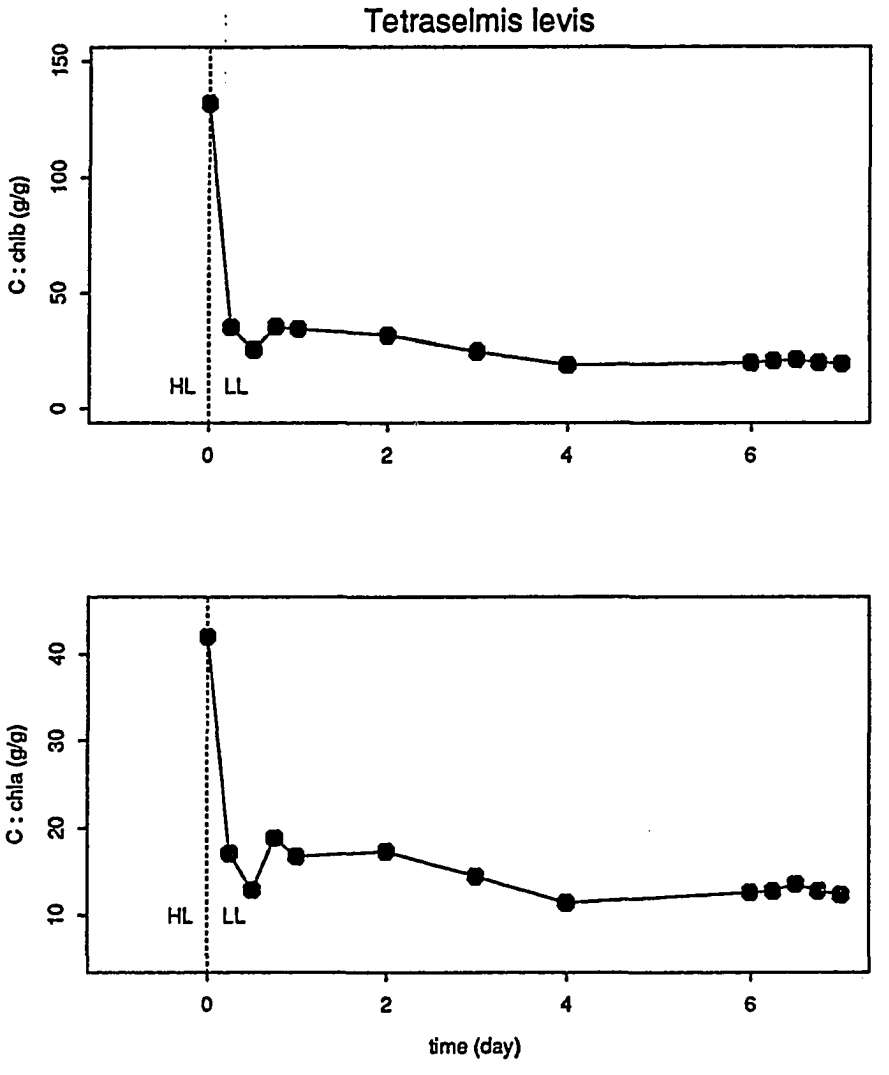


Appendix 2.G2

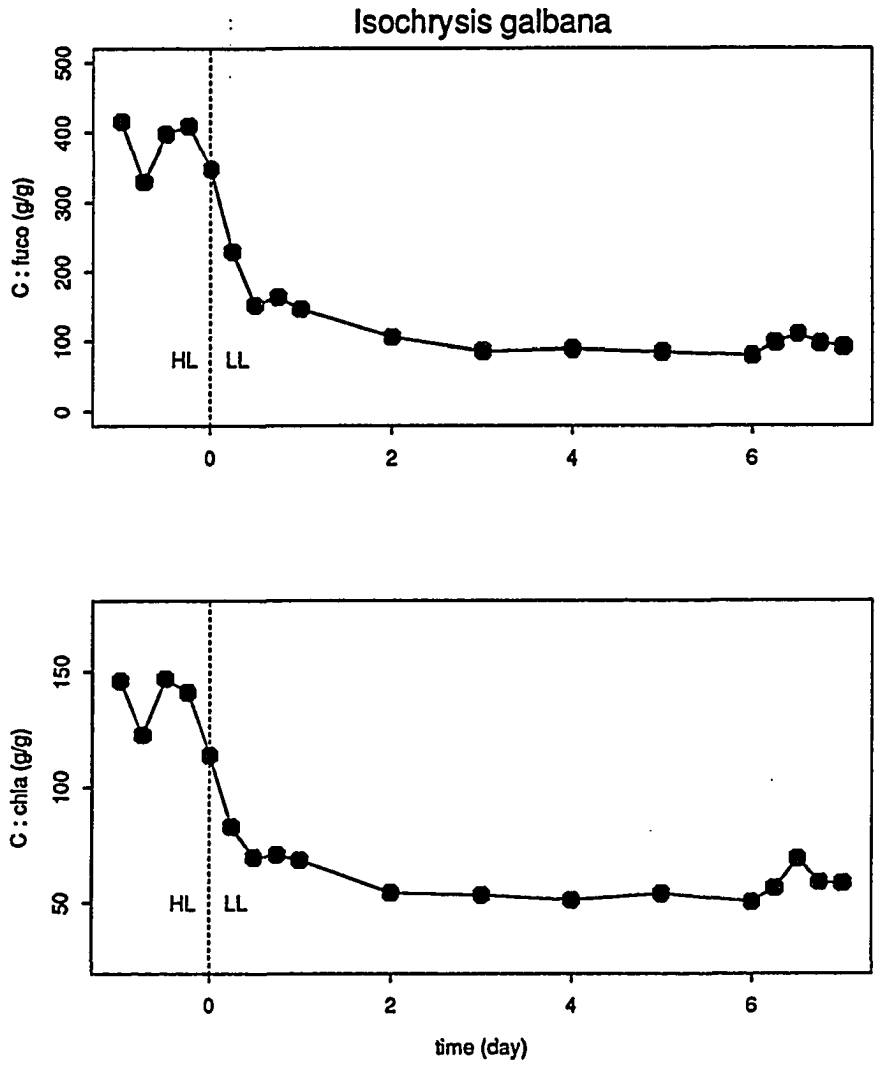


Appendix 3. Time course changes in C : pigment ratios in marine phytoplankton. (A) *Tetraselmis levis,* (B) *Isochrysis galbana,* (C) *Thalassiosira oceanica,* (D) *Skeletonema costatum,* and (E) *Skeletonema costatum* (f/60), following one–step changes in light intensity from high light to low light (HL–>LL) during the semi–continuous culture experiments. Nutrient enrichments are f/10 media unless otherwise noted. Light transitions occurred at time zero, which coincided with the beginning of the light period in a 12:12 L:D light regime. The interval of diurnal sampling during the first day (time 0~1) and last day (time 6~7) after light transition and one day before it (time –1~0), was 0.25 d (6 hr). All other sampling took place at the beginning of each light period. The ratios are in g g^{-1}.

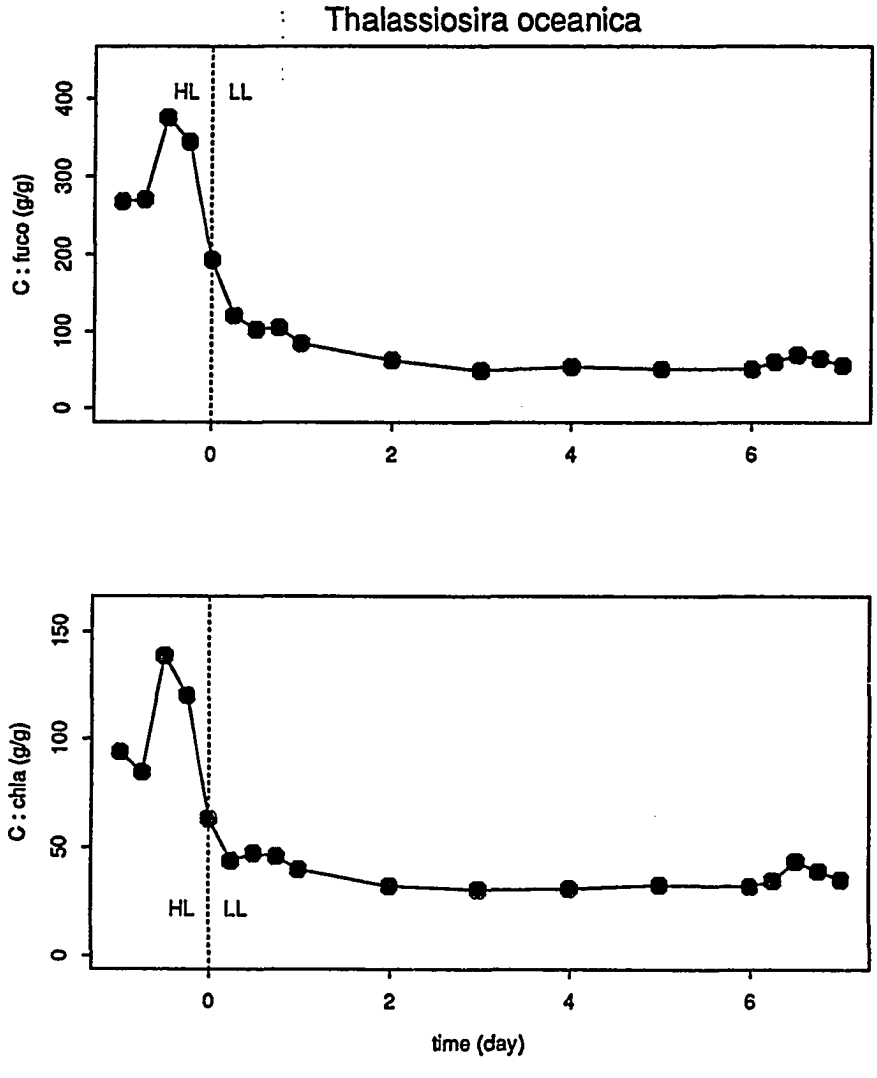
Appendix 3A



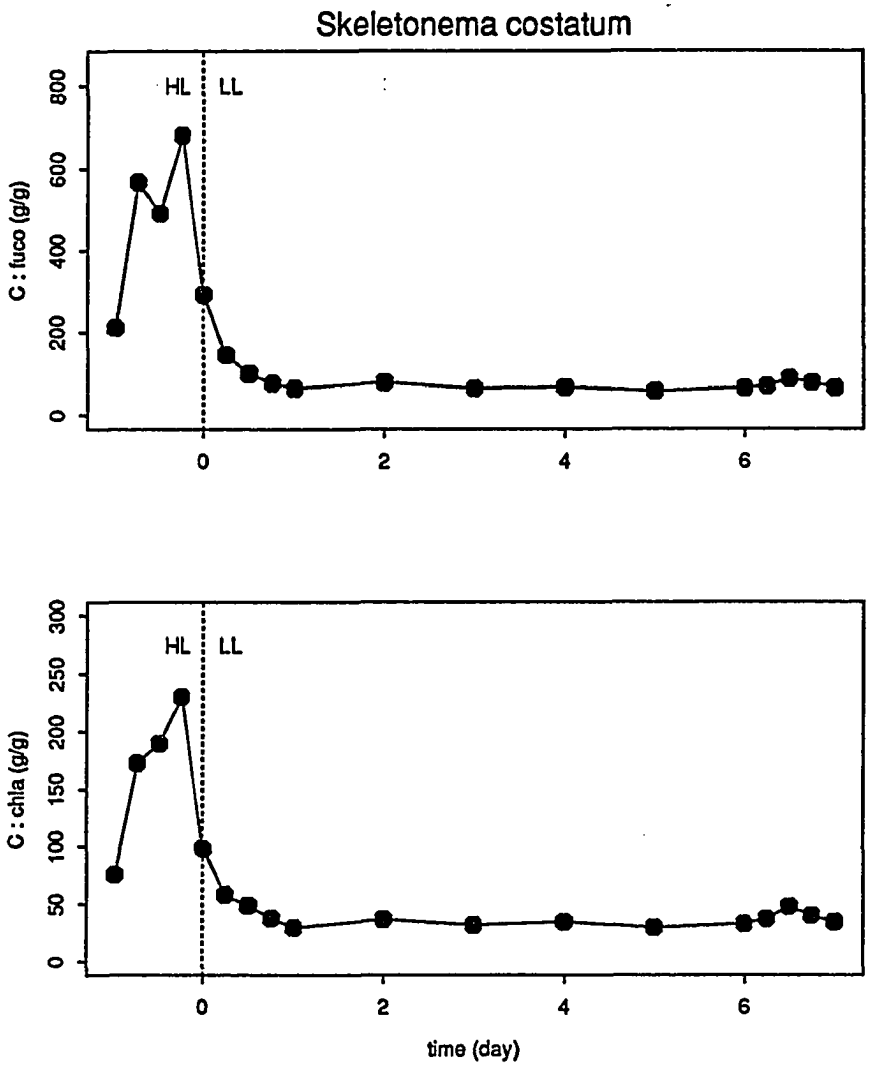
Appendix 3B



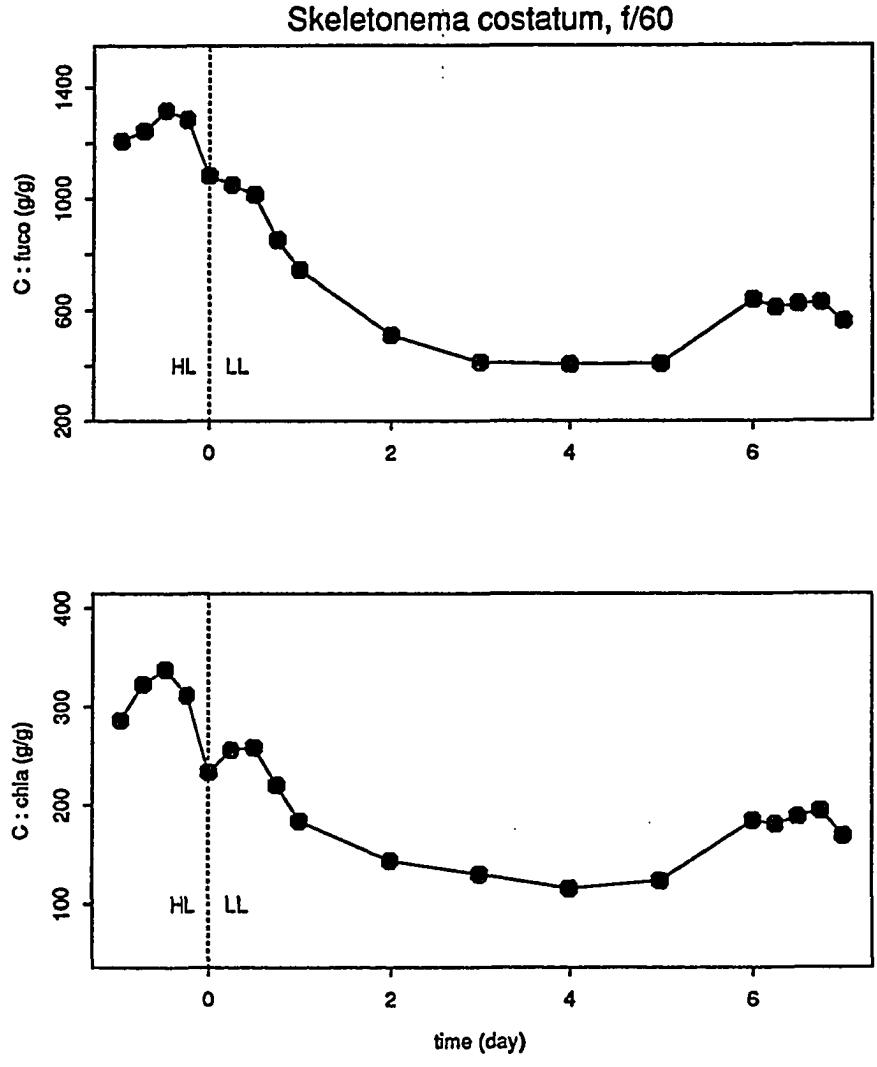
Appendix 3C



Appendix 3D



Appendix 3E



AUTOBIOGRAPHICAL STATEMENT

Chunzhi Guo was born on March 27, 1962 in Fujian Province of the People's Republic of China to Qingju Zhang and Wenjiao Guo.

Mr. Guo received his B.S. from Shanghai Fisheries College in July 1982 and was appointed immediately afterwards as an Assistant Lecturer at the very same college. He worked there for three years before leaving for the United States in 1985 to further his education. He first attended the University of Michigan at Ann Arbor, MI, for graduate studies in Natural Resources. He transferred in 1986 to Old Dominion University, Norfolk, VA, to study Biological Oceanography.

Mr. Guo is a member of American Geophysical Union, American Society of Limnology and Oceanography, and Phycological Society of America. He is also a member of the National Honor Society of Phi Kappa Phi.

Mr. Guo is awarded a postdoctoral fellowship from the National Research Council as a Resident Research Associate at the Southeast Fisheries Science Center, NMFS/NOAA, in Beaufort, N.C.

In 1988, Mr. Guo married Beirong Zhang, of Shanghai, China. They have a daughter, Ashley Zhang Guo, born in April, 1992.